DATA EVALUATION RECORD

STUDY 7

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CAS No. 141517-21-7
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44496731
Fackler, P. 1997. Metabolism of [phenyl(A)-U-^{14}C]-CGA-279202 in a typical loam soil under aerobic conditions. Laboratory Project ID: 1781.1295.6518.760. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA; and submitted by Novartis Crop Protection, Inc., Greensboro, NC.

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REVIEWED BY: M. T. Holdsworth, M.S. Signature: Mark Holdsworth
TITLE: Scientist Date: 3/23/99

EDITED BY: C. A. Little, Ph.D. Signature: Paul Clifton for C.A. Little
TITLE: Sr. Scientist/Asst. Project Manager Date: 3/23/99

APPROVED BY: P. H. Howard 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 Soil

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

2. The study meets Subdivision N Guidelines for the fulfillment of EPA data requirements on aerobic soil metabolism.

3. Uniformly phenyl ring-labeled [^{14}C]trifloxystrobin, at a nominal concentration of 0.5 mg/kg, degraded with a registrant-calculated half-life of 2.0 days ($r^2 = 0.97$) in loam soil maintained at 75% of 0.33 bar moisture content and incubated in darkness at 25 ± 1 °C for up to 365 days. The degradation of trifloxystrobin was biphasic, with an apparent half-life of <1 day. The parent compound rapidly converted to the acid equivalent (CGA-321113) of trifloxystrobin. Degradate data are reported in parent eqivalents. All data, designated as percentages of the applied, are percentages of the nominal application. The parent compound was initially present at 98.6% (493 ppb) of the applied radioactivity, decreased to 43.5% (218 ppb) by 1 day posttreatment and 7.7% (38.3 ppb) by 7 days, and was last detected at 1.2% (5.9 ppb) of the applied at 210 days. The major degradate CGA-321113 was a maximum of 84.7% (424 ppb) of the applied radioactivity at 28 days posttreatment and was 39.5% (197 ppb) at 365 days. The minor degradate CGA-357276 was present at a maximum of 5.6% (27.9 ppb) of the applied radioactivity at 365 days posttreatment. The minor degradates CGA-373466, CGA-357261, CGA-331409, and CGA-320299 were each present at ≤1.0% (≤5 ppb) of the applied radioactivity throughout the incubation period. Nonextractable [^{14}C]residues were 27.4% of the applied radioactivity at 365 days posttreatment; radioactivity associated with fulvic acid, humic acid, and humin fractions were 4.2%, 2.0%, and 15.6% of the applied, respectively. Total [^{14}C]volatiles (detected as $^{14}CO_2$ only) were 5.3% of the applied radioactivity at 90 days posttreatment, increased to 10.0% by 151 days, and were a maximum of 17.9% at 365 days

METHODOLOGY

Samples (100 g) of sieved (2 mm) loam soil (collected from Grand Forks, North Dakota; 40% sand, 40% silt, 20% clay, 4.9% organic matter, pH 6.8, CEC 29.7 meq/100 g; Table 3, p. 57) were placed in aluminum trays, adjusted to 50% of the soil moisture content at 0.33 bar, and pre-incubated at 23 °C for 7 days (pp. 23, 24). Subsamples (10 g) of the pre-incubated soil were placed in an aluminum pan and treated with uniformly phenyl ring-labeled [^{14}C]trifloxystrobin {CGA-279202; methyl (E,E)-α-(methoxyimino)-2-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl] benzeneacetate; radiochemical purity 99.1%, specific activity 30.1 μCi/mg; p. 21}, dissolved in acetonitrile, at a nominal

3

application rate of 0.5 mg/kg. The treated soil samples were placed in amber bottles and mixed with additional subsamples (90 g) of nontreated, preincubated soil; the homogenized soil was adjusted to 75% of the soil moisture content at 0.33 bar. The soil moisture content was maintained at 75% of 0.33 bar by the addition of sterile water throughout the incubation period as necessary (p. 41). Amber bottles containing treated samples were placed horizontally in a rack and were incubated in darkness at 25 ± 1°C for up to 365 days (p. 26); temperature was measured daily (Table 7, pp. 61-71). Filtered, humidified CO_2-free air was pumped through the test bottles and into a series of volatile traps: a polyurethane foam plug followed by an ethylene glycol trap, two 10% KOH traps, and a Tenax®/charcoal tube (p. 27; Figure 3, p. 96). Duplicate samples were removed for analysis at 0, 0.25, 1, 7, 14, 28, 46, 90, 151, 210, 270 and 365 days posttreatment. An additional set of soil samples was treated with the test compound at a nominal application rate of 3 mg/kg; samples were incubated as described above and were removed for analysis at 0, 46, 324, and 365 days posttreatment to determine whether a dose rate response occurred.

At each sampling interval, soil samples were extracted twice by shaking with acetonitrile:water (8:2, v:v) and centrifuged (pp. 20, 28; Figure 4, p. 97). The supernatants were decanted and combined, and triplicate aliquots were analyzed by LSC; the limit of detection was 0.00014-0.00024% of the applied radioactivity (pp. 34). An aliquot of the combined extracts was concentrated by rotary evaporation and analyzed by HPLC (Hibar Lichrosorb RP-18 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 (p. 30); the limits of detection and quantitation were 120 dpm and 0.009-0.018 μg/mL, respectively. Fractions were collected at one-minute intervals and analyzed for total radioactivity by LSC; the limits of detection and quantitation were 27 dpm and 0.002-0.004 μg/mL, respectively (p. 34). To confirm compound identities, extracts were analyzed by two-dimensional TLC on silica gel plates developed with toluene:chloroform:ether:formic acid (60:34:5:1; v:v:v:v) followed by toluene:ethyl acetate:acetic acid (70:30:1.5, v:v:v; p. 31); [^{14}C]residues on the plates were quantified by radiomage scanning. Radioactive areas were scraped from the plates, extracted with methanol, and analyzed by LSC. Samples were co-chromatographed with nonradiolabeled reference standards which were visualized under UV (254 nm) light. To identify unknown metabolites, extracts were analyzed by LC/MS (Lichrosorb RP-18 column) using a mobile phase gradient of 0.1% acetic acid:acetonitrile (80:20 to 20:80; v:v) with UV (250 nm) detection (p. 32); the limit of detection was 0.001-0.005 μg/mL.

Post-extracted soil samples were Soxhlet extracted with acetonitrile:water (9:1; v:v) and triplicate extracts were analyzed by LSC (p. 20). Acetonitrile was removed from the sample extracts by rotary evaporation and the remaining aqueous phase was partitioned three times with ethyl acetate. Triplicate aliquots of the combined ethyl acetate partitions were analyzed by LSC. Post-extracted soil samples were subjected to a second Soxhlet extraction with methanol and triplicate aliquots of the extracts were analyzed by LSC. To

determine nonextractable [^{14}C]residues, post-extracted soil samples were analyzed by LSC following combustion (p. 29); data were corrected for combustion efficiency (82.6-96.6%; p. 34). In an attempt to remove bound residues, selected post-extracted soil samples (day 90) were refluxed with 0.1 *N* HCl and shaken (p. 28). The sample was centrifuged and triplicate aliquots of the extract were analyzed by LSC. To determine bound residues associated with humic acid, fulvic acid and humin fractions, subsamples of selected post-extracted soil samples were extracted by shaking overnight with $CaCl_2$ and 3 *N* NaOH, centrifuged, and the supernatant decanted (p. 29; Figure 5, p. 98). The supernatant was acidified to pH 1 with concentrated HCl and centrifuged. The supernatant was decanted, filtered and analyzed by LSC to determine the radioactivity associated with the fulvic acid fraction. The precipitate was combusted and analyzed by LSC to determine the radioactivity associated with the humic acid fraction. The post-extracted soil was analyzed by LSC following combustion to determine radioactivity associated with the humin fraction.

Volatile traps were removed for analysis at each sampling interval except 0 and 0.25 days (p. 27); traps were replaced biweekly beginning at day 7. The polyurethane foam plugs were extracted with methanol and triplicate aliquots were analyzed for total radioactivity by LSC. The Tenax®/charcoal traps were extracted with carbon disulfide:propanol (95:5, v:v) and triplicate aliquots of the extract analyzed by LSC. Triplicate aliquots of ethylene glycol and potassium hydroxide traps were analyzed by LSC. $^{14}CO_2$ was confirmed in selected KOH traps (46, 223, and 365 days) by precipitation with $BaCl_2$ (Table 10, p. 75).

Soil viability was determined at 0, 1, 46 and 365 days posttreatment by enumeration of fungi, actinomycetes and heterotrophic bacteria on peptone dextrose agar with rose bengal and streptomycin, malt agar, actinomycete agar, and nutrient agar plates supplemented with soil extract (Appendix III, pp. 187-191); data indicated that the soil was viable (Table 4, p. 58).

DATA SUMMARY

Uniformly phenyl ring-labeled [^{14}C]trifloxystrobin (radiochemical purity 99.1%), at a nominal concentration of 0.5 mg/kg, degraded with a registrant-calculated half-life of 2.0 days ($r^2 = 0.97$; 0-7 day data) in loam soil maintained at 75% of 0.33 bar moisture content and incubated in darkness at 25 ± 1° C for up to 365 days (Figure 8, p. 102; Table 14, p. 84). All data, designated as percentages of the applied, are percentages of the nominal application. The parent compound was initially present at 98.6% (493 ppb) of the applied radioactivity, decreased to 43.5% (218 ppb) of the applied by 1 day posttreatment and 7.7% (38.3 ppb) by 7 days, was 0.0-1.1% (0-5.3 ppb) at 46-151 days, and was last detected at 1.2% (5.9 ppb) at 210 days (Table 12, pp. 79, 80). The major degradate

(E,E)-α-methoxyimino-2-[[[[1-[3-trifluoromethyl)phenyl]ethylidene]amino]

oxy]methyl]acetic acid methyl ester (CGA-321113)

was initially present at 3.0% (14.8 ppb) of the applied radioactivity, increased to 52.5% (263 ppb) of the applied by day 1, and was a maximum of 84.7% (424 ppb) at 28 days posttreatment, and was 39.5% (197 ppb) at 365 days. The minor degradate (E)-α-2[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl]-benzonitrile (CGA-357276) was first detected at 1.0% (5 ppb) of the applied radioactivity at 7 days posttreatment, was 2.9-5.1% (14.6-25.5 ppb) at 28-270 days, and was a maximum of 5.6% at 365 days. The minor degradates (Z,E)-α-methoxyimino-2-[[[[1-[3-trifluoromethyl)phenyl]ethylidene] amino]oxy]methyl]acetic acid (CGA-373466), CGA-357261, CGA-331409, and CGA-320299 were each present at ≤1.0% (≤5 ppb) of the applied radioactivity throughout the incubation period (Tables 12 and 13, pp. 81-83). Uncharacterized [^{14}C]radioactivity was ≤4.4% of the applied radioactivity. Nonextractable [^{14}C]residues were a maximum of 27.7% of the applied radioactivity at 151 days, and were 27.4% of the applied at 365 days (Table 9, p. 73). [^{14}C]Residues associated with fulvic acid, humic acid, and humin fractions of the soil organic matter were 4.2%, 2.0%, and 15.6% of the applied radioactivity, respectively, at 365 days posttreatment (Table 15, p. 85). Total [^{14}C]volatiles (detected as evolved $^{14}CO_2$ only; p. 42) were first detected at 0.1% of the applied radioactivity at 1 day posttreatment, increased to 10.0% by 151 days, and were a maximum of 17.9% at 365 days.

Material balances (for individual replicates), based on LSC analysis, were 90.4-102.0% (with the exception of 107.4-120.1% at 28 days) of the applied radioactivity throughout the incubation period (Table 9, p. 73).

Additional data were reported for a separate set of samples treated at a nominal application rate of 3.0 mg/kg (see Comment #2).

COMMENTS

1. The registrant-calculated half-life of 2.0 days was greater than the apparent half-life of <1 day. By 1 day posttreatment, only 43.5% of the applied radioactivity was present as parent compound (Table 12, p. 79).

2. A second study was conducted at an application rate of 3.0 mg/kg and utilized only four sampling intervals (0, 46, 324, and 365 days posttreatment); these data were not reported by the reviewer. The reviewer reported the results from the 0.5 mg/kg study only. The reviewer notes that the percentages of applied radioactivity present respectively as parent compound and the degradate CGA-321113 at 365 days posttreatment were similar between the two studies, but that the degradate CGA-357276 was present at a greater percentage of the applied radioactivity (14.5% *vs.* 5.6%) in the 3.0-ppm treatment rate study (Table 16, pp. 86, 87). The study author stated that a dose rate response was not

observed (p. 48).

3. The study was conducted using uniformly phenyl ring-labeled [^{14}C]trifloxystrobin labeled on the α-ring. Two additional studies were done using uniformly phenyl ring-labeled [^{14}C]trifloxystrobin labeled on the α-ring (MRID 44496732) and the β-ring (MRID 44496730).

4. The study author stated that the concentration of the parent applied in this study was equivalent to the highest single application rate for soil (0.5 lb/A; p. 35). The reviewer notes that in an additional aerobic soil metabolism study (MRID 44496732), the study author stated that the nominal treatment rate in that study (3.0 ppm) was the approximate proposed maximum label rate for a single application (p. 22). Clarification by the registrant is necessary.

5. Frozen storage stability data were inadequate. Storage stability was assessed by analyzing samples collected at 1- and 151-days posttreatment and comparing the data with data from samples stored frozen for 11 months (day 1 sample) or 6 and 8.5 months (151 day sample; Table 11, p. 78). The study author stated that comparison of the original HPLC peaks with subsequent re-analysis confirmed compound stability (p. 42). A storage stability study should be conducted using soils collected from the test site that have been fortified separately with the test compound and its degradates and stored for a duration equal to the longest storage interval utilized for the test samples. The reviewer notes that storage stability studies are only required for samples stored for longer than 30 days. The maximum duration for which samples were stored was not reported.

6. The soil series name was not reported.

7. An extraction efficiency study was conducted (p. 19); data indicated that the extraction method was adequate for the loam soil (Table 1, p. 51).

8. To confirm the presence of aerobic conditions, redox potential (E_h), pH and dissolved oxygen were measured from a slurry of soil (5 g) and reagent water (10 mL) at each sampling interval (p. 26). The reviewer is unclear as to whether this method was adequate to demonstrate that aerobic conditions were maintained throughout the incubation, but notes that the redox potential values for the all replicates from the final three sampling intervals indicated that moderately reducing conditions were present (Table 8, p. 72). The length of time between the preparation of the slurry and the measurement of the redox potential was not reported.

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