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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

DATE: 1/13/00

SUBJECT: **TI-435** -- New Insecticide Seeking Use on Corn, Canola, Apple, and Pear. **Briefing Memorandum for Meeting of Metabolism Assessment Review Committee.**

DP Barcode: D260059
Submission #: S569419
Chemical#: XXXX
Trade Name: XXX
40 CFR: 180.XXX

PRAT Case#:292353
Caswell#:None
Class: Insecticide
EPA Reg#: Not Registered
MRID: None

TO: George Kramer, Chemist
MARC Executive Secretary
RAB1/HED (7509C)

FROM: Yan Donovan, Chemist
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INTRODUCTION

TI-435 is a new insecticide which was discovered by Takeda, a Japanese company. Takeda will register the active ingredient and the foliar uses of the compound, and Bayer will register the compound for seed treatment uses. For initial submissions, Takeda will submit a US registration for foliar applications on apples and pears, and Bayer will submit the registrations in the US and Canada for corn and canola seed treatment. Sugar beet registration is only intended for Europe.

At a pre-registration meeting with RD, representatives from the two companies presented the residue chemistry data and plant and animal metabolism studies, in the hope to seek EPA agreement and/or guidance on residue definition and crop residue methodology for TI-435. Information below are from that presentation. RAB2 seeks the Committee's opinion on the residue of concern for this active ingredient, which has not yet been submitted for registration. It is understood that any decision made at this stage is contingent upon complete review of the studies confirming the information presented in this memo.

1. Residue Chemistry Section

a. Use Information/Identification of Chemical

For seed treatment, TI-435 600 FS formulation will be used

For **canola**, the amount of ai/lb of seed would be 600 g ai/220 lb of seeds. The application rate would be 16.36 g ai/acre (**0.036 lb ai/acre**) based on a seeding rate of 6 lb. seeds/acre. For **corn**, the treatment rate is 2 mg ai/kernel. Based on a maximum planting rate of 35,000 seeds (kernels)/acre, the application rate would be 70 g ai/acre (**0.154 lb ai/acre**).

For Foliar Spray, TI-435 WDG50% (water dispersible granular) formulation will be used

For **apple/pear**, three foliar spray applications at 14-day interval, each application rate would be 75 g ai/ha, total 225 g ai/ha/year (**0.2 lb ai/acre/year**).

b. Summary of Plant, Livestock Metabolism Studies and

c. Rotational Crop Studies

Plant Metabolism Studies:

In the plant metabolism studies, TI-435 was applied as a foliar spray on apple and tomato, in a planting hole for tomato, and as a seed treatment for corn and sugar beet. In all plant metabolism studies (with the exception of the guanidine-label corn metabolism study), TI-435 was applied at rates which were 1.1X to 2.1X the recommended label use rate of TI-435.

Confined rotational crops study was performed using wheat, Swiss chard, and turnip planted in sets at 1, 5.1, and 10.5 months after treatment of the soil at a 1X rate.

A proposed metabolic path way in plants, along with chemical names and structures of TI-435 and its metabolite identified, is in attachment 1(electronically not available).

Total radioactive residues (TRR) and extractability of crop residues are presented in Attachment 2. Summary of the characterized and identified ¹⁴C-residues in plant matrices is presented in Attachment 3. Details of the plant metabolism studies are in Attachment 4. (Electronically not available).

TI-435 was the major residue found in all plant matrices (except for the sugar beet leaves and some samples from the rotational crop study). In the rotational crop study, TI-435 was a major residue in all matrices except the wheat grain. Although MG was found in significant levels in sugar beet leaves following treatment with TI-435, literature review had shown that MG was also naturally occurring in citrus seed, soybean, tea plant, fresh and smoked fish, chicken, pork, and as an oxidative degradation product in mammals.

Although TZNG and MNG (both metabolites probably also formed in soil) were found in wheat grain and in Swiss chard at levels of 23% (0.03 ppm) and 28% (0.07 ppm), respectively, these two metabolites were present at very low levels in human consumables (<0.001 to 0.07 ppm).

TMG was only found in significant amounts in sugar beet leaves (27% of the TRR, 0.239 ppm). TZMU, NTG, TZU, and UK5 were minor plant metabolites.

Based on the results of the metabolism study, the two companies proposed that TI-435 be considered as the only residue of concern in plants and, hence, the only analyte to be measured in the TI-435 crop residue analytical method, for the following reasons:

1. TI-435 was the predominant residue in crops.
2. Although MG, MNG, and TZNG were found at >10% of the TRR in several crops, these metabolites would not pose an unreasonable health risk since their levels in human consumables were quite low (<0.001 to 0.07 ppm). In the case of MG, the maximum levels found in human food following the use of TI-435 (at the maximum label use rates) would be approximately 17,800 times less than the naturally occurring levels of MG in smoked fish.

RAB2 notes that some of the metabolites (TZNG, MG, and MNG) were found at significant levels (expressed as percent of total radioactive residues) in rotational crops, especially in rotated sugar beets. Even though the registrants do not intend to initially register TI-435 for use on sugar beet in the USA and Canada, the significant levels of MG and TMG in sugar beet leaves

could indicate a potential for significant levels of these metabolites in succeeding (rotational) crops.

Nature of the Residue in Rats

In the rat metabolism experiments, >90% of the orally administered dose was excreted in urine within 24 hours. Radioactivity remaining in tissues and organs at sacrifice (72 hours for the low dose experiment) was $\leq 0.1\%$ of the administered dose. The major residues identified in the excreta were TI-435, MNG, and TZNG. The metabolic profile of TI-435 in rats was similar to the profile found in the plant metabolism studies. The proposed metabolic pathway in rat is depicted in Attachment 5. (Electronically not available).

In a preliminary rat metabolism experiment, 8-week, male Wistar rats were given a single oral dose of [^{14}C] TI-435 at a rate of 5 mg/kg body weight. Blood, heart, kidney, liver, lung, spleen, and urine were taken (at sacrifice) at 4 hours following oral dosing. MG and TMG (both plant metabolites which were found as minor metabolites in rat excreta) were found in significant quantities in the liver. Table 1 shows the Percentage distribution of radioactivity in tissues of male rats 4 hours¹ after a single oral administration of nitroimino- ^{14}C -TI-435 at the rate of 5 mg/kg. MG and TMG (both plant metabolites which were found as minor metabolites in rat excreta) were found in significant quantities in the liver.

Table 1. Percentage distribution of radioactivity in tissues of male rats 4 hours¹ after a single oral administration of nitroimino- ^{14}C -TI-435 at the rate of 5 mg/kg

Metabolite	Percent of TRR in Sample at 4 Hours						
	Blood	Heart	Kidney	Liver	Lung	Spleen	Urine
TI-435	86.1	88.7	87.3	47.1	89.0	89.2	78.2
TZNG	3.1	5.9	6.1	4.6	4.0	4.2	8.8
TZMU	0.1	ND ²	ND ²	0.4	0.7	0.3	ND ²
MNG	6.1	4.8	5.2	2.0	5.7	6.0	9.7
NTG	ND ²	ND ²	ND ²	ND ²	ND ²	ND ²	1.7
TMG	ND ²	ND ²	ND ²	24.3	ND ²	ND ²	ND ²
MG	ND ²	ND ²	ND ²	7.4	ND ²	ND ²	ND ²
UK-Ni-1	ND ²	ND ²	ND ²	0.3	ND ²	ND ²	ND ²
Origin ³	ND ²	ND ²	ND ²	5.5	ND ²	ND ²	1.6
Extractable- ^{14}C	95.4	99.4	98.6	91.6	99.4	99.7	100.0
Nonextractable- ^{14}C	4.6	0.6	1.4	8.4	0.6	0.3	-
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

¹ The maximum concentration of ^{14}C in blood occurred at 4 hours following oral administration of [^{14}C] TI-435.

² ND: not detected in this matrix

³ Radioactivity at the origin on the TLC.

Summarizing the plant and rat metabolism studies, the residue levels of all the plant metabolites in human consumables are very low (<0.01 ppm to 0.07 ppm; Table 2). Only MNG and TZNG (both of which are major rat metabolites) would be expected to occur at levels between 0.03 and 0.07 ppm. All other plant metabolites are expected to be found at levels equal to or less than 0.01 ppm in human consumables which would be below the limit of quantitation of the crop residues analytical method.

Table 2. Residues in rats and human consumables from corn, confined rotational crops, tomato (with soil application), sugar beet, and apple, following treatment with TI-435.									
Crop/Matrix	Ppm Residues Expressed as TI-435 Equivalents								
	TRR at 1X ¹	TI-435	MNG	TZNG	MG	TZMU	NTG	TMG	UK-5
CORN Kernels	0.01 - 0.03	0.01	<0.01	<0.01	<0.01	<0.01	n.d. ²	<0.01	n.d. ²
ROT. CROP Wheat Grain	0.04 - 0.12 ³	<0.01	<0.01	0.03	<0.01	n.d. ²	<0.01	<0.01	n.d. ²
Swiss Chard	0.12 - 0.26 ³	0.05	0.07	0.03	0.01	0.01	0.01	0.01	n.d. ²
Turnip Roots	0.01 - 0.02 ³	0.01	<0.01	<0.01	n.d. ²	<0.01	n.d. ²	n.d. ²	n.d. ²
Tomato Foliar	0.54	0.52	n.d. ²	n.d. ²	n.d. ²	n.d. ²	n.d. ²	n.d. ²	n.d. ²
Soil	0.01	0.01	<0.01	<0.01	n.d. ²	n.d. ²	n.d. ²	n.d. ²	n.d. ²
Sugar Beet Root	0.03	0.01	<0.01	<0.01	<0.01	<0.01	n.d. ²	<0.01	n.d. ²
Apple	0.06	0.03	n.d. ²	<0.01	n.d. ²	0.01	n.d. ²	n.d. ²	<0.01
RAT⁴		55 to 73	14	11	0.4	0.5	4	0.3	0.2

¹ Total radioactive residues extrapolated to 1X rate.

² Metabolite was not detected in this matrix.

³ Total radioactive residues for all three plant-backs (rotations).

⁴ Rat data are expressed as percent of administered dose

Conclusion

Based on the residue chemistry, rat metabolism, and available toxicology data, Bayer and Takeda would like to propose to measure only TI-435, the major plant residue, as the residue of concern in crops matrices. In the event of misuse of TI-435 on crops, TI-435 will be the predominant residues in most crop matrices.

d. Residue Analytical Methods

The registrants had developed a plant residue analytical method with liquid chromatography-mass spectrometer/mass spectrometer (lc-ms/ms) detection that requires less than 24 hours to analyze a set of sample, uses very small quantities of solvent, and employs the same extraction scheme as were used in the plant metabolism studies. Attachment 6 shows the detailed steps of this residue method. This method detects the parent compound (TI-435) only. At the pre-registration meeting, RAB2 stated that for the determination of other metabolites, HED might consider a marker compound concept where a multiplication factor could be used to obtain total residues of concern for risk assessment purposes. Subsequent to the meeting, the registrant calculated some correction factors for different crops for total residue determination, which are shown in Table 3 below.

Table 3. Percent of TRR in crop matrices that will be measured by the proposed TI-435 Crop residue analytical method and the correction factors needed to obtain total residues of TI-435 in crops.		
Crop	Percent of TI-435	Correction Factor
Rotational Crops ¹		
Wheat		
Wheat Forage	21 - 46	2.2 - 4.8
Wheat Hay	13 - 17	5.9 - 7.7
Wheat Straw	7 - 12	8.3 - 14.3
Wheat Grain	2 - 3	33.3 - 50.0
Swiss Chard	21 - 35	2.9 - 4.8
Turnips		
Turnip Tops	21 - 32	3.1 - 4.8
Sugar Beet ²		
Leaves	4 - 61	1.6 - 25.0
Roots	24 - 68	1.5 - 4.2
Corn		
Forage	42 - 62	1.6 - 2.4
Stover	18 - 38	2.6 - 5.6
Kernels	18 - 43	2.3 - 5.6
Apple	62	1.6
Tomatoes		
Spray Application	97	1.0
Soil Application	66	1.5

¹ Percent values from samples taken from all three rotations.

² Residues from samples taken at 43, 50, and 143 days posttreatment

Residue Analytical Methods - Animal Commodities No residue analytical method for animal tissues, milk, and eggs is available at this time.

e. Multiresidue Method

TI-435 has not been evaluated according to Protocol A and C.

f. Crop Field Trials and Livestock Feeding studies

Not available.

g. International Considerations

The Codex Alimentarius Commission, Mexico, and Canada have not established maximum residue limits (MRLs) for residues of TI-435 in/on plant and animal commodities.

2. Toxicology Section

Summary of Toxicology Data for the Major TI-435 Plant Metabolites

1.0 Reverse Mutation in five Histidine-requiring strains of *Salmonella typhimurium*

1.1 MG: Reverse Mutation in five Histidine-requiring strains of *Salmonella typhimurium*

Methyl guanidine was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments.

An initial toxicity range-finder experiment was carried out in strain TA100 only, using final concentrations of Methyl guanidine at 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. Following these treatments, no evidence of toxicity (as normally indicated by a thinning of the background bacterial lawn and/or a marked reduction in revertant numbers) was observed either in the presence or in the absence of S-9. These range-finder results were used to provide the TA100 mutagenicity data for Experiment 1. Experiment 1 treatments of the remaining test strains were performed using the same dose-range. Following these treatments once again no evidence of toxicity was observed.

Experiment 2 treatments were performed with the maximum treatment concentration of 5000 µg/plate retained. Narrowed dose intervals were employed in order to more closely investigate those doses of Methyl guanidine considered most likely to induce any mutagenic response. In addition, all treatments in the presence of S-9 employed a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay. Following these Experiment 2 treatments, once again no evidence of toxicity was apparent in any of the test strains.

The test article was completely soluble in the aqueous assay system at all concentrations treated, in each of the experiments performed.

Negative (solvent) and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments.

No Methyl guanidine treatment of any of the test strains produced an increase in revertant numbers sufficient to be considered as indicative of mutagenic activity.

It was concluded that Methyl guanidine did not induce mutation in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102), when tested under the conditions employed for this study, which included treatments up to 5000 µg/plate, both in the absence and in the presence of a rat liver metabolic activation system (S-9).

1.2 TZMU: Reverse Mutation in five Histidine-requiring strains of *Salmonella typhimurium*

TZMU was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments.

An initial toxicity range-finder experiment was carried out in strain TA100 only, using final concentrations of TZMU at 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. Following these treatments, no evidence of toxicity (as normally indicated by a thinning of the background bacterial lawn and/or a marked reduction in revertant numbers) was observed either in the presence or in the absence of S-9. These range-finder results were used to provide the TA100 mutagenicity data for Experiment 1. Experiment 1 treatments of the remaining test strains were performed using the same dose-range. Following these treatments once again no evidence of toxicity was observed.

Experiment 2 treatments were performed with the maximum treatment concentration of 5000 µg/plate retained. Narrowed dose intervals were employed in order to more closely investigate those doses of TZMU considered most likely to induce any mutagenic response. In addition, all treatments in the presence of S-9 employed a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay. Following these Experiment 2 treatments, once again no evidence of toxicity was apparent in any of the test strains.

The test article was completely soluble in the aqueous assay system at all concentrations treated, in each of the experiments performed.

Negative (solvent) and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments.

No TZMU treatment of any of the test strains produced an increase in revertant numbers sufficient to be considered as indicative of mutagenic activity.

It was concluded that TZMU did not induce mutation in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102), when tested under the conditions employed for this study, which included treatments up to 5000 µg/plate, both in the absence and in the presence of a rat liver metabolic activation system (S-9).

1.3 TZNG: Reverse Mutation in five Histidine-requiring strains of *Salmonella typhimurium*

TZNG was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments.

An initial toxicity range-finder experiment was carried out in strain TA100 only, using final concentrations of TZNG at 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. Following these treatments, no evidence of toxicity (as normally indicated by thinning of the background bacterial lawn and/or a marked reduction in revertant numbers) was observed either in the presence or in the absence of S-9. These range-finder results were used to provide the TA100 mutagenicity data for Experiment 1. Experiment 1 treatments of the remaining test strains were performed using the same dose-range as employed in the range-finder experiment. Following these treatments evidence of toxicity, manifest as slight thinning of the background bacterial lawn, was observed at the maximum treatment concentration solely with strain TA98 in the absence of S-9.

Experiment 2 treatments were performed with the maximum treatment concentration of 5000 µg/plate retained. Narrowed dose intervals were employed in order to more closely investigate those doses of TZNG considered most likely to induce any mutagenic response. In addition, all treatments in the presence of S-9 employed a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay. Following these Experiment 2 treatments, evidence of toxicity was apparent at the maximum treatment concentration with all the test strains both in the absence and in the presence of S-9 (except strain TA1535 where no evidence of toxicity was observed).

The test article was completely soluble in the aqueous assay system at all concentrations treated, in each of the experiments performed.

Negative (solvent) and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments.

No TZNG treatment of any of the test strains produced an increase in revertant numbers sufficient to be considered as indicative of mutagenic activity.

It was concluded that TZNG did not induce mutation in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102), when tested under the conditions employed for this study, which included treatments up to 5000 µg/plate, both in the absence and in the presence of a rat liver metabolic activation system (S-9).

1.4 TMG: Reverse Mutation in five Histidine-requiring strains of *Salmonella typhimurium*

TMG was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments.

An initial toxicity range-finder experiment was carried out in strain TA100 only, using final concentrations of TMG at 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. Following

these treatments, no evidence of toxicity (as normally indicated by a thinning of the background bacterial lawn and/or a marked reduction in revertant numbers) was observed either in the presence or in the absence of S-9. These range-finder results were used to provide the TA100 mutagenicity data for Experiment 1. Experiment 1 treatments of the remaining test strains were performed using the same dose-range as employed for the range-finder experiments. Following these treatments, once again, no evidence of toxicity was apparent.

Experiment 2 treatments were performed with the maximum treatment concentration of 5000 µg/plate retained. Narrowed dose intervals were employed in order to more closely investigate those doses of TMG considered most likely to induce any mutagenic response. In addition, all treatments in the presence of S-9 employed a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay. Following these Experiment 2 treatments, evidence of toxicity was apparent (manifest as a slight thinning of the background bacterial lawn) at the maximum treatment concentration with test strains TA98, TA100 and TA1537 in the presence of S-9.

The test article was completely soluble in the aqueous assay system at all concentrations treated, in each of the experiments performed.

Negative (solvent) and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments.

No TMG treatment of any of the test strains produced an increase in revertant numbers sufficient to be considered as indicative of mutagenic activity.

It was concluded that TMG did not induce mutation in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102), when tested under the conditions employed for this study, which included treatments up to 5000 µg/plate, both in the absence and in the presence of rat liver metabolic activation system (S-9).

2.0 Acute Oral Toxicity Studies in the Rat

2.1 MG: Acute Oral Toxicity study in the Rat

This study was conducted to assess the acute oral toxicity of methyl guanidine (MG) in the fasted rat. The method followed was in compliance with that described in Japan Ministry of Agriculture, Forestry and Fisheries, Testing Guidelines for Toxicology Studies, Acute Oral Toxicity and equivalent US, EU and OECD guidelines.

Preliminary investigations were conducted by subjecting groups of two male and two female fasted rats to a single oral administration of methyl guanidine (MG) at 150, 500, 2000 or 5000 mg/kg. There were deaths among rats treated at dose levels of 500 mg/kg and above. The deaths occurred between ½ hour and 3 hours after dosing.

In the main study, groups of five male and five female fasted rats were given the test article as a single dose by oral gavage on Day 1 at dose levels of 260, 355, 435, 530 or 650 mg/kg. The test article was dispersed in 5% aqueous gum arabic and administered at a dose volume of 20 mL/kg. All animals surviving treatment were killed on Day 15 and subsequently underwent a full necropsy.

Three female rats were found dead following a single oral dose of methyl guanidine (MG) at 435 mg/kg; one male and four females died following dosing at 530 mg/kg and in the group dosed at 650 mg/kg there were four male and five female decedents. One male dosed at 650 mg/kg was killed *in extremis* due to the severity of clinical signs. Deaths occurred at intervals from within one hour after dosing until Day 2.

Principal clinical signs of reaction to treatment were staining of the snout, salivation, unkempt appearance and arched/hunched posture apparent on the day of treatment. Other reactions to treatment included lethargy, piloerection, ataxia, tremor, twitching and arched gait. Isolated cases of tachypnoea, vocalisation and hypothermia were seen throughout the dose groups. Palpebral closure, chromodacryorrhoea, anogenital soiling, prone, clonic convulsions and emprostotonus were seen in animals treated at 650 mg/kg. Several females dosed at 355 mg/kg showed rigid extension of the tail associated with the observation of arched gait.

Onset of the principal clinical signs was generally between one and two hours after treatment and recovery of surviving animals was advanced by Day 3 and complete by Day 4.

All surviving rats, with the exception of one female treated at 435 mg/kg, gained body weight during the first and second weeks of the observation period.

Principal necropsy findings of decedents included inflated and dark/red lungs, distended/pale stomach with abnormal contents and distension of the small intestine with abnormal contents.

Other findings included dark areas on the spleen, red fluid in the thoracic cavity, enlarged mesenteric lymph nodes and renal pelvic dilatation.

The terminal necropsy on Day 15 of one male treated at 355 mg/kg revealed renal pelvic dilatation. All other terminal kill animals showed no macroscopic changes.

The observed macroscopic changes were not considered to be consistent with an effect of methyl guanidine (MG).

The acute median lethal oral doses (LD_{50}) and the 95% confidence limits for methyl guanidine (MG) were estimated to be as follows:

Combined male and female rats 498 (454 to 549) mg/kg.

Male rats 550 mg/kg (fiducial limits could not be calculated)

Female rats 446 (376 to 522) mg/kg.

2.2 TZMU: Acute Oral Toxicity study in the Rat

This study was conducted to assess the acute oral toxicity of TZMU in the fasted rat. The method followed was in compliance with that described in Japan Ministry of Agriculture, Forestry and Fisheries, Testing Guidelines for Toxicology Studies, Acute Oral Toxicity and equivalent US, EU and OECD guidelines.

Preliminary investigations were conducted by subjecting groups of two male and two female fasted rats to a single oral administration of TZMU at 200, 800, 1500, 4000 or 5000 mg/kg. There were deaths among rats treated at dose levels of 1500 mg/kg and above. The deaths occurred between two hours after treatment and Day 2.

In the main study, groups of five fasted male and five fasted female rats were given the test article as a single dose by oral gavage at dose levels of 920, 1152, 1440, 1800 or 2250 mg/kg on Day 1. The test article was dispersed in 5% m/v aqueous gum arabic and administered at a dose volume of 20 mL/kg. All animals surviving treatment were killed on Day 15 and subsequently underwent a full necropsy.

Rats were found dead following a single oral dose of TZMU among males and females dosed at 1800 mg/kg (four males and three females) and 2250 mg/kg (four males and five females). Three male and two female rats were found dead and one male and two female rats were killed *in extremis* following treatment at 1440 mg/kg. Decedents amongst animals treated at 1152 mg/kg (one male and three females) were killed *in extremis*. Deaths occurred at intervals from within 4½ hours of dosing up to Day 2.

Principal signs of reaction to treatment were lethargy and palpebral closure seen in all animals in all groups. Other reactions included low gait, piloerection, ataxia, prone, flaccidity, vasodilatation, unconscious, salivation, hunched posture and lachrymation. Less common observations were dyspnoea, bradypnoea, tachypnoea, rales, sunken flanks, limp, twitching, staining of the snout, exophthalmus, chromodacryorrhoea, unkempt appearance and hypothermia.

Onset of principal clinical signs was between ¼ and ½ hour after treatment and recovery of surviving rats was advanced by Day 2 and complete by Day 3.

All surviving rats achieved body weight gains during the first and second weeks of the study.

Principal necropsy findings of decedents included inflated and dark lungs, pale/mottled liver, yellow colouration to the mucosal surfaces of the stomach and impaction of the caecum. Less common findings included renal pelvic dilatation, pale kidneys, red fluid in the abdominal/thoracic cavity and small intestine, red jejunum, distension of the small intestine, yellow coloured/large mesenteric lymph nodes and uterine distension.

No macroscopic changes were found at the necropsy of all surviving animals on Day 15.

The acute median lethal oral dose (LD₅₀) and the 95% confidence limits for TZMU were estimated to be as follows:

Combined male and female rats 1351 (1145 to 1565) mg/kg.

Male rats 1424 (1104 to 1824) mg/kg.

Female rats 1282 (912 to 1613) mg/kg.

2.3 TZNG: Acute Oral Toxicity study in the Rat

This study was conducted to assess the acute oral toxicity of TZNG, a metabolite of TI-435, in the fasted rat. The method followed was in compliance with that described in Council Directive 91/414/EEC as amended by Commission Directive 94/79/EC, Annex to Commission Directive 92/69/EEC, Method B1, OECD Guidelines for Testing of Chemicals, Method 401, US EPA Health Effect Test Guidelines OPPTS 870.1100 and is understood to meet the requirements of the Japan Ministry of Agriculture, Forestry and Fisheries.

Preliminary investigations were conducted by subjecting groups of two fasted female rats to a single oral administration of TZNG at 1000, 1200, 1400, 1800 or 2000 mg/kg. There were deaths at 1200 mg/kg and above. The deaths occurred between Day 2 and Day 6.

In the main study, groups of five fasted female rats were given the test article as a single dose by oral gavage at dose levels of 1125, 1350 or 1620 mg/kg. The test article was dispersed in 5% m/v aqueous gum arabic and administered at a dose volume of 20 mL/kg on Day 1. An additional group of five fasted male rats was dosed at 1450 mg/kg to confirm that males were not markedly more susceptible to the toxic effects of the test article than the females. All animals surviving treatment were killed on Day 15 and subsequently underwent a full necropsy.

Three rats were found dead on Day 3 and one on Day 4 following treatment at 1620 mg/kg. One rat dosed at 1350 mg/kg was found dead on Day 5. There were no deaths amongst rats dosed at 1125 or 1450 mg/kg.

Principal clinical signs of reaction to treatment were lethargy and palpebral closure seen in all animals in all groups. Other reactions included arched gait, hunched posture, piloerection, wasted appearance, staining of the snout and an unkempt appearance. Isolated cases of anogenital soiling, bradypnoea, ataxia, straub tail, tremor and hypothermia were also apparent among the treated rats. Clinical signs were first apparent between one and four hours after dosing.

Recovery of the majority of animals was complete within four days of treatment. Most females treated at 1125 mg/kg appeared unkempt until Day 11.

Among surviving rats, some marked body weight losses were recorded between Day 1 and Day 8. All treated rats gained weight from Day 8 to Day 15 and gained weight overall between Day 1 and Day 15.

No macroscopic changes were observed at necropsy of the female rats killed on Day 15.

Macroscopic examination of decedents treated at 1620 mg/kg included dark and red lungs, distended and pale stomach and dark foci and red areas also affecting the stomach. Necropsy of one rat found dead after treatment at 1350 mg/kg revealed a small spleen and pale stomach.

Necropsy of males dosed at 1450 mg/kg revealed small testes affecting all rats and soft testes apparent in two of the same animals.

Following a single administration of TZNG to female fasted rats, the acute median lethal oral dose (LD₅₀) and its 95% confidence limits were estimated to be 1481 (1257 to 1882) mg/kg.

A confirmatory dose of 1450 mg/kg, given to male fasted rats, resulted in no death indicating that males were not markedly more susceptible to the toxicity of TZNG than were the females.

2.4 TMG: Acute Oral Toxicity study in the Rat

This study was conducted to assess the acute oral toxicity of TMG, a metabolite of TI-435, in the fasted rat. The method followed was in compliance with that described in Council Directive 91/414/EEC as amended by Commission Directive 94/79/EC, Annex to Commission Directive 92/69/EEC, Method B1, OECD Guidelines for Testing of Chemicals, Method 401, US EPA Health Effects Test Guidelines OPPTS 870.1100 and is understood to meet the requirements of the Japan Ministry of Agriculture, Forestry and Fisheries.

Preliminary investigations were conducted by subjecting groups of two fasted female rats to a single oral administration of TMG at 400, 570, 800, 1120 or 2000 mg/kg. All rats died following treatment at 1120 or 2000 mg/kg and one rat was found dead in each group treated at 570 or 800 mg/kg. Both rats survived treatment at 400 mg/kg. Deaths occurred between ¼ and 3 hours after treatment.

In the main study, groups of five fasted female rats were given the test article as a single dose by oral gavage at dose levels of 225, 650 or 1100 mg/kg on Day 1. The test article was dispersed in 5% m/v aqueous gum arabic and administered at a dose volume of 20 mL/kg. An additional group of five fasted male rats was dosed at 550 mg/kg to confirm that males were not markedly more susceptible to the toxic effects of the test article than the females. All animals surviving treatment were killed on Day 15 and subsequently underwent a full necropsy.

Rats were found dead following treatment at 550 mg/kg (three males), 650 mg/kg (four females or 1100 mg/kg (five females). Deaths occurred at intervals from within 1 hour of dosing until Day 2. There were no deaths following treatment at 225 mg/kg.

Principal clinical signs of reaction to treatment were lethargy and palpebral closure seen in all animals dosed at 550 mg/kg and above and a number of rats treated at 225 mg/kg. Other reactions included tremor, twitching, tachypnoea, prone and dyspnoea. Isolated cases of vasoconstriction, ataxia, salivation, piloerection, hunched posture and hypothermia were also seen in treated groups. Clinical signs were first apparent between ¼ and 4 hours after treatment.

Recovery of surviving rats, as judged by external appearance and behaviour, was underway four hours after treatment and complete by Day 2.

All surviving rats gained weight during the first and second weeks of the observation period.

Principal necropsy findings of decedents included dark and inflated lungs, darkened livers and distention and reddening of the jejunum. Less common findings included mottling of the lung and liver and a yellow jejunum.

No macroscopic changes were apparent at necropsy of the majority of animals killed on Day 15. Necropsy of one rat revealed mottled kidneys.

Following a single administration of TMG to female fasted rats, the acute median lethal oral doses (LD₅₀) was estimated to be 567 mg/kg. No confidence limits could be calculated.

A confirmatory dose of 550 mg/kg administered to male rats resulted in three deaths indicating that males were not markedly more susceptible to the toxic effects of TMG than were the females.

TI-435: Summary of Mutagenicity Tests and Acute Oral Toxicity Study in the Rat

1. TI-435: Mutagenicity Tests

Test System	Concentration/Dose	Results
Bacterial reverse mutation test (TA 1535, 100, 1537, 98) +/- S9 <i>E. coli</i> WP2 uvrA +/- S9	313-5000 µg/pl	Negative
Ames test	50-5000 µg/pl	Positive ¹
Ames test (TA 1535)	up to 7000 µg/pl	Negative
Ames test (TA 1535)	up to 5000 µg/pl	Negative
Ames test (TA 1535, 100, 1537, 98, 102)	up to 5000 µg/pl	Negative
Mouse lymphoma test (L5178Y TK +/- mouse lymphoma cells)	1 st exp.: 312.5 - 2500 µg/ml 2 nd exp.: 300 - 2400 µg/ml	Positive ¹
HPRT/V79	156-5000 µg/ml	Negative
CYT/Chinese Hamster lung cells	up to 937.5 (-S9) 1875 (+S9) µg/ml	Positive ¹
Micronucleus Test in Mice ²	25, 50, 100 mg/kg b.w. p.o.	Negative
DNA repair in <i>Bacillus subtilis</i> H17+M45	375-6000 µg/disk	Negative
UDS/hepatocytes (<i>ex vivo</i>)	2500, 5000 mg/kg b.w.	Negative (4h, 16h)

¹ With and without S9

² Clinical signs at all doses, increased mortality at 100 mg/kg b.w.

Positive results of mouse lymphoma test checked by HPRT

Positive results of CYT/CHL test 'overruled' by MNT

2. TI-435: Acute Oral Toxicity Study in the Rat

This study was conducted to assess the acute oral toxicity of TI-435 in the fasted rat. The method followed was in compliance with that described in Japan Ministry of Agriculture, Forestry and Fisheries, Testing Guidelines for Toxicology Studies, Acute Oral Toxicity and equivalent US, EU, and OECD guidelines.

Preliminary investigations were conducted by subjecting groups of two male and two female fasted rats to a single oral administration of the test article at 2000, 3500, 4000, 4200, and 4800 mg/kg body weight. There were deaths among rats treated at dose levels of 4000 mg/kg and above. The deaths occurred at intervals from the day after treatment (Day 2) until Day 9.

In the main study, groups of five male and five female fasted rats were given TI-435 as a single dose by oral gavage at dose levels of 1758, 2283, 2965, 3850, and 5000 mg/kg body weight. The test article was dispersed in 5% m/v aqueous gum arabic and administered at a dose volume of 10 mL/kg on Day 1. All animals surviving treatment were killed on Day 15 and subsequently underwent a full necropsy.

Single female rats were found dead on Day 2 after single oral administration of the test article at 2965 mg/kg and on Day 4 after treatment at 5000 mg/kg. Two rats were killed on humane grounds on Day 12. The male (No. 800; 5000 mg/kg) and the female (No. 821; 3850 mg/kg) had shown progressive losses of body weight during the observation period and they were considered to be moribund.

The principal signs of reaction to treatment were palpebral closure, decreased activity and tremor followed at later stages of the observation period by a wasted appearance and hair loss. Less common clinical signs apparent at all dose levels included ataxia and hunched posture.

Onset of principal clinical signs was not consistent between the groups. Tremor and palpebral closure were first apparent between one hour and four hours after dosing and decreased activity was generally observed from Day 2. Hair loss was particularly evident among female rats and commenced at various intervals from Day 2 to Day 10. Several rats eventually developed bald patches on the pelt because of persistent hair loss. A progressive loss of body weight was associated with onset of a wasted appearance that was first apparent between Day 4 and Day 6.

Cases of decreased activity, palpebral closure, lethargy, hunched posture, hair loss, wasted appearance, staining of the snout, and soiling of the anogenitalia continued to occur during the second week of the observation period. Rats dosed at 1758, 2283 or 2965 mg/kg were recovered from all clinical signs except hair loss by Day 15. A few cases of palpebral closure and hunched posture and more common cases of wasted appearance and hair loss persisted at Day 15 among rats dosed at 3850 and 5000 mg/kg but the improving condition of these animals was demonstrated by marked increases in the body weights between Days 8 and 15.

The great majority of the rats lost weight between Day 1 and Day 4. Further losses of body weight occurred between Day 4 and Day 8 among females dosed at 1758, 2283, 3850 or 5000 mg/kg and in a single male dosed at 5000 mg/kg. By Day 12 one female (No. 821) dosed at 3850 mg/kg and one male (No. 800) dosed at 5000 mg/kg had lost sufficient body weight that they were considered moribund and

were killed on humane grounds. Large gains of body weight between Day 8 and Day 15 were recorded for all rats excepting a single female treated at the lowest dose level.

Necropsy of two rats found dead on Day 2 (2965 mg/kg) or day 4 (5000 mg/kg) revealed reddening of the lungs of the rat treated at the lower dose level. No other macroscopic changes were apparent during the *post-mortem* examination.

Two rats were killed on humane grounds on Day 12. Necropsy revealed a large liver and large mandibular lymph nodes in the female (3850 mg/kg) and one small testis, large and firm mandibular lymph nodes, thickening of the lower mandible, and a pale focus on one kidney of the male rat (5000 mg/kg).

Surviving rats were killed at completion of the fourteen days observation period. No macroscopic changes were apparent during necropsy of the great majority of these rats. There were isolated cases of renal pelvic dilatation among rats treated at the lower dose levels and a single case of uterine distension (1758 mg/kg).

As no group showed a mortality of greater than 20%, either in terms of single sex groups or combined sex groups, it was concluded that the acute median lethal oral doses (LD_{50}) of TI-435 were greater than 5000 mg/kg body weight in both male and female fasted rats.

Questions for the Committee:

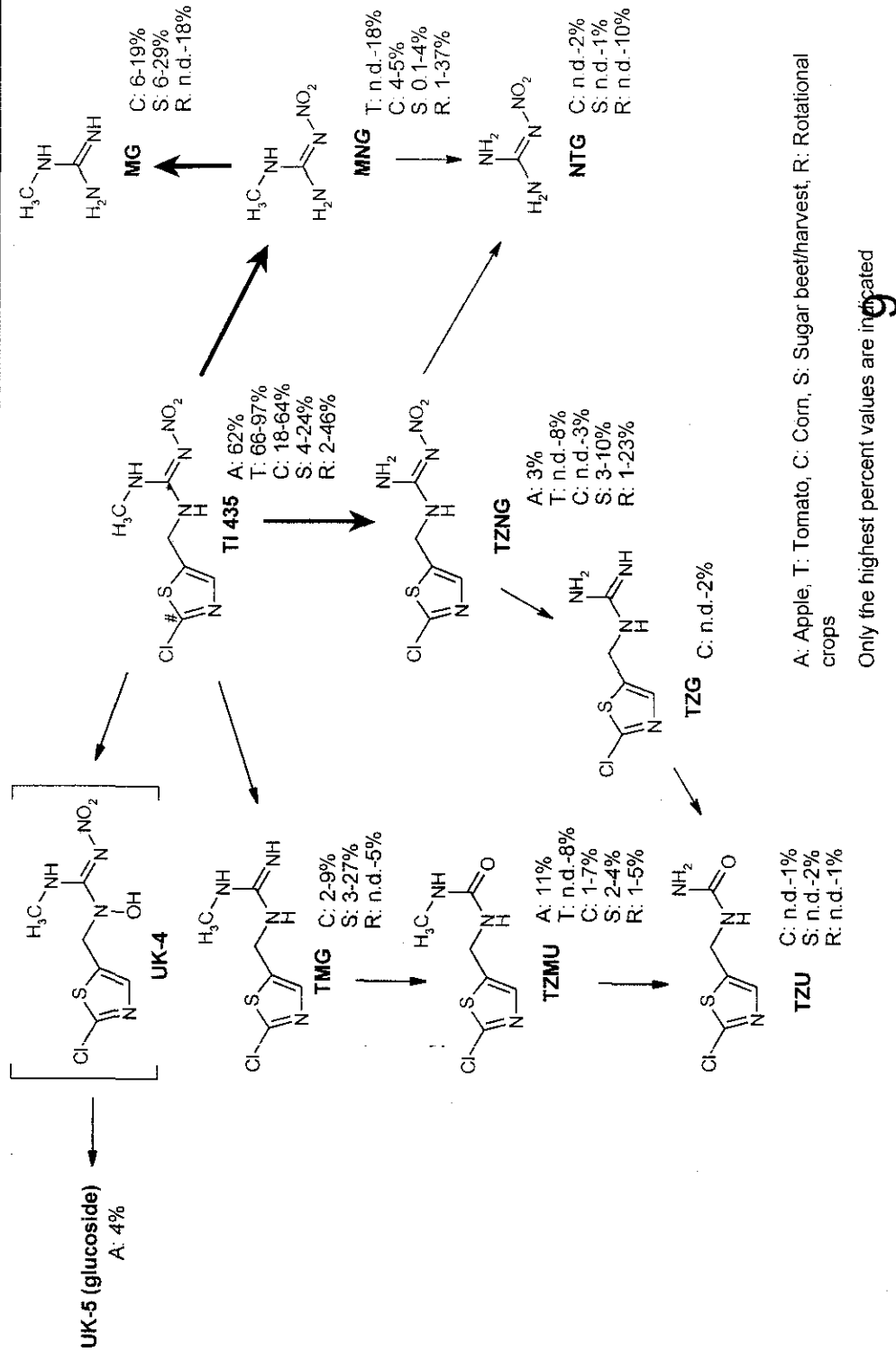
1. Does the Committee concur that the parent compound is the residue of concern in plants and in animals for purposes of tolerance enforcement and risk assessment?

Not available electronically:

- Attachments: 1. Proposed Metabolic Pathways of TI-435 in Plants,
- Attachments: 2. TRR and Extractability of Crop Residues,
- Attachments: 3. Metabolites Identified in Plants,
- Attachments: 4. Additional Information on Plant Metabolism Studies,
- Attachments: 5. Proposed Metabolic Pathways of TI-435 in Rats,
- Attachments: 6. Residue Methodology.

cc: with attachment: Yan Donovan; [REDACTED]; Reading file.

Proposed metabolic pathway for TI-435 in plants



Attachment 2

TRR and Extractability of Crop Residues

Crop	TRR (ppm)	Extractability (%)	Identified (%)
Apple	0.076	93.1	78.6
Tomato (spray)	0.57	99.9	96.6
Tomato (soil)	0.01	98.2	92.1
Corn 1 st (seed), Forage	0.136	97.0	69.1
Stover	0.175	92.5	64.5
kernels	0.006	87.4	52.0
Corn 2 nd (seed), Forage	0.89	93.8	81.5
Stover	3.06	91.5	68.1
kernels	0.063	94.9	61.7
Sugar beet, Leaf	0.886	93.3	74.6
Root	0.034	87.9	46.0
Rotational crops* 1st	0.02 – 2.65	80.4 – 97.0	43.5 – 78.1
2nd	0.01 – 1.23	79.8 – 98.2	30.4 – 79.9
3rd	0.01 – 1.24	80.1 – 97.1	32.1 – 82.8

*: human consumables: 0.01 – 0.26 ppm

Metabolites Identified in Plants

% of the TRR: only the highest percent values are indicated

	RAC	TI-435	TZNG	TZMU	TMG	TZU	MG	MNG	NTG	UK5	Identifie
Spray	apple	61.5	2.8	10.6	n.d.	n.d.	n.d.	n.d.	n.d.	3.7	78.6
	tomato	96.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	96.6
Soil	tomato	66.1	8.4	n.d.	n.d.	n.d.	n.d.	17.7	n.d.	n.d.	92.1
Seed Corn (1 st)	forage	41.5	2.0	5.7	8.6	n.d.	6.1	3.7	1.5	n.d.	69.1
	stover	18.0	1.1	6.5	7.3	n.d.	10.9	5.4	2.4	n.d.	62.3
	kernels	18.3	n.d.	3.9	5.9	n.d.	18.9	5.0	n.d.	n.d.	63.3
Corn (2nd)	forage	64.3	3.1	4.9	7.2	1.0	-	-	-	n.d.	80.5
	stover	40.6	2.8	9.4	8.1	2.8	-	-	-	n.d.	63.7
	kernels	58.3	0.7	0.7	1.9	n.d.	-	-	-	n.d.	61.6
Sugar beet	leaf	4.3	3.3	4.3	27.0	1.7	28.6	4.1	1.3	n.d.	74.6
	root	24.4	9.8	1.8	3.1	n.d.	6.2	0.7	n.d.	n.d.	46.0
Rot. crops	Rot: 1st	46.0	22.7	5.1	5.3	0.8	9.3	19.7	8.0	n.d.	43.5-78.
	Rot: 2nd	37.7	14.8	4.7	4.2	0.5	11.4	27.6	8.4	n.d.	30.4-79.
	Rot: 3rd	27.3	8.0	2.6	4.9	0.4	17.5	37.4	10.1	n.d.	32.1-82.
Rat		73	11	0.5	0.3	0.7	0.4	14	4	0.2	

n.d.: not detected; -: not applicable

Additional Information

Details of Plant Metabolism Studies

Apple, spray, 1st label
Tomato, spray, 1st label
Tomato, soil, 1st label
Corn, seed, 1st label
Corn, seed, 2nd label
Sugar Beet, seed, 1st label
Rotational Crop, 1st label

Apple, spray application (1st label)

Appl. rate: 202 g a.i./ha × 2 applications (99 and 14 days before harvest)

TRR: 0.076 ppm

Extractability: 93.1%. Surface wash with methanol/water 1:1 followed by extraction with acetonitrile (ACN)/water, 1:1 (2×) and ACN

Metabolites identified in apples

Metabolite	%	Ppm
TI435	61.5	0.046
TZNG (†)	2.8	0.002
TZMU	10.6	0.009
N-OH-Conjugate	3.7	0.003
TOTAL	78.6	0.060

†: tentatively identified

Tomato, spray application (1st label)

Appl. rate: 158 g a.i./ha x 2 (17 and 3 days before harvest)

TRR: 0.57 ppm

Extractability: 99.9%. Surface wash with methanol/water 1:1, followed by extraction with ACN/water, 1:1 (3x)

Metabolites identified in tomatoes

Metabolite	%	Ppm
TI435	96.6	0.55
TOTAL	96.6	0.55

Tomato, planting hole (1st label)

Appl. rate: 15 mg a.i./plant (97 days before harvest)

TRR: 0.01 ppm

Extractability: 98.2%. Extraction with ACN/water (1:1) and (4:1)

Metabolites identified in tomatoes

Metabolite	%	Ppm
TI435	66.1	0.01
TZNG	8.4	<0.01
MNG	17.7	<0.01
TOTAL	92.1	0.01

Corn, seed treatment (1st label)

Appl. rate: 1.06 mg a.i./seed, 80000 seeds/ha, 85 g a.i./ha

TRR: Forage: 0.136 ppm Stover: 0.175 ppm Kernels: 0.006 ppm

Extractability: Forage: 97.0% Stover: 92.5% Kernels: 87.4%

All corn matrices were extracted with ACN/water 1:1 (2x) followed by ACN and microwave extraction with ACN/water (1:1)

Metabolites identified in corn RACs

Metabolite	Forage day 60		Stover day 144		Kernels day 144	
	%	ppm	%	ppm	%	ppm
TI435	41.5	0.057	18.0	0.031	18.3	0.001
TZNG(t)	2.0	0.003	1.1	0.002	n.d.	n.d.
TZMU	5.7	0.008	6.5	0.011	3.9	<0.001
TMG	8.6	0.012	7.3	0.013	5.9	<0.001
MG	6.1	0.008	10.9	0.019	18.9	0.001
MNG	3.7	0.005	5.4	0.009	5.0	<0.001
NTG (t)	1.5	0.002	2.4	0.004	n.d.	n.d.
TOTAL	69.1	0.094	64.5	0.090	52.0	0.003

t: tentatively identified, n.d.: not detected

Corn, seed treatment (2nd label)

Appl. rate: 3.2 mg a.i./seed, 81500 seeds/ha, 261 g a.i./ha

TRR: Forage: 0.89 ppm Stover: 3.06 ppm Kernels: 0.063 ppm

Extractability: Forage: 93.8% (extraction with ACN/water 1:1, 2× followed by ACN and microwave extraction with ACN/water 1:1)

Stover: 74.8% (extraction with ACN/water 1:1, 2× followed by ACN; exhaustive (microwave) extraction will be conducted)

Kernels: 54.8% (extraction with ACN/water 1:1, 2× followed by ACN; exhaustive (microwave) extraction will be conducted)

Metabolites identified in corn RACs

Metabolite	Forage day 63		Stover day 162		Kernels day 162	
	%	Ppm	%	ppm	%	ppm
TI435	64.3	0.57	40.6	1.24	58.3	0.037
TZNG	3.1	0.03	2.8	0.09	0.7	<0.001
TZMU	4.9	0.04	9.4	0.29	0.7	<0.001
TMG	7.2	0.06	8.1	0.25	1.9	0.001
TZU	1.0	0.01	2.8	0.09	n.d.	n.d.
TOTAL	80.5	0.71	63.7	1.96	61.6	0.039

n.d.: not detected

Sugar beet (leaf), seed treatment (1st label)

Appl. rate: 1.58 mg a.i./seed, 120000 plants/ha, 190 g a.i./ha

TRR (leaf): day 48: 1.750 ppm day 55: 0.522 ppm harvest: 0.886 ppm

Extractability: day 48: 98.9% day 55: 94.4% harvest: 93.3%

All samples were extracted with ACN (2x) followed by ACN/water 1:1 (2x), ACN/1 M HCl 1:1, ACN/1 M NH₃ 1:1, and reflux with 0.1 M NaOH

Metabolites identified in sugar beet leaf

Metabolite	Leaf day 48		Leaf day 55		Leaf/harvest day 144	
	%	ppm	%	ppm	%	ppm
TI435	49.3	0.863	60.5	0.316	4.3	0.038
TZNG	5.6	0.098	10.3	0.054	3.3	0.029
TZMU	3.6	0.063	2.9	0.015	4.3	0.038
TMG	9.7	0.170	6.0	0.031	27.0	0.239
TZU	1.7	0.030	1.4	0.007	1.7	0.015
MG	6.5	0.114	3.2	0.017	28.6	0.253
MNG	4.3	0.075	4.5	0.023	4.1	0.036
NTG	1.5	0.026	1.4	0.007	1.3	0.012
TOTAL	82.2	1.439	90.2	0.470	74.6	0.660

Sugar beet (root), seed treatment (1st label)

Appl. rate: 1.58 mg a.i./seed, 120000 plants/ha, 190 g a.i./ha

TRR (Root): day 48: 0.860 ppm day 55: 0.202 ppm harvest: 0.034 ppm

Extractability: day 48: 98.4% day 55: 93.8% harvest: 87.9%

All samples were extracted with ACN (2×) followed by ACN/water 1:1 (2×), ACN/1 M HCl 1:1, ACN/1 M NH₃ 1:1, and reflux with 0.1 M NaOH

Metabolites identified in sugar beet root

Metabolite	Root day 48		Root day 55		Root/harvest day 144	
	%	ppm	%	ppm	%	ppm
TI435	50.0	0.430	67.9	0.137	24.4	0.008
TZNG	4.9	0.042	9.1	0.018	9.8	0.003
TZMU	1.4	0.012	1.3	0.003	1.8	0.001
TMG	5.9	0.051	1.0	0.002	3.1	0.001
TZU	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MG	10.4	0.089	4.7	0.009	6.2	0.002
MNG	3.4	0.030	1.4	0.003	0.7	<0.001
NTG	0.3	0.003	n.d.	n.d.	n.d.	n.d.
TOTAL	76.3	0.657	85.4	0.172	46.0	0.015

n.d.: not detected

Rotational crop (1st label)

Appl rate: 300 g a.i./ha (2 × 150 g a.i./ha)

TRR:

Rotational crop	Total Radioactive Residue (TRR)					
	first rotation sowing: day 29		second rotation sowing: day 153		third rotation sowing: day 314	
	day*	ppm	day*	ppm	day*	ppm
Wheat forage	70	0.28	200	0.39	362	0.34
Wheat hay	106	0.54	259	0.38	404	0.37
Wheat straw	152	2.65	314	1.23	462	1.24
Wheat grain	152	0.12	314	0.05	462	0.04
Swiss chard	70	0.16	210	0.26	375	0.12
Turnip leaf	106	0.37	237	0.23	389	0.11
Turnip root	106	0.02	237	0.01	389	0.01

day*: days after application

Extractability: Wheat forage, hay, Swiss chard, Turnip leaves, roots: 85-98%
(extraction with ACN/water 1:1 (2×) followed by ACN)
Wheat straw, grain: 80-94%
(extraction with ACN/water 1:1 (2×) followed by ACN and
microwave extraction with ACN/water 1:1)

Rotational crop: first rotation (1st label)

Metabolites identified in the first rotational crops

Metabolite	Wheat, forage		Wheat, hay		Wheat, straw		Wheat, grain		Swiss chard		Turnip, leaf		Turnip, root	
	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm
TI 435	46.0	0.13	17.1	0.09	12.4	0.33	2.6	< 0.01	35.0	0.05	31.6	0.12	40.0	0.01
TZNG	6.0	0.02	11.8	0.06	10.7	0.28	22.7	0.03	16.2	0.03	6.6	0.02	1.8	< 0.01
TZMU	3.4	0.01	2.4	0.01	2.9	0.08	1.0	< 0.01	4.2	0.01	5.1	0.02	1.2	< 0.01
TMG	3.2	0.01	3.0	0.02	4.0	0.11	0.8	< 0.01	1.4	< 0.01	5.3	0.02	n.d.	n.d.
TZU	0.2	< 0.01	0.2	< 0.01	0.8	0.02	0.3	< 0.01	0.2	< 0.01	0.4	< 0.01	0.2	< 0.01
MG	3.0	0.01	6.8	0.04	9.3	0.25	3.1	< 0.01	1.9	< 0.01	4.8	0.02	n.d.	n.d.
MNG	13.8	0.04	11.4	0.06	9.1	0.24	5.6	0.01	19.7	0.03	11.7	0.04	0.6	< 0.01
NTG	2.5	0.01	5.1	0.03	5.6	0.15	8.0	0.01	6.3	0.01	2.8	0.01	n.d.	n.d.
TOTAL	78.1	0.22	57.9	0.32	54.8	1.45	44.0	0.06	85.0	0.13	68.4	0.25	43.8	0.01

n.d.: not detected

Rotational crop: second rotation (1st label)

Metabolites identified in the second rotational crops

Metabolite	Wheat, forage		Wheat, hay		Wheat, straw		Wheat, grain		Swiss chard		Turnip, leaf		Turnip, root	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
TI 435	32.4	0.13	16.4	0.06	11.4	0.14	2.0	< 0.01	21.3	0.06	31.3	0.07	37.7	< 0.01
TZNG	6.1	0.02	7.9	0.03	7.9	0.10	11.0	0.01	14.8	0.04	7.4	0.02	1.1	< 0.01
TZMU	3.2	0.01	3.1	0.01	2.7	0.03	1.3	< 0.01	3.2	0.01	4.7	0.01	1.7	< 0.01
TMG	2.4	0.01	2.5	0.01	3.6	0.04	2.1	< 0.01	2.4	0.01	4.2	0.01	n.d.	n.d.
TZU	n.d.	n.d.	n.d.	n.d.	0.5	0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MG	4.9	0.02	7.7	0.03	11.4	0.14	2.3	< 0.01	2.2	0.01	5.1	0.01	n.d.	n.d.
MNG	22.4	0.09	16.7	0.06	13.3	0.16	5.6	< 0.01	27.6	0.07	21.9	0.05	2.2	< 0.01
NTG	4.5	0.02	5.4	0.02	6.4	0.08	6.6	< 0.01	8.4	0.02	5.4	0.01	n.d.	n.d.
TOTAL	75.9	0.29	59.7	0.22	57.1	0.70	30.8	0.02	79.9	0.21	79.9	0.18	42.8	< 0.01

n.d.: not detected

Rotational crop: third rotation (1st label)

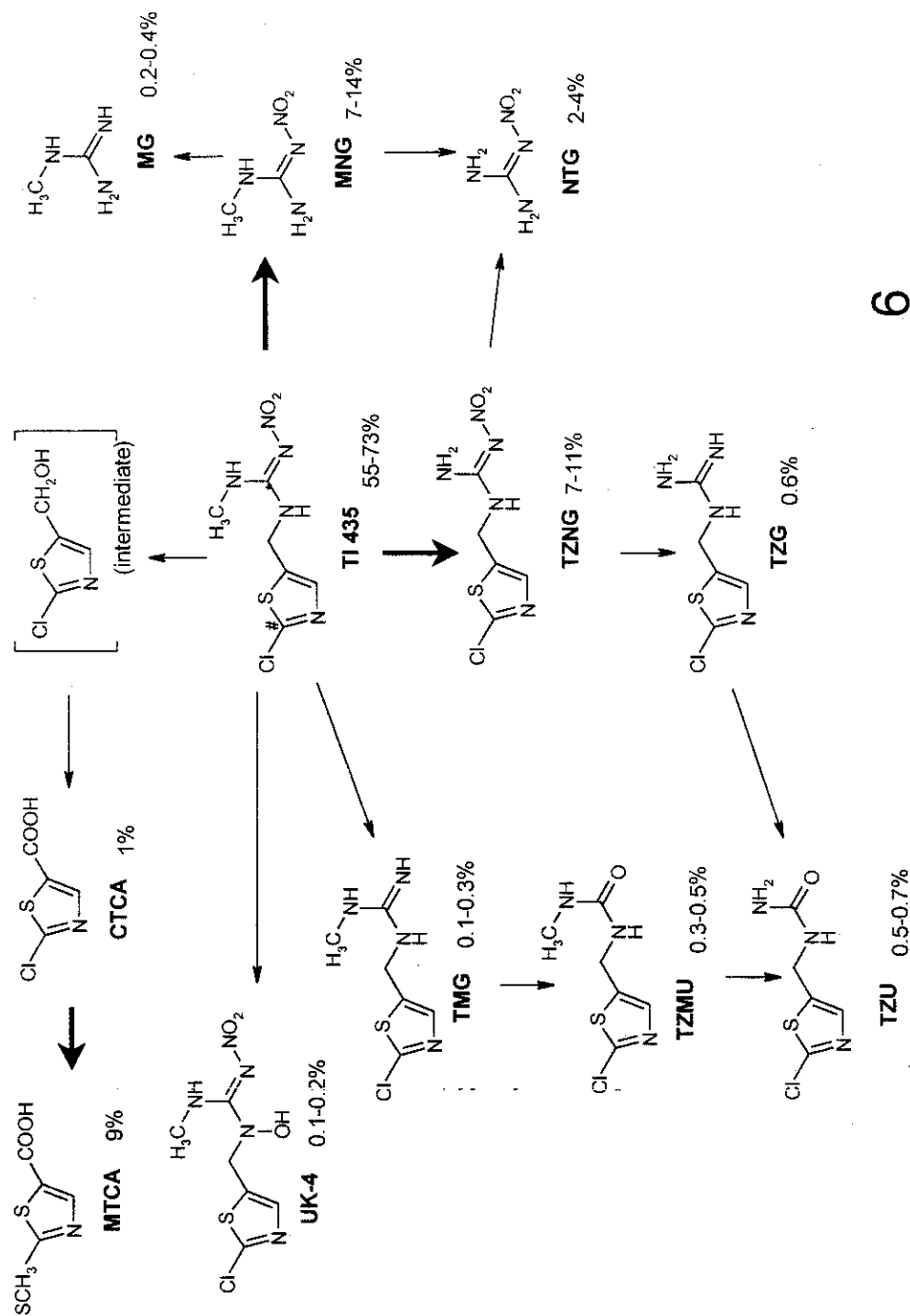
Metabolites identified in the third rotational crops

Metabolite	Wheat, forage		Wheat, hay		Wheat, straw		Wheat, grain		Swiss chard		Turnip, leaf		Turnip, root	
	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm
TI 435	21.0	0.07	12.5	0.05	7.4	0.09	2.4	<0.01	21.6	0.02	21.2	0.02	27.3	<0.01
TZNG	5.9	0.02	7.6	0.03	8.0	0.10	6.7	<0.01	7.0	0.01	5.1	0.01	1.5	<0.01
TZMU	1.9	0.01	2.2	0.01	2.0	0.02	1.4	<0.01	2.6	<0.01	1.6	<0.01	1.0	<0.01
TMG	3.0	0.01	3.4	0.01	4.9	0.06	3.4	<0.01	1.4	<0.01	3.5	<0.01	n.d.	n.d.
TZU	n.d.	n.d.	n.d.	n.d.	0.4	0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MG	7.0	0.02	11.4	0.04	17.5	0.22	2.5	<0.01	2.8	<0.01	7.8	0.01	n.d.	n.d.
MNG	26.9	0.09	18.4	0.07	9.1	0.11	7.5	<0.01	37.4	0.04	28.9	0.03	2.3	<0.01
NTG	6.2	0.02	6.6	0.02	5.1	0.06	7.2	<0.01	10.1	0.01	6.9	0.01	n.d.	n.d.
TOTAL	72.0	0.24	62.1	0.23	54.4	0.68	40.7	0.02	82.8	0.10	75.0	0.08	32.1	<0.01

n.d.: not detected

Attachment 5

Proposed biotransformation pathway in the rat



Residue methodology

Sample material (5 g)



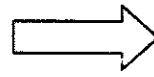
1. Add 30 mL of ACN/water (2:1 v/v for high water content sample
1:1 v/v for dry sample materials)

Maceration



1. Rinse blender with 2 mL of water and 2 mL of ACN
2. Add 2 g of Celite 545
3. Filter
4. Wash filter cake with 20 mL of ACN/water
5. Evaporate to aqueous remainder
6. Apply sample to 20 mL ChemElut column

ChemElut Clean-up



1. Allow sample to adsorb to column material for 15 min
2. Elute with 60 mL of cyclohexane/ethyl acetate (1:1, v/v)
3. Evaporate to dryness
4. Dissolve in 20 mL of ACN/water (1:4, v/v)

TI-435, LC-MS/MS Detection

LOQ: 0.02 ppm