Anaerobic Aquatic Metabolism of Pymetrozine

Report: Das, Y. (1996) CGA-215944: Metabolism of (Triazinyl-6- (carbon-14)) CGA-215944 Under Anaerobic Aquatic Soil Conditions: Lab Project Number: 94014: 48-94. Unpublished study prepared by Innovative Scientific Services, Inc. 606 p.

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Classification: A study classification was not provided on the attached Data Evaluation Record, therefore, this study is now classified as **Supplemental**. This study is scientifically valid and provides useful information on the anaerobic aquatic metabolism of pymetrozine in flooded sandy loam soil; however, the pattern of decline of several degradates were not clearly demonstrated, and the test soils were flooded with reagent grade water rather than natural water. The second extract contained a maximum of 18.5% of the applied and were not characterized.

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DATA EVALUATION RECORD

STUDY 5b

CHEM: 101103 Pymetrozine §162-3
CAS No. 123312-89-0
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44024968
Das, Y. T. 1996. Metabolism of [triazinyl-6-^{14}C]CGA-215944 under anaerobic aquatic soil conditions. ISSI Study No: 94014; Sponsor Study No: 48-94. Unpublished study performed by Innovative Scientific Services, Inc., Piscataway, NJ; and submitted by Ciba-Geigy Corporation, Greensboro, NC.

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CONCLUSIONS

Metabolism - Anaerobic Aquatic

1. This study is scientifically valid and provides useful information on the anaerobic aquatic metabolism of pymetrozine in flooded sandy loam soil.

2. These studies can be used to fulfill the Subdivision N Guidelines of EPA data requirements on anaerobic aquatic metabolism. However; note the following:

   (i) no clear pattern of decline of the major degradate CGA-180777 was observed by the end of study. However, no further action is needed as the studies were conducted to the maximum required period (approximately one year).

   (ii) the test soils were flooded with reagent grade water rather than natural water. Although Subdivision N Guidelines recommend the use of natural water, studies using reagent water should produce half-lives longer than those reported in natural water, since the microbial activity in reagent water are presumably much less than in natural water.

3. Triazinyl ring-labeled [6-^{14}C]pymetrozine, at nominal concentrations of 1.5 and 10.3 ppm, degraded with registrant-calculated half-lives of 88 (r^2 = 0.98) and 108 (r^2 = 1.00) days, respectively, in anaerobic flooded sandy loam soil incubated in darkness at 25 °C for up to 176 and 357 days, respectively. All data, designated as percentages of the applied, were reported as percentages of the nominal application.

   In the water phase of the 1.5 ppm test system, the parent compound was 83.8% of the applied radioactivity at day 0, decreased to 44.6% of the applied by 7 days, and was 7.1% at 176 days. The major degradate 4-amino-4,5-dihydro-5-hydroxy-6-methyl-1,2,4-triazin-3(2H)-one was a maximum of 17.0-17.6% of the applied radioactivity at 120-176 days. The minor degradates CGA-294849, CGA-215525, GS-23199, CGA-249257, CGA-313124, CGA-359009 ("2U" in data tables), and fourteen unidentified degradates were each detected at ≤6.6% of the applied radioactivity. In the acetonitrile:water soil extract, the parent compound was 12.2% of the applied radioactivity at day 0, was a maximum of 45.4% of the applied at 3 days, and was 16.0% at 176 days. The minor degradates CGA-294849, CGA-215525, GS-23199, CGA-249257, CGA-313124, CGA-359009, and ten unidentified degradates were each detected at ≤3.2% of the applied radioactivity. The NaOH soil extract contained a maximum of 18.5% of the applied radioactivity at 58 days; [^{14}C]residues were not characterized. Evolved $^{14}CO_2$ accounted for 3.6% of the applied radioactivity at 176 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase from 0 to 3 days and from 120 to 176 days posttreatment.

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In the water phase of the 10.3 ppm test system, the parent compound was 88.4% of the applied radioactivity at day 0, decreased to 48.4% of the applied by 14 days, and was 1.9% at 357 days posttreatment. The major degradate GS-23199 was a maximum of 13.4% of the applied radioactivity at 357 days posttreatment. The major degradate CGA-249257 was a maximum of 11.5% of the applied radioactivity at 273 days posttreatment and was 10.8% at 357 days. The major degradate 4-amino-4,5-dihydro-5-hydroxy-6-methyl-1,2,4-triazin-3(2H)-one was a maximum of 17.8% of the applied radioactivity at 176 days posttreatment, and was 10.4% at 357 days. The minor degradates CGA-294849, CGA-215525, CGA-313124, CGA-359009, and thirteen unidentified compounds were each detected at ≤5.8% of the applied radioactivity. In the acetonitrile:water soil extract, the parent compound was detected at 9.2% of the applied radioactivity at day 0, was a maximum of 39.4% of the applied at 7 days posttreatment, and was 9.0% at 357 days. The minor degradates CGA-294849, GS-23199, CGA-249257, CGA-313124, CGA-359009, and ten unidentified compounds were each detected at ≤2.4% of the applied radioactivity. The NaOH soil extract contained a maximum of 12.5% of the applied radioactivity at 58 days posttreatment; [^{14}C]residues were not characterized. Evolved $^{14}CO_2$ accounted for 6.1% of the applied radioactivity at 357 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase throughout the incubation (except at day 58).

METHODOLOGY

Samples (5 g) of sieved (2 mm) Hanford sandy loam soil (71% sand, 20% silt, 9% clay, 0.3% organic matter, pH 7.4, CEC 4.6 meq/100 g; Table II, p. 58) collected from Sanger, CA, were placed into borosilicate glass bottles and flooded with reagent-grade water (water characteristics were not reported); the final soil:water ratio was 1:2 (w:v, pp. 30-31). The soil/water systems were purged with hydrogen and nitrogen, and pre-incubated anaerobically (nitrogen atmosphere) in sealed test vessels in darkness at 25 °C for approximately two weeks (Appendix I, p. 237). Following the pre-incubation period, the soil/water samples were treated with triazinyl ring-labeled [6-^{14}C]pymetrozine (6-methyl-4-(pyridin-3-yl-methylenamino)-4,5-dihydro-1,2,4-triazin-3(2H)-one; radiochemical purity 97.5%; specific activity 55.4 µCi/mg; p. 99), dissolved in acetonitrile, at a nominal rate of 1.5 or 10.3 ppm (p. 33). Treated flasks were purged with hydrogen and nitrogen, and incubated anaerobically (nitrogen atmosphere) in sealed test vessels in darkness at 25 ± 1 °C for up to 176 or 357 days, respectively. To trap volatiles, a 2.0 *N* potassium hydroxide volatile trap was used inside each test vessel (Figures 5, 7; pp. 103, 105). Soil/water samples treated at 1.5 ppm were removed for analysis at 0, 1, 3, 7, 14, 30, 58, 92, 120, and 176 days posttreatment; samples treated at 10.3 ppm were removed for analysis at 0, 1, 3, 7, 14, 30, 58, 92, 120, 176, 273, and 357 days posttreatment (p. 34). Triplicate samples (one for pH and redox potential determinations only) were analyzed for each test system at each sampling interval. To determine soil viability, additional treated samples were analyzed at 0, 30, 58, 176, and 357 (10.3 ppm treatment only) days

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posttreatment for total anaerobic microbial populations in flooded soil. Microbial populations were reported as CFU/g dry soil; data indicated that both test systems were viable at each sampling interval (Table VIII, p. 66).

Soil/water samples were centrifuged, and the water phase was decanted and analyzed in triplicate for total radioactivity by LSC; the limit of detection was 0.2 ng (as parent pymetrozine; pp. 34, 36). Aliquots of the water phase were filtered (0.45 μm) and concentrated by evaporation and/or lyophilization, and analyzed by reverse-phase HPLC (Zorbax RX-C8 column) using a mobile phase gradient of water:methanol (both with 0.05% trifluoroacetic acid, v:v; 100:0 to 95:5 to 0:100, v:v) with radioactive flow detection; the limit of quantitation was ≥0.01% of the injected radioactivity (p. 37). Samples were co-chromatographed with non-radiolabeled reference standards which were detected by UV light (220 nm). HPLC column recoveries (for water samples) based on LSC analysis of collected fractions were 95.9% to 103.5% (1.5 ppm test system) and 96.8% to 102.7% (10.3 ppm test system; p. 38). To confirm compound identities, samples were analyzed by reverse-phase HPLC/MS (Zorbax RX-C8 column) with a mobile phase gradient of water:methanol, (100:0 to 95:5 to 50:50, v:v) with the mass spectrometer in the atmospheric pressure chemical ionization, E.I. or D.C.I. modes (pp. 39, 44). Additional confirmational analyses were performed using two-dimensional TLC on silica gel plates developed perpendicularly with chloroform:methanol:ammonium hydroxide:water (80:30:2:4, v:v:v:v) and ethyl acetate:methanol:water (75:15:10, v:v:v) or with isopropanol and chloroform:methanol:formic acid:water (75:25:4:2, v:v:v:v; p. 38); reverse-phase two-dimensional TLC (C8 silica gel plates; solvents as described above; p. 49); NMR spectrometry (p. 48); and GC/MS (E.I. or D.C.I. mode; pp. 40, 49). Samples analyzed by TLC were co-chromatographed with nonradiolabeled reference standards which were visualized with UV (254 nm) light; radiolabeled parent and degradates were located using a radioimaging scanner (p. 39). Radioactivity was further quantified by scraping areas of radioactivity on TLC plates into vials, and dissolving the gel in deionized water and acetonitrile; triplicate aliquots of each solution were analyzed for total radioactivity by LSC.

Soil samples were extracted three times by shaking and sonicating with acetonitrile:water (8:2, v:v); samples were centrifuged and the extract was decanted and combined (p. 36). Triplicate aliquots were analyzed for total radioactivity by LSC. Extracts were filtered, concentrated, and analyzed by HPLC as described above for the water samples. Column recoveries were 95.0-104.0% (1.5 ppm test system) and 95.7-104.4% (10.3 ppm test system). Parent and degradate compound identities were confirmed in selected samples by HPLC/MS or TLC as described above. Soil samples were further extracted by agitating and sonicating at 80 °C with 1.0 *N* aqueous NaOH; samples were centrifuged and the extract decanted. The extraction was repeated and triplicate aliquots of the combined NaOH extracts were analyzed for total radioactivity by LSC. Selected NaOH extracts were analyzed by reverse-phase HPLC as described above; several polar compounds were separated, but were not identified (pp. 50-51). The water phase and the acetonitrile:water extracts were analyzed within 1 day of sampling; test samples were stored up to 45 days prior to the NaOH extraction (Tables VI, VII, pp. 64-65). Triplicate subsamples of

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post-extracted soil were analyzed for total radioactivity by LSC following combustion. At each sampling interval, the head space of each test vessel was analyzed for total radioactivity by LSC following combustion (p. 35). Volatile traps (2.0 *N* potassium hydroxide) were removed for analysis at 100 (1.5 ppm test systems) and 100 and 191 (10.3 ppm test systems) days posttreatment (pp. 31); triplicate aliquots were analyzed for total radioactivity by LSC. Precipitation with barium carbonate confirmed that all volatile compounds present were $^{14}CO_2$ (p. 43).

To confirm the presence of anaerobic conditions, the redox potential was measured at each sampling interval in both test systems; pH was measured at each sampling interval following 58 days posttreatment (p. 33). The redox potentials were measured using a platinum electrode (E_o) and were corrected to normal hydrogen electrode potentials (E_{nhe}); data for both test systems indicated that anaerobic conditions were maintained throughout the incubation. In the 1.5 ppm test system, redox potentials were -411 to -738 mV (E_o) and -212 to -539 mV (E_{nhe}; Table IX, p. 67). In the 10.3 ppm test system, redox potentials were -361 to -760 mV (E_o) and -162 to -561 mV (E_{nhe}). The pH was 8.3 to 8.9 (1.5 ppm test system) and 8.2 to 8.9 (10.3 ppm test system; Table X, p. 68).

DATA SUMMARY

Triazinyl ring-labeled [6-^{14}C]pymetrozine (radiochemical purity 97.5%), at nominal concentrations of 1.5 and 10.3 ppm, degraded with respective registrant-calculated half-lives of 88 (r^2 = 0.98) and 108 (r^2 = 1.00) days in anaerobic flooded sandy loam soil incubated in darkness at 25 °C for up to 176 and 357 days, respectively (Tables XXVII, XXVIII, pp. 97-98; Figures 101, 102, pp. 213-214). All data, designated as percentages of the applied, were reported as percentages of the nominal application.

Low Treatment Rate System (1.5 ppm)

In the water phase of the 1.5 ppm test system, the parent compound was 83.8% of the applied radioactivity at 0 days posttreatment, decreased to 44.6% of the applied by 7 days, was 14.0% at 92 days posttreatment, and was 7.1% at 176 days posttreatment (Table XVII, pp. 79-80). The major degradate

> 4-amino-4,5-dihydro-5-hydroxy-6-methyl-1,2,4-triazin-3(2H)-one (common name not specified; designated as "Unknown III" in data tables)

was initially detected at 0.3% (single replicate) of the applied radioactivity at 3 days posttreatment, increased to 11.4% of the applied by 58 days posttreatment, and was 17.0-17.6% at 120-176 days posttreatment. The minor degradates 4-amino-6-methyl-1,2,4-triazine-3,5(2H, 4H)-dione-methyl-1,2,4 (CGA-294849); 4-amino-4,5-dihydro-6-methyl-1,2,4-triazin-3(2H)one (CGA-215525); GS-23199 (chemical name not specified; structure reported in Figure 100, p. 212); 4,5-dihydro-6-methyl-1,2,4-triazin-3(2H)-one (CGA-249257); CGA-313124 (chemical

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name not specified; structure reported in Figure 100, p. 212); 1,2,4-triazine-3,5(2H, 4H)-dione, dihydro-6-methyl-4-[(3-pyridinylmethylene)amino]- (CGA-359009; designated as "2U" in data tables); and fourteen unidentified degradates were each detected at ≤6.6% of the applied radioactivity. Uncharacterized [^{14}C]residues were a maximum of 1.6% of the applied radioactivity.

In the acetonitrile:water soil extracts of the 1.5 ppm test system, the parent compound was initially detected at 12.2% of the applied radioactivity at 0 days posttreatment, increased to a maximum of 45.4% of the applied by 3 days posttreatment, was 25.4% at 120 days, and was 16.0% at 176 days posttreatment (Table XXII, p. 88-89). The minor degradates CGA-294849, CGA-215525, GS-23199, CGA-249257, CGA-313124, CGA-359009 (designated as "2U" in data tables), "Unknown III," (see water results above) and nine unidentified degradates were each detected at ≤3.2% of the applied radioactivity. Uncharacterized [^{14}C]residues were a maximum of 1.2% of the applied radioactivity. The NaOH soil extract contained a maximum of 18.5% of the applied radioactivity at 58 days posttreatment and was 15.3% of the applied at 176 days posttreatment (Table XI, pp. 69-70); [^{14}C]residues were not characterized. Nonextractable [^{14}C]residues were a maximum of 3.0% of the applied radioactivity at 0 and 176 days posttreatment.

Evolved $^{14}CO_2$ accounted for a maximum of 3.6% of the applied radioactivity at 176 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase from 0 to 3 days and 120 to 176 days posttreatment.

Material balances were 98.9-106.4% of the applied radioactivity throughout the incubation period.

High Treatment Rate System (10.3 ppm)

In the water phase of the 10.3 ppm test system, the parent compound was 88.4% of the applied radioactivity at 0 days posttreatment, decreased to 48.4% of the applied by 14 days posttreatment and 12.6% by 120 days, and was 1.9% at 357 days posttreatment (Table XVIII, pp. 81-82). The major degradate

GS-23199 (chemical name was not reported; structure reported in Figure 100, p. 212)

was initially detected at 0.2% of the applied radioactivity at 30 days posttreatment, increased to 3.6% of the applied by 120 days posttreatment, and was a maximum of 13.4% at 357 days posttreatment. The major degradate

4,5-dihydro-6-methyl-1,2,4-triazin-3(2H)-one (CGA-249257)

was initially detected at 0.2% of the applied radioactivity at 30 days posttreatment,

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increased to 6.4% of the applied by 120 days posttreatment, and was 10.8-11.5% at 273-357 days posttreatment. The major degradate

4-amino-4,5-dihydro-5-hydroxy-6-methyl-1,2,4-triazin-3(2H)-one (common name not specified; designated as "Unknown III")

was initially detected at 0.2% of the applied radioactivity at 3 days posttreatment, was a maximum of 17.6-17.8% at 120-176 days posttreatment, and was 10.4% at 357 days posttreatment. An unidentified major degradate (designated as Unknown I) was detected at a maximum of 11.5% at 273 days posttreatment and was 5.9% of the applied at 357 days posttreatment. Selected extracts (day 273 and 357) further analyzed by TLC were determined to contain three unidentified minor degradates plus the minor degradate 4-amino-4,5-dihydro-6-methyl-1,2,4-triazin-3-one at 2.3-5.7% (p. 52; Table XXVI, p. 96), each at ≤4.0% of the applied radioactivity. The minor degradates 4-amino-6-methyl-1,2,4-triazine-3,5(2H, 4H)-dione-methyl-1,2,4 (CGA-294849); 4-amino-4,5-dihydro-6-methyl-1,2,4-triazin-3(2H)one (CGA-215525); CGA-313124 (chemical name not specified; structure reported in Figure 100, p. 212); 1,2,4-triazine-3,5(2H, 4H)-dione, dihydro-6-methyl-4-[(3-pyridinylmethylene)amino]- (CGA-359009; designated as "2U" in data tables); and thirteen unidentified compounds were each detected at ≤5.8% of the applied radioactivity. Uncharacterized [^{14}C]residues were a maximum of 1.8% of the applied radioactivity.

In the acetonitrile:water soil extract of the 10.3 ppm test system, the parent compound was initially detected at 9.2% of the applied radioactivity at 0 days posttreatment, increased to a maximum of 39.4% of the applied by 7 days posttreatment, was 19.4% at 176 days posttreatment, and was 9.0% at 357 days posttreatment (Table XXIII, pp. 90-91). The minor degradates CGA-294849, CGA-2155255, GS-23199, CGA-249257, CGA-313124, CGA-359009 (designated as "2U" in data tables), and ten unidentified compounds were each detected at ≤2.4% of the applied radioactivity. Uncharacterized [^{14}C]residues were a maximum of 0.8% of the applied radioactivity. The NaOH soil extract contained a maximum of 12.5% of the applied radioactivity at 58 days posttreatment and was 11.9% of the applied at 357 days posttreatment (Table XII, pp. 71-72); [^{14}C]residues were not characterized. Nonextractable [^{14}C]residues were a maximum of 3.1% of the applied radioactivity at 357 days posttreatment.

Evolved $^{14}CO_2$ accounted for 6.1% of the applied radioactivity at 357 days posttreatment. The distribution of [^{14}C]residues between the soil and water fractions was not reported; however, the majority of the [^{14}C]residues was observed in the water phase throughout the incubation with the exception of day 58.

Material balances were 101.0-105.0% of the applied radioactivity throughout the incubation period.

COMMENTS

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1. The patterns of decline of several degradates were not clearly demonstrated in either test system studied. In the water phase of the 1.5 ppm test system, "Unknown III" (identified in the data summary) was a maximum of 17.0-17.6% of the applied at the final sampling intervals (120-176 days posttreatment); the degradates GS-23199, CGA-249257 and Unknown IV were respective maximums of 5.2%, 6.6% and 4.2% of the applied at 176 days. In the water phase of the 10.3 ppm test system (see Comment #2), CGA-249257 was a maximum of 10.8-11.5% of the applied at the final sampling intervals (273-357 days posttreatment); the degradates GS-23199 and Unknown II were respective maximums of 13.4% and 5.8% of the applied at 357 days. Subdivision N Guidelines require that the study be conducted until the formation and decline of degradates is clearly demonstrated, or for one year. Although the high treatment rate study (10.3 ppm) was conducted for approximately one year, exaggerated rate studies may not be used to determine kinetics information, as discussed in Comment #2 below.

2. The reviewer notes that the low treatment rate study was performed at the maximum seasonal label rate. Data obtained from the high (exaggerated) treatment rate study may be used for degradate identification purposes, but may not be used to provide kinetics information (USEPA. 1993. *Pesticide Reregistration Rejection Rate Analysis: Environmental Fate*. EPA 738-R-93-010, p. 67).

3. The test soil was flooded with reagent grade water rather than natural water. The microbial and chemical composition of natural water may alter the pattern or rate of degradation of pymetrozine determined in this study; the use of reagent grade water is not recommended as it does not adequately represent a natural sediment/water system.

4. The test soil was not flooded for 30 days prior to treatment with pymetrozine; however, redox measurements indicated that the systems were anaerobic at the time of treatment (Table IX, p. 67).

5. The registrant only reported residue data in the form of percentages of the applied or injected radioactivity. In future studies submitted to the EPA, it is necessary that residue data also be submitted in units of concentration, such as ppm.

6. The reported water solubility of pymetrozine is 270 mg/mL (20 ^{o}C, pH 7.35; p. 99).

7. The maximum recommended use rate for pymetrozine is 5 oz. a.i./A/application, with a maximum of 10 applications per growing season (p. 52). The low dose rate of 1.5 ppm used in the study is approximately equal to the soil concentration in the 0- to 6-inch depth following the application of 50 oz. a.i./A, which is the maximum total seasonal application rate.