DATA EVALUATION RECORD

STUDY 9

CHEM 129112 Trifloxystrobin §162-4
CAS No. 141517-21-7
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44496734
Atkins, R. H., W. B. Nixon, and P. N. Coody. 1997. Aerobic aquatic metabolism of [phenyl(A)-U-^{14}C]-CGA-279202. PTRL Project No. 1057. Novartis Study No. 290-96. Unpublished study performed by PTRL East, Inc., Richmond, KY; and submitted by Novartis Crop Protection, Inc., Greensboro, NC.

DIRECT REVIEW TIME = 43.5 Hours

REVIEWED BY:	D. A. Saccone, B.S.	Signature: M C Mahoney
TITLE:	Scientist M. Mahoney, M.S. Scientist	Date: 3/23/99
EDITED BY:	C. A. Little, Ph.D.	Signature: Paul Clarke for C.A.Little
TITLE:	Sr. Scientist/Asst. Project Manager	Date: 3/23/99
APPROVED BY:	P. H. Howard, Ph.D.	Signature: Philip H Howard
TITLE:	Project Manager	Date: 3/22/99
ORG:	Syracuse Research Corp. Arlington, VA 22202	
TEL:	703/413-9369	

APPROVED BY:	Raanan Bloom
TITLE:	Environmental Scientist
ORG:	ERB III/EFED/OPP
TEL:	703/305-6464

SIGNATURE:

CONCLUSIONS

Metabolism - Aerobic Aquatic

1. This study is scientifically valid and provides useful information on the aerobic aquatic metabolism of trifloxystrobin.

2. This study does not meet Subdivision N Guidelines for the fulfillment of EPA data requirements on aerobic aquatic metabolism for the following reasons:

   (i) the pesticide was not applied at the same time the soil was flooded; and

   (ii) redox potential data were not provided for the sediment phase.

3. Uniformly phenyl ring-labeled [^{14}C]trifloxystrobin, at a nominal application rate of 0.61 ppm, degraded with a registrant-calculated half-life of 14.5 hours (0-2 day data only; r^2 = 0.89) in aerobic flooded soil that was incubated in darkness at 25 ± 1 °C for up to 99 days. The apparent half-life was <8 hours. However, the data may not accurately depict the metabolism of the parent under aerobic aquatic conditions because a 4-day preincubation period was used, during which the soil was flooded (prior to treatment with the pesticide); redox potential data were not provided for the soil and aerobicity at treatment was not confirmed. All data, designated as percent of the applied radioactivity based on HPLC analysis, are percentages of the nominal application. In the aqueous phase, the parent compound was initially present at 24.6% of the applied radioactivity (reviewer-calculated mean of two replicates) and was last detected at 1.3% at 8 hours posttreatment. The major degradate CGA-321113 was initially (day 0) 7.5% of the applied, was a maximum of 82.3% at 15 days, and was 53.5% of the applied at 99 days. In the soil extracts, the parent compound was initially 54.5% of the applied radioactivity, decreased to 27.4% by 8 hours, and was 0.3-0.9% of the applied from 15 to 99 days. The major degradate CGA-321113 was initially (day 0) 2.4% of the applied radioactivity, was a maximum of 23.3% at 61 days, and was 21.1% at 99 days. The minor degradate CGA-331409 was a maximum of 0.4% of the applied radioactivity at day 0. Nonextractable [^{14}C]residues were a maximum of 13.8% of the applied radioactivity at 99 days; radioactivity associated with the fulvic acid, humic acid, and humin fractions (99 days) was 55%, 23%, and 10% of the nonextractable radioactivity, respectively. Evolved $^{14}CO_2$ accounted for a maximum of 2.5% of the applied radioactivity at 99 days posttreatment; [^{14}C] organic volatiles were negligible.

METHODOLOGY

Subsamples (20 g) of sieved (2 mm) sandy loam soil (78% sand, 10% silt, 12% clay, 1.4% organic matter, pH 6.6, CEC 5.5 meq/100 g; Table II, p. 45) collected from

Wareham, MA, were placed in flasks and flooded with 100 mL of HPLC grade water (pp. 16, 17). The soil/water systems were pre-incubated in darkness at 25°C for 4 days prior to treatment; humidified, CO_2-free air was drawn through the flasks. Following the pre-incubation period, the soil/water systems were treated with uniformly phenyl ring-labeled [^{14}C]trifloxystrobin {CGA-279202; methyl (E, E)-α-(methoxyimino-Z-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl]benzeneacetate; radiochemical purity 98.5%, specific activity 38.4 μCi/mg; pp. 16, 36}, dissolved in acetonitrile, at a nominal application rate of 0.61 ppm (based on total sample weight; p. 19). Flasks containing the soil/water systems were sealed, mixed by swirling, and incubated in darkness at 25 ± 1°C; the temperature was monitored daily and the data were provided in Table V (p. 48). To determine microbial activity, two untreated soil/water systems were prepared and incubated under similar conditions as the test samples (p. 17). A vacuum source was utilized to draw humid, CO_2-free air through the systems and into an ethylene glycol trap and two 10% aqueous KOH volatile traps (p. 19; Figure 2, p. 67). Duplicate soil/water samples were removed for analysis at 0, 0.33 (8 hours), 1, 2, 5, 15, 30, 61 and 99 days posttreatment (p. 17).

At each sampling interval, the soil/water systems were transferred to centrifuge bottles and the soil and water phases were separated by centrifuging (p. 20). The supernatant was decanted and filtered (Whatman No. 1 paper), and triplicate aliquots were analyzed for total radioactivity by LSC; the limit of quantitation was two times the background level (p. 24). The aqueous phase was concentrated and analyzed by reverse-phase HPLC (Ultrasphere C-18 ODS column) using a mobile phase gradient of 0.1% aqueous formic acid:acetonitrile (100:0 to 35:65 to 0:100, v:v) with UV (250 nm) and radioactive flow detection (pp. 22, 24); the limit of quantitation was 0.004 μg/mL (p. 34). Samples were co-chromatographed with nonradiolabeled reference standards. To confirm compound identities, sample extracts were concentrated and analyzed by two-dimensional TLC on silica gel plates developed sequentially in toluene:chloroform:ethyl ether:88% formic acid (60:34:5:1, v:v:v:v) and toluene:ethyl acetate:17.4 *N* glacial acetic acid (70:30:1.5, v:v:v); areas of radioactivity on the plates were quantitated by radioimage scanning (p. 26). Samples were co-chromatographed with nonradiolabeled reference standards which were visualized by UV (254 nm) light. Areas of radioactivity on the plates were scraped, dissolved in methanol, and analyzed for total radioactivity by LSC (p. 28).

Soil samples were extracted by shaking three times with acetonitrile:acidified water (glacial acetic acid, pH 4; 8:2, v:v) and centrifuged. The supernatants were decanted, filtered (Whatman No. 1 paper) and combined, and aliquots were analyzed for total radioactivity by LSC (p. 21). Post-extracted soil samples were air dried, ground to powder, and analyzed for total radioactivity by LSC following combustion (p. 23). Selected post-extracted soil samples (day 99) were re-extracted with acetonitrile:acidified water (pH 4) using Soxhlet extraction; extracts were concentrated by rotary evaporation and analyzed by LSC and HPLC as previously described. The limit of quantitation for HPLC was 0.012 μg/g (p. 34). To determine radioactivity associated with the humic acid,

fulvic acid and humin fractions of soil organic matter, selected post-extracted soil samples (day 99) were further extracted by refluxing with 0.1 *N* NaOH for 4 hours and centrifuged. The residual soil was extracted by shaking with HPLC grade water and centrifuged. The supernatants were decanted, filtered (Whatman No. 1 paper), and combined with the reflux solutions. The caustic extract was acidified to pH 2 (2 *N* HCl), stored overnight to precipitate humic acids, and centrifuged. The supernatant (fulvic acid fraction) was analyzed for total radioactivity by LSC. The precipitate and air-dried, post-extracted soil were analyzed by LSC following combustion to determine the radioactivity associated with the humic acid and humin fractions, respectively. All filters used in the analytical phase were combusted and analyzed by LSC (p. 23).

Triplicate aliquots of the volatile trap solutions were analyzed for total radioactivity by LSC at each sampling interval and at 43 days posttreatment for the 61-day sample, and at 43 and 82 days posttreatment for the 99-day sample (p. 23); the limits of quantitation for LSC were 48 dpm/mL for ethylene glycol and 158 dpm/mL for KOH (p. 24). The presence of $^{14}CO_2$ in the KOH traps was confirmed using $BaCl_2$ precipitation.

To confirm the presence of aerobic conditions in the aqueous phase, the redox potential (E_h), pH, and dissolved oxygen content were measured at each sampling interval (p. 20). Conditions were moderately oxidizing to oxidizing in the aqueous phase, with redox potentials of 206-281 mV (with the exception of 149-179 at 5 days) and dissolved oxygen contents of 4.0-8.0 mg/L throughout the incubation period (Table VI, p. 51). The pH was 7.0 ± 0.90 throughout the incubation period.

To confirm the microbial viability of the soil/water systems, control samples were incubated for 6 or 35 days (to simulate 4 days prior to dosing and approximately 30 days posttreatment) and aerobic bacteria, actinomycetes, and fungi were enumerated by plate count using selective media (p. 17); data indicated that the soils were viable (Table IV, p. 47).

DATA SUMMARY

Uniformly phenyl ring-labeled [^{14}C]trifloxystrobin (radiochemical purity 98.5%), at a nominal application rate of 0.61 ppm, degraded with a registrant-calculated half-life of 14.5 hours (0-2 day data only; $r^2 = 0.89$) in aerobic flooded soil that was incubated in darkness at 25 ± 1 °C for up to 99 days (Table XVI, p. 64; Figure 22, p. 87). The apparent half-life of the compound was less than the reported half-life; the parent degraded from 79.1% to 28.7% of the applied radioactivity between 0 and 8 hours posttreatment. However, the data may not accurately depict the metabolism of the parent under aerobic aquatic conditions because a 4-day preincubation period was used, during which the soil was flooded (prior to treatment with the pesticide). All data, designated as percent of the applied radioactivity based on HPLC analysis, are percentages of the nominal application.

In the aqueous phase, the parent compound was initially present at 24.6% of the applied radioactivity (reviewer-calculated mean of two replicates) and was last detected at 1.3% of the applied at 8 hours posttreatment (Table VIII, p. 54). The major degradate

(E,E)-α-(methoxyimino)-Z-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]amino] oxy]methyl]benzeneacetic acid (CGA-321113)

was initially (day 0) present at 7.5% of the applied radioactivity, increased to 59.2% of the applied by 8 hours and 72.7% by 1 day posttreatment, was a maximum of 82.3% at 15 days posttreatment, and was 53.5% of the applied at 99 days posttreatment.

In the soil extracts, the parent compound was initially present at 54.5% of the applied radioactivity, decreased to 27.4% by 8 hours and 2.5% by 5 days posttreatment, and was 0.3-0.9% of the applied from 15 to 99 days posttreatment. The major degradate

CGA-321113

was initially (day 0) present at 2.4% of the applied radioactivity, increased to 13.4% of the applied by 2 days, was a maximum of 23.3% of the applied at 61 days posttreatment, and was 21.1% of the applied at 99 days posttreatment; based on replicate data, a clear pattern of degradation was not observed by the end of the incubation period. The minor degradate (E,Z)-α-(methoxyimino)-Z-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]amino] oxy]methyl] benzeneacetic acid (CGA-331409) was present at a maximum of 0.4% of the applied radioactivity at day 0 and was last detected at 0.1% of the applied (one replicate) at 5 days posttreatment. Two unidentified minor degradates were each detected at ≤0.3% of the applied radioactivity.

Nonextractable [^{14}C]residues were 0.5-6.6% of the applied radioactivity through 61 days and were 13.8% of the applied at 99 days posttreatment. At 99 days posttreatment, 55%, 23%, and 10% of the nonextractable radioactivity was associated with the fulvic acid, humic acid, and humin fractions, respectively (p. 38).

Evolved $^{14}CO_2$ was ≤0.2% through 61 days posttreatment and accounted for 2.5% of the applied radioactivity at 99 days posttreatment; [^{14}C]organic volatiles were 0.6% of the applied at 99 days (Table VII, p. 52).

Material balances (for individual replicates) were 91.0-97.4% of the applied radioactivity throughout the incubation period (Table VII, p. 52).

COMMENTS

1. The soil was not treated with [^{14}C]trifloxystrobin at the same time the system was

flooded. Soil samples were flooded and preincubated for four days prior to treatment. Subdivision N Guidelines require that the pesticide be applied to the soil at the same time the soil is flooded so that both aerobic and anaerobic conditions exist in the soil and the predominant microorganism population is aerobic. Redox potential data were not submitted for the soil phase (also see Comment #3). Based on the rapid degradation rate observed for the parent compound, it is unlikely that an additional study would provide new information on the half-life of the parent compound. However, the effects of the preincubation period on the metabolism of the major degradate are unclear.

2. The study authors reported that the test water was HPLC grade water (p. 17). Subdivision N Guidelines require that the test water be representative of the intended use site.

3. Redox potential data were provided only for the aqueous phase; the redox potential was not measured in the soil phase. Because data were not provided, the reviewer was unable to confirm that aerobic conditions were present in the soil at the time of treatment (four days following flooding).

4. The study authors did not submit concentration data for the parent compound and its degradates in the water and soil phases; data were reported only as percentages of the applied radioactivity. In future studies submitted to the EPA, the registrant should include concentration data.

5. The limits of detection were not reported for LSC and HPLC. Method detection limits should be reported to allow the reviewer to assess the adequacy of the methods for the determination of the parent and its degradates.

6. The origin and soil series name of the soil utilized in this study were not reported. The study author reported that the soil was provided by Springborn Laboratories located in Wareham, MA (p. 16).

7. The proposed metabolic pathway of [^{14}C]trifloxystrobin under aerobic aquatic conditions was provided in Figure 23 (p. 88).

8. The study was conducted using uniformly phenyl ring-labeled [^{14}C]trifloxystrobin (CGA-279202). The compound contained an additional phenyl ring structure that was not radiolabeled (Figure 1, p. 65). An additional study with the second ring labeled may not be necessary because the total recovery of parent compound and degradates exceeded 90%, suggesting that no new degradates greater than 10% of the dose rate would likely be formed from this unlabeled ring.

9. Sterilized soil/water test systems were not prepared as controls and incubated identically to the treated test systems.

10. The aqueous solubility of the parent compound is 0.6 ppm at 20 °C (pH not specified; MRID 44496733).

11. The proposed maximum label rate use (one application) for the parent compound was reported as 0.5 lb a.i./A , or 0.5 ppm based on the surface three inches of soil (p. 34). The reviewer notes that an application rate of 0.61 ppm was utilized in this study, while a rate of 0.17 ppm was utilized in the anaerobic aquatic metabolism study (MRID 44496733).

12. The reviewer notes that the nominal application rate (0.61 ppm) was calculated based on the total weight of the samples and that the initial aqueous concentration was not reported. Generally, the reported application rate is based on the aqueous concentration (or the expected aqueous concentration) at the time 0 and does not take into account the mass of the soil.

13. The reviewer notes that, based on the study authors' statements on page 35 which indicate that compounds present in this study at ≥2% of the applied radioactivity (0.0122 ppm) must be identified, the registrant is likely not aware of the current EPA requirements/policy concerning the identification of major degradates. Only compounds present at levels approaching 10% of the applied radioactivity are considered to be major degradates in the laboratory studies.

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Pages 8 through 33 are not included.

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