Data Evaluation Record on the column leaching of clothianidin from soil planted with treated corn seeds

PMRA Submission Number {.....} EPA MRID Number 47483002

Data Requirement: PMRA Data Code:
EPA DP Barcode: 357014
OECD Data Point:
EPA Guideline: Non-guideline

Test material:

Common name: Clothianidin.

Chemical name:

IUPAC name: (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine.
(E)-N-(2-chloro-1,3-thiazol-5-yl)methyl]-N-[oxido(oxo)hydrazono]methanediamine.
Chloro-1,3-thiazol-5-yl)methyl]-N-{(E)-(methylamino)[oxido(oxo)hydrazono]methyl}amine.

CAS name: [C(E)]-N-[(2-chloro-5-thiazolyl)methyl]-N′-methyl-N″-nitroguanidine.

CAS No.: 210880-92-5 (formerly 205510-53-8).

Synonyms C-1015; C-908; TI435; K-1142.

Smiles string: CNC(=N[N+](=O)O)NCc1cnc(Cl)s1 (Online SMILES Translator and Structure File Generator at http://cactus.nci.nih.gov/services/translate/).
[O-][N+](=O)N=C(NCc1cnc(s1)Cl)NC.

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CITATION: Arthur, E.L., J. Shepherd, and A.R. Dominic. 2006. [Thiazole-2-^{14}C]clothianidin: seed leaching study. Unpublished study performed by Bayer CropScience, Stilwell, Kansas; sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina. Bayer CropScience Study Number: METIX050. Bayer CropScience Report Number: METIX050-1. Experimental start date July 23, 2004 and completion date June 2, 2005 (p. 6). Final report issued April 30, 2006 (report amendment date May 7, 2008).

EXECUTIVE SUMMARY

The leaching characteristics of [thiazole-2- ^{14}C]-labeled (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine (clothianidin; purity 98.4%) were studied in a silt loam soil [pH 6.1, organic carbon 1.8%] from Nebraska. The study was conducted in compliance with USEPA 40 CFR Part 160. No guidelines exist for this study. Soil columns consisting of PVC pipe (10 x 31 inches) were packed with silt loam soil to a depth of 24 inches, saturated using distilled water, and allowed to drain to field capacity. Seventeen corn seeds were treated with a slurry of [thiazole-2-^{14}C]clothianidin, polymer, and water at an application rate of 1.17 ± 0.02 mg a.i./seed. Talc was added and the seeds were shaken and placed on wax paper to dry. For leaching, ten prepared soil columns were each planted with one treated seed. Fertilizer (Osmocote slow release, 14-14-14) was added to the surface of the soil and the seeds were planted at a depth of 1.5 inches. The bottom of each column was covered with plastic sheeting to protect from light. The soil columns were stored in a heated greenhouse maintained at 9-45°C under a 12-hour light:12-hour dark cycle. Fourteen leaching events were conducted throughout the leaching phase. Approximately 130 cm (51 inches) of water were added to the soil columns over a 4-month period, which exceeded the target of the 30-year annual rainfall for Branchton, Ontario, Canada.

Leachate was collected daily for the first week and then per leaching event thereafter, and analyzed for total radioactivity using LSC. The seeds were removed from the soil, extracted with acetonitrile, followed by extraction on an Accelerated Solvent Extractor (ASE) with acetonitrile. The roots were removed from the soil, weighed, and extracted by shaking with acetonitrile. The seed and root extracts were analyzed for total radioactivity using LSC. The extracts were filtered and the seeds and roots were dried, combusted, and analyzed using LSC. Plant material was removed from the surface of the soil columns, frozen, homogenized, then combusted and analyzed using LSC to determine total radioactivity within the plant. Duplicate soil columns were sacrificed at 2, 4, 8, and 16 weeks post corn-spike (3, 5, 9, and 17 week post-planting), frozen, and cut into segments (*ca*. 15 cm). The soils were partially dried and homogenized, and aliquots were extracted on an ASE with acetonitrile, followed by water, and then acetonitrile at 100°C. The extracts were combined prior to analysis using LSC. The identities of [thiazole-2-^{14}C]clothianidin and its transformation products were confirmed by HPLC using the following reference standards: clothianidin, desmethyl, thiazolyl methylurea, and thiazolyl urea. LC-ESI/MS was used to confirm the identity of the parent compound and its transformation products.

Mean mass balances were 116.1% of the applied at 2 weeks, 91.8% at 4 weeks, 85.7% at 8 weeks, and 80.5% at 16 weeks.

In seeds treated with [thiazole-2-^{14}C]clothianidin, total [^{14}C]residues decreased from an average of 54.9 ±17.5% of the applied at 2 weeks to 28.7 ± 0.3% at 4 weeks, and were 0.5 ± 0.1% at 16 weeks. The seed was difficult to retrieve in soil after 8 weeks due to decomposition. Extractable [^{14}C]residues decreased from an average of 52.3 ± 16.3% of the applied at 2 weeks to <0.05%

after 16 weeks. Nonextractable [^{14}C]residues increased from an average of 2.5 ± 1.1% of the applied at 2 weeks to an average of 3.0 ± 0.4% of the applied at 4 weeks, then decreased to 0.5 ± 0.1% at 16 weeks.

In the plants, total [^{14}C]residues increased from an average of 1.1 ± 0.1% of the applied at 2 weeks to 5.7 ± 0.3% at 16 weeks.

In the roots, total [^{14}C]residues increased from an average of 0.4 ± 0.0% of the applied at 2 weeks to 2.7 ± 1.3% at 4 weeks, then decreased to 1.5 ± 0.9% at 8 weeks and 0.9 ± 0.1% at 16 weeks at a depth of 0-15 cm. For the remaining 15-30 cm and 30-61 cm depths, total [^{14}C]residues were <0.05% of the applied. Extractable [^{14}C]residues were a maximum average of 0.3 ± 0.1% of the applied and nonextractable [^{14}C]residues were a maximum average of 2.4 ± 1.3% of the applied, each at 4 weeks.

In the soil, the majority of [^{14}C]residues were found in the surface soil (0-15 cm). Total [^{14}C]residues increased from an average of 59.8 ± 2.1% of the applied at 2 weeks to 76.2 ± 14.0% at 8 weeks, and were 70.2 ± 0.6% at 16 weeks. [^{14}C]Residues averaged ≤0.05% of the applied at the 15-61 cm depths for the 2- and 4-week intervals. At 8 and 16 weeks, total [^{14}C]residues were an average 1.5 ± 0.9% and 1.8 ± 1.0% of the applied, respectively, in the 15-30 cm depth; 0.7 ± 0.5% and 1.0 ± 0.6%, respectively, in the 30-46 cm depth; and 0.3 ± 0.1% and 0.7 ± 0.3%, respectively, in the 46-61 cm depth. Extractable [^{14}C]residues were a maximum average of 64.4 ± 12.0% of the applied at 8 weeks (0-15 cm). Nonextractable [^{14}C]residues were a maximum average of 14.2 ± 1.1% of the applied at 16 weeks (0-15 cm). No transformation products were identified, as no degradation of [thiazole-2-^{14}C]clothianidin was observed in the soil. HPLC analysis of the surface soil extracts confirmed the identity of clothianidin.

Using nonlinear degradation kinetics (GraphPad™ PRISM), a DT_{50} of 165 days ($k = 0.0042$ day^{-1}; $r^2 = 0.66$) was determined.

No radioactivity was recovered in the leachate for the first eight leaching events. At the ninth leaching event, an average of 0.05% of the applied radioactivity was recovered. The percentage of applied radioactivity leached from the soil declined in the subsequent leaching events, and was <0.01% of the applied at the final leaching event. A cumulative 0.17% of the applied radioactivity was leached. HPLC analysis showed that clothianidin was the primary residue in the leachate, accounting for a maximum of 0.055% of the applied. Thiazolyl methyl urea and an unidentified polar transformation product accounted for maximums of 0.014% and 0.016% of the applied, respectively.

The study authors proposed that the route of dissipation for clothianidin was through formation of bound residues and movement into the plant.

Study Acceptability: This study is classified as [to be filled in by EFED reviewer]. No significant deviations from good scientific practices were noted.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: No guidelines exist for this study.

COMPLIANCE: The study was conducted in compliance with USEPA 40 CFR Part 160 (1989; p. 3). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Certification statements were provided (pp. 2-5).

A. MATERIALS:

1. Test Material [Thiazole-2-^{14}C]-clothianidin (p. 16; Figure 1, p. 48).

Chemical Structure: See DER Attachment 1.

Description: Technical grade.

Purity:

Radiolabeled
- Radiochemical purity: 95.7% (Vial No. C-1015).
  98.9% (Vial No. C-908; p. 16).
  98.4%, range 98.0-98.7% (TLC; Figure 8, pp. 56-57).
- Analytical purity: Not reported.
- Specific radioactivity: 26.4 mCi/mmole (Vial No. C-1015).
  26.4 mCi/mmole (Vial No. C-908).
- Location of the label: Carbon 2 of the thiazole ring.

Storage conditions of test chemicals: The test substance was stored frozen (≤-13.6°C) in acetonitrile (2.96 µg/µL; p. 16).

Physico-chemical properties of clothianidin:

Parameter	Value	Comment
Molecular weight	249.7 g/mole	
Molecular formula	Not reported	
Water Solubility	0.327 g/L	At 20°C.
Vapor Pressure/Volatility	1.3×10^{-10} Pa. 3.8×10^{-11} Pa.	At 25°C. At 20°C.
UV Absorption	Not reported.	
pKa	11.09	At 20°C.
K_{ow}/log K_{ow}	Not reported.	
log P_{ow}	0.7	At 25°C.
Stability of compound at room temperature, if provided	Not reported.	

Data were obtained from p. 17 and Figure 1, p. 48 of the study report.

2. Soil Characteristics

Table 1: Description of soil collection and storage.

Description	Details
Geographic location	Bayer Research Farm, Springfield, NE.
Pesticide use history at the collection site	Never been treated with a chloronicotinyl insecticide.
Collection procedures	Not reported.
Sampling depth	0-15.2 cm.
Storage conditions	Stored outside at ambient temperature in 30-gallon containers.
Storage length1	*ca*. 2 months.
Soil preparation	Not reported.

Data were obtained from p. 18 and Appendix 2, p. 77 of the study report.
1 Storage length was determined by the reviewer as the interval between the date of collection (May 10, 2004) to experimental study initiation (July 23, 2004).

Table 2: Properties of the soils.

Property	Details
Soil texture (USDA)[1]	Silt loam
% Sand	24
% Silt	53
% Clay	23
pH (saturated paste)	6.1
Organic carbon (%)	1.8
Organic matter (%)	3.1
CEC (meq/100 g soil)	22.7
Moisture at 1/3 atm (%)	28.3
Bulk density (g/cc)	1.14
Biomass (mg microbial C/100 g or CFU or other)	Not reported.
Soil taxonomic classification	Fine-silty, mixed, superactive, mesic Typic Hapludoll.
Soil series	Marshall.
Soil mapping unit (for EPA)	Latitude: 41.01° Longitude: -96.09°

Data were obtained from Table 1, p. 32 and Appendix 2, p. 77 of the study report.
1 Textural classifications were confirmed by the reviewer using the NRCS soil texture calculator http://soils.usda.gov/technical/aids/investigations/texture/ which calculates texture based on the percent sand and clay.

C. STUDY DESIGN:

1. Preliminary study: No preliminary studies were reported.

2. Definitive study experimental conditions: The mobility of [thiazole-2-^{14}C]-labeled (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine (clothianidin; purity 98.4%; nominal application rate of 1.25 mg/seed; measured application rate of 1.17 ± 0.02 mg a.i./seed; p. 27; Table 2, p. 33; Table 8, p. 39) was investigated using a silt loam soil planted with treated corn seeds (Pioneer® brand corn hybrid 3223 with CRM 116; pp. 17, 19; Appendix 4, p. 79). The soil microbial biomass was not determined.

Soil columns were prepared by packing PVC pipe (10 x 31 inches) with silt loam soil to a depth of 24 inches (p. 17; Table 2, p. 33; Figure 3, p. 51). The bottom of each column was capped with a PVC cap modified with thirteen 1-inch diameter holes. Mesh (4 mm) was placed on top of the holes, followed by a layer of glass wool and a layer of sand (5 cm). The columns were saturated using distilled water and were allowed to drain to field capacity.

Prior to planting the seeds in the soil columns, a bromide ion breakthrough test was conducted to demonstrate column flow (pp. 18-19). Filter paper was used to protect the edges of the surface of each soil column from the tracer solution. Bromide solution (2.5 mL), prepared by adding KBr (10 g; purity 99.2%; K-1333) to deionized water (35 mL), was added to the surface of each soil column using a glass pipette (Figure 6, p. 54). Following application, glass wool was added to the soil surface, and each column was leached with deionized water (unspecified volume) for 6 days. Leachate was collected in stainless steel pans and derivatized by mixing aliquots (100 µL) with 100 µL each of phosphate buffer (pH 6.4), sodium 2-iodosobenzoate, and 2,6-dimethyl-phenol solutions. The samples were stored for 5 minutes at room temperature. Sulfuric acid solution (100 µL) was added, and the samples were again stored for 5 minutes at room temperature prior to the addition of acetonitrile:water (45:55, v:v; 0.5 mL). A series of bromide standards (5-100 ppm) was also derivatized. The samples were analyzed by HPLC under the following operating conditions: REXGHROM S5-100-ODS C_{18} (2) column (250 mm x 4.5 mm; particle size not reported), isocratic mobile phase of acetonitrile:0.1% H_3PO_4 in water (45:55, v:v), flow rate of 1.5 mL/minute with UV detection (220 nm; p. 24). Bromide ion was also measured with a probe. Readings obtained from a range of standards were used to derive a standard curve (linear regression analysis) which was used to extrapolate concentrations from readings of samples. Bromide ion breakthrough was observed in all soil columns, thereby demonstrating column flow prior to planting the treated seeds (p. 27).

For treatment of the seeds, an aliquot (9.01 mL) of [thiazole-2-^{14}C]clothianidin (purity 98.4%; Figure 8, pp. 56-57), prepared in acetonitrile at a concentration of 2.59 mg/mL, was added to a round bottom flask (25 mL; pp. 19-20; Table 3, p. 34). The solution was rotary evaporated just to dryness, the flask was rinsed with acetone (2 x 300 µL), and the solution was evaporated under nitrogen. An aliquot (25 µL) of formulation blank was added and the sample was sonicated prior to the addition of polymer (10 µL; Poncho/Polymer PRECISE™ Seed Finisher 1003; commercial application rate of 1.5 fluid oz/80,000 seeds; Appendix 4, p. 79). Water (40 µL) was added to produce a slurry, 17 corn seeds (Pioneer® brand corn hybrid; relative maturity 116 days) were added, and the mixture was shaken by hand for *ca*. 5 minutes. Talc (9 mg; Gustafson; treatment rate of 1.5 oz/80,000 seeds) was added and the flask was shaken by hand for *ca*. 3 minutes. The seeds were removed and placed on wax paper to dry. To determine whether the target application rate was achieved, five seeds were extracted with acetone (3 x 5 mL) under ambient conditions, the extracts were pooled, and aliquots (3 x 1 mL) were analyzed for total radioactivity using LSC. The seeds were extracted using an Accelerated Solvent Extraction (ASE) and acetonitrile (100%) at 100°C and 1500 psi (Table 4, p. 35). The extracted seeds were analyzed using LSC following combustion.

For leaching, ten prepared soil columns were each planted with one treated seed (pp. 17, 21; Table 2, p. 33). Prior to planting, a spatula was used to till the soil to a 6-inch depth. Fertilizer (Osmocote slow release, 14-14-14) was added to the surface of the soil and the seeds were planted at a depth of 1.5 inches. The bottom of the columns were covered with plastic sheeting to protect from light, and the columns were stored in a heated greenhouse maintained at 9-45°C under a 12-hour light:12-hour dark cycle (pp. 18, 21; Figure 4, p. 52; Figure 5B, p. 53). Fourteen

leaching events were conducted throughout the leaching phase. The targeted rainfall amount was equivalent to the average monthly rainfall in Branchton, Ontario, Canada (Table 5, p. 36). Leachate was collected in stainless steel pans daily for the first week and then per leaching event thereafter (Figure 5A, p. 53). Duplicate soil columns were sacrificed at 2, 4, 8, and 16 weeks post corn-spike (3, 5, 9, and 17 week post-planting). Samples were analyzed within one week of sampling, then were stored frozen (<0°C) for a maximum of 30 days.

3. Description of analytical procedures:

Extraction/clean up/concentration methods: Frozen soil columns were cut into segments (*ca*. 15 cm; p. 22; Table 7, p. 38; Figure 9, p. 58). The soils were partially dried at room temperature and homogenized using a jar mill. Aliquots were extracted on an ASE at ambient conditions with acetonitrile, followed by water, and then acetonitrile at 100°C. The extracts were combined prior to analysis. Extraction efficiency, monitored on representative samples, averaged 102% (p. 28).

The seeds were removed from the soil and extracted by shaking for 20 minutes with acetonitrile under ambient conditions, followed by extraction on an Accelerated Solvent Extractor (ASE) with acetonitrile (p. 22; Figure 9, p. 58). The roots were removed from the soil, weighed, and extracted by shaking with acetonitrile.

Total ^{14}C measurement: The soil, seed, and root extracts were analyzed for total radioactivity using LSC (p. 22; Figure 9, p. 58).

Non-extractable residues, if any: Plant material was removed from the surface of the soil columns, weighed, and frozen (p. 22; Figure 9, p. 58). The frozen tissues were homogenized using a blender, combusted, and analyzed using LSC to determine total radioactivity within the plant.

The extracts were filtered and the seeds and roots were dried, combusted, and analyzed using LSC (p. 22; Figure 9, p. 58). At early intervals, the entire plants and roots were combusted. At later intervals, the tissues were homogenized with a robocup using dry ice prior to combustion.

Derivatization method, if used: A derivatization method was not employed in this study.

Identification and quantification of parent compound: Aliquots of the soil extracts were analyzed for [thiazole-2-^{14}C]clothianidin using HPLC under the following conditions: YMC ODS-AQ SN 042544567 column (dimensions and particle size not reported), gradient mobile phase combining (Solvent System A) 0.1% phosphoric acid in water and (Solvent System B) acetonitrile [percent A:B (v:v) at 0-1 min., 100:0; 26 min., 10:90; 27-30 min., 0:100], flow rate of 1.0 mL/minute with UV (254 nm) and radioactive flow detection (pp. 22-23). The retention time of [thiazole-2-^{14}C]clothianidin was *ca*. 18 minutes (Figure 10A, p. 59). A second HPLC method was used as a confirmatory method. HPLC Method II was coducted under the following

operating conditions: Phenomenex Aqua C18 125A column (250 x 4.6 mm; 5 µm), gradient mobile phase combining (Solvent System A) 0.1% formic acid in water and (Solvent System B) acetonitrile [percent A:B (v:v) at 0-3 min., 100:0; 25-28 min., 5:95; 30 min., 100:0], flow rate of 1.0 mL/minute with UV (254 nm) and radioactive flow detection. The retention time of [thiazole-2-^{14}C]clothianidin was *ca*. 19 minutes (Figure 10B, p. 59). HPLC recoveries, monitored on representative samples, averaged 95.2% (p. 28).

LC-ESI/MS was performed in positive ion mode (capillary temperature 350°C, spray voltage 4.5 kV, nitrogen sheath gas (40 psi) and auxillary gas (5 mL/min; p. 24). The scanned mass range was 50-750 amu with a scan time of 1 second. LC was performed using a Nucleodur column (250 x 2 mm; 5 µm), mobile phase combining (A) water in 0.1% formic acid and (B) methanol [percent A:B (v:v) at 0-1 min., 95:5; 15-21 min., 0:100; 21.5-26 min., 95:5], flow rate of 250 µL/minute (p. 25).

Identification and quantification of transformation products, if appropriate: Samples were analyzed for transformation products as described for the parent compound. The following reference standards were used:

Common name (Synonyms)	IUPAC name	CAS name	CAS number	Molecular weight (g/mole)	SMILES Code
Desmethyl (TZNG; K-1177)	N-[(2-Chloro-1,3-thiazol-5-yl)methyl][oxido(oxo)-hydrazono]methanediamine.	N-[(2-Chloro-5-thiazolyl)methyl]-N'-nitroguanidine.	135018-15-4	235.6	[O-][N+](=O)N=C(NCc1cnc(s1)Cl)N
Thiazolyl methylurea (TZMU; K-1178)	N-[(2-Chloro-1,3-thiazolyl-5-yl)methyl]-N'-methylurea.	N-[(2-Chloro-5-thiazolyl)methyl]-N'-methylurea.	634192-72-6	205.7	CNC(=O)NCc1cnc(s1)Cl
Thiazolyl urea (TZU; K-1192)	N-[(2-Chloro-1,3-thiazolyl-5-yl)methyl]urea.	[(2-Chloro-5-thiazolyl)methyl]urea.	635283-92-0	191.6	NC(=O)NCc1cnc(s1)Cl

Data were obtained from Figure 1, pp. 48-49 of the study report.

Detection limits (LOD, LOQ) for the parent compound: The minimum sensitivity for LSC analysis was 0.0036% of the applied radioactivity for the leachate samples (Appendix 5, p. 80). The minimum sensitivity for LSC analysis was 0.11%, 2.9 x 10^{-5}%, and 1.4 x 10^{-2}% of the applied radioactivity for the soil, seed, and plant extracts, respectively (p. 22; Appendix 6, p. 81). The Limit of Detection (LOD) for HPLC analysis was 1000 dpm; the minimum sensitivity for detection was 0.002% of the applied radioactivity (p. 24; Appendix 7, p. 82). The Limit of Quantification (LOQ) for HPLC analysis was not reported.

Detection limits (LOD, LOQ) for the transformation products, if appropriate: The LOD and LOQ were as described for the parent compound.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: The samples were stored in a greenhouse maintained at a mean temperature of 24°C (range 9-45°C; p. 27; Appendix 8, p. 83). Relative humidity was a mean of 30% (range 22-80%). Approximately 130 cm (51 inches) of water were added to the soil columns over a 4-month period, which exceeded the target of the 30-year annual rainfall for Branchoton, Ontario, Canada (pp. 28, 30; Table 9, p. 40; Figure 12, p. 61).

Normal agricultural practices were used to maintain plant health during the study (p. 28). Fertilizer (Osmocote) was added to surface soil prior to seed planting and on day 37 post corn-spike. Fertilizer (Peters Professional 20:20:20; rate 1.5 g fertilizer/L deionized water) was also added with the leaching water on days 69, 78, and 79 post-corn spike. A foliar application of Kelthane was applied on days 21 and 75 to control for spider mites, and Orthene was applied on day 36 for aphids and on day 84 for mites.

B. MASS BALANCE: Mean mass balances were 116.1% of the applied at 2 weeks, 91.8% at 4 weeks, 85.7% at 8 weeks, and 80.5% at 16 weeks (p. 29; Tables 12-15, pp. 43-46).

C. LEACHING: In the seeds treated with [thiazole-2-^{14}C]clothianidin, total [^{14}C]residues decreased from an average of 54.9 ±17.5% of the applied at 2 weeks to 28.7 ± 0.3% at 4 weeks, and were 0.5 ± 0.1% at 16 weeks (p. 29; Tables 12-16, pp. 43-47; Figure 16, p. 66; DER Attachment 2). The seed was difficult to retrieve in soil after 8 weeks due to decomposition. Extractable [^{14}C]residues decreased from an average of 52.3 ± 16.3% of the applied at 2 weeks to <0.05% after 16 weeks. Nonextractable [^{14}C]residues increased from an average of 2.5 ± 1.1% of the applied at 2 weeks to an average of 3.0 ± 0.4% of the applied at 4 weeks, then decreased to 0.5 ± 0.1% at 16 weeks.

In the plants, total [^{14}C]residues increased from an average of 1.1 ± 0.1% of the applied at 2 weeks to 5.7 ± 0.3% at 16 weeks (p. 29; Tables 12-16, pp. 43-47; Figure 16, p. 66).

In the roots, total [^{14}C]residues increased from an average of 0.4 ± 0.0% of the applied at 2 weeks to 2.7 ± 1.3% at 4 weeks, then decreased to 1.5 ± 0.9% at 8 weeks, and 0.9 ± 0.1% at 16 weeks, at a depth of 0-15 cm (p. 29; Tables 12-16, pp. 43-47; Figure 16, p. 66; DER Attachment 2). For the remaining 15-30 cm and 30-61 cm depths, total [^{14}C]residues were <0.05% of the applied. Extractable [^{14}C]residues were a maximum average of 0.3 ± 0.1% of the applied and nonextractable [^{14}C]residues were a maximum average of 2.4 ± 1.3% of the applied, each at 4 weeks.

In the soil, the majority of [^{14}C]residues were found in the surface soil (0-15 cm; p. 29; Tables 12-16, pp. 43-47; Figure 17, p. 67; DER Attachment 2). Total [^{14}C]residues increased from an average of 59.8 ± 2.1% of the applied at 2 weeks to 76.2 ± 14.0% at 8 weeks, and were 70.2 ± 0.6% at 16 weeks. [^{14}C]Residues averaged ≤0.05% of the applied at the 15-61 cm depths for the

2- and 4-week intervals. At 8 and 16 weeks, total [^{14}C]residues were an average 1.5 ± 0.9% and 1.8 ± 1.0% of the applied, respectively, in the 15-30 cm depth; 0.7 ± 0.5% and 1.0 ± 0.6%, respectively, in the 30-46 cm depth; and 0.3 ± 0.1% and 0.7 ± 0.3%, respectively, in the 46-61 cm depth. Extractable [^{14}C]residues were a maximum average of 64.4 ± 12.0% of the applied at 8 weeks (0-15 cm; Figure 18, p. 68). Nonextractable [^{14}C]residues were a maximum average of 14.2 ± 1.1% of the applied at 16 weeks (0-15 cm). No transformation products were identified, as no degradation of [thiazole-2-^{14}C]clothianidin was observed in the soil. HPLC analysis of the surface soil extracts confirmed the identity of clothianidin (Figures 19-21, pp. 69-74).

Using nonlinear degradation kinetics (GraphPad™ PRISM), a DT_{50} of 165 days ($k = 0.0042$ day^{-1}; $r^2 = 0.66$) was determined (pp. 26, 30; Figure 22, p. 75).

No radioactivity was recovered in the leachate for the first eight leaching events (p. 28; Table 10, p. 41; Figure 13, p. 62; Appendices 9-10, pp. 84-88). At the ninth leaching event, an average of 0.05% of the applied radioactivity was recovered. The percentage of applied radioactivity leached from the soil declined in the remaining leaching events, and was <0.01% at the final leaching event. A cumulative 0.17% of the applied radioactivity was leached. HPLC analysis showed that clothianidin was the primary residue in the leachate, accounting for a maximum of 0.055% of the applied (Table 11, p. 42). Thiazolyl methyl urea and an unidentified polar transformation product accounted for maximums of 0.014% and 0.016% of the applied, respectively (Figures 14-15, pp. 63-65).

The study authors proposed that the route of dissipation for clothianidin was through formation of bound residues and movement into the plant (p. 30).

III. STUDY DEFICIENCIES

No guidelines exist for this study. No study deficiencies were noted.

IV. REVIEWER'S COMMENTS

1. The maximum annual application rate for clothianidin seed treatment uses on corn and canola is 0.105 kg/ha, based on the corn application rate of 1.25 mg/seed and 84,000 seeds/hectare (p. 16). The target application rate for use in this study was based on the maximum annual application rate for clothianidin seed treatment of 1.25 mg/seed.

2. An excessive amount of water was required to generate enough leachate for the leaching study, due to intense evapotranspiration of the plants (p. 28; Appendix 9, pp. 84-87).

3. Plant and soil extracts were stored frozen and leachate samples were refrigerated prior to analysis (p. 30). All samples were analyzed within 30 days. It was stated that no storage stability data are required.

4. Table 2 of the study report incorrectly states that the soil columns were 24 inches in length (p. 33). The soil columns were 31 inches in length, and were packed with the test soil to a depth of 24 inches.

V. REFERENCES

1. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 163-1. Mobility studies. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.

2. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

3. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738.

4. U.S. Environmental Protection Agency. 2003. Guidance for Calculating Sorption Coefficients in Batch Equilibrium Studies.

Attachment 1: Structures of Parent Compound and Transformation Products

Clothianidin [C-1015, C-908, TI435, K-1142, TI-435, TI-435 50 WDG, TI-435 50WDG]

IUPAC Name: (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine.
(E)-N-(2-chloro-1,3-thiazol-5-yl)methyl]-N-[oxido(oxo)hydrazono]methanediamine.
Chloro-1,3-thiazol-5-yl)methyl]-N-{(E)-(methylamino)[oxido(oxo)hydrazono]methyl}amine.

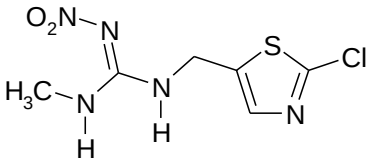
CAS Name: [C(E)]-N-[(2-chloro-5-thiazolyl)methyl]-N′-methyl-N″-nitroguanidine.

CAS Number: 210880-92-5 (formerly 205510-53-8).

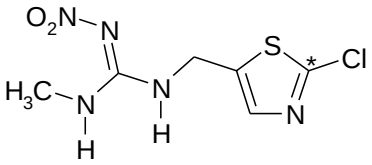
SMILES String: CNC(=N[N+](=O)O)NCc1cnc(Cl)s1 (Online SMILES Translator and Structure File Generator at http://cactus.nci.nih.gov/services/translate/).
[O-][N+](=O)N=C(NCc1cnc(s1)Cl)NC.

Empirical formula: $C_6H_8ClN_5O_2S$ **Molecular formula:** $C_6H_8ClN_5O_2S$

Unlabeled



[Thiazole-2-^{14}C]Clothianidin



* = Location of the radiolabel.

Identified Compounds

Clothianidin [C-1015, C-908, TI435, K-1142, TI-435, TI-435 50 WDG, TI-435 50WDG]

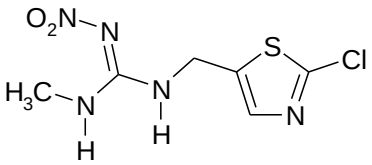
IUPAC Name: (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine.
(E)-N-(2-chloro-1,3-thiazol-5-yl)methyl]-N-[oxido(oxo)hydrazono]methanediamine.
Chloro-1,3-thiazol-5-yl)methyl]-N-{(E)-(methylamino)[oxido(oxo)hydrazono]methyl}amine.

CAS Name: [C(E)]-N-[(2-chloro-5-thiazolyl)methyl]-N′-methyl-N″-nitroguanidine.

CAS Number: 210880-92-5 (formerly 205510-53-8).

SMILES String: CNC(=N[N+](=O)O)NCc1cnc(Cl)s1 (Online SMILES Translator and Structure File Generator at http://cactus.nci.nih.gov/services/translate/).
[O-][N+](=O)N=C(NCc1cnc(s1)Cl)NC.

Empirical formula: $C_6H_8ClN_5O_2S$ **Molecular formula:** $C_6H_8ClN_5O_2S$

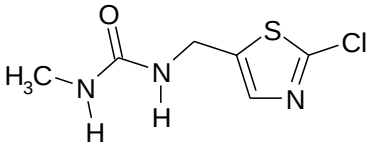


Thiazolyl methylurea [TZMU, K-1178]

IUPAC Name: 1-(2-Chlorothiazol-5-ylmethyl)-3-methylurea.
N-[(2-Chloro-1,3-thiazolyl-5-yl)methyl]-N'-methylurea.

CAS Name: N-[(2-Chloro-5-thiazolyl)methyl]-N'-methylurea.

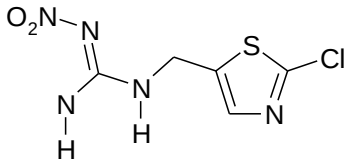
CAS Number: 634192-72-6.



Unidentified Reference Compounds

Desmethyl [TZNG, K-1177]

IUPAC Name: N-[(2-chloro-1,3-thiazol-5-yl)methyl][oxido(oxo)hydrazono]methanediamine.
CAS Name: N-[(2-chloro-5-thiazolyl)methyl]-N'-nitroguanidine.
CAS Number: 135018-15-4.



Thiazolyl Urea [TZU, K-1192]

IUPAC Name: N-[(2-Chloro-1,3-thiazol-5-yl)methyl]urea.
CAS Name: [(2-Chloro-5-thiazolyl)methyl]urea.
CAS Number: Not reported.