DATA EVALUATION RECORD

STUDY 6

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FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44496730
Pike, S. L. 1997. Metabolism of [phenyl(B)-U-^{14}C]-CGA-279202 in a typical sandy loam soil under aerobic conditions. SLI Study No.: 1781.1295.6520.760. Novartis Study No.: 670-95. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA; and submitted by Novartis Crop Protection, Inc., Greensboro, NC.

DIRECT REVIEW TIME = 50 Hours

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CONCLUSIONS

Metabolism - Aerobic Soil

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

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

3. Uniformly phenyl ring-labeled [^{14}C]trifloxystrobin (radiochemical purity 98.7%), at a nominal application rate of 0.5 mg/kg, degraded with a registrant-calculated half-life of 2.4 days (r^2 = 0.82; 0-7 days data) in sandy 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. The parent compound was initially 98.2% (491 ppb) of the applied radioactivity, decreased to 53.8% (269 ppb) by 6 hours posttreatment and 24.9% (125 ppb) by 1 day, and was 0.7-1.8% (3.3-9.0 ppb) at 210-365 days. The major degradate CGA-321113 was a maximum of 85.2% (426 ppb) at 7 days posttreatment and was 43.4% (217 ppb) at 365 days. The minor degradate CGA-357276 was a maximum of 4.9% (24.5 ppb) of the applied radioactivity at 270 days posttreatment. The minor degradates CGA-373466 and CGA-357262 were each ≤4.1% (20.6 ppb) of the applied radioactivity from 90 to 365 days posttreatment; data from 0 to 43 days were not reported. Nonextractable [^{14}C]residues were a maximum of 22.4% of the applied radioactivity at 365 days posttreatment. [^{14}C]Residues associated with the fulvic acid, humic acid, and humin fractions (365 days) were 0.3%, <0.1%, and 0.2% of the applied radioactivity, respectively. Radiolabeled [^{14}C]volatiles were 1% of the applied radioactivity at 14 days posttreatment, increased to 8.7% by 150 days, and were a maximum of 19.5% at 365 days. Most of the [^{14}C]volatiles were identified as $^{14}CO_2$. Additional data (for only four sampling intervals) were reported for a separate set of samples treated at a nominal application rate of 3.0 mg/kg.

METHODOLOGY

Samples (100 g) of sieved (2 mm) sandy loam soil (collected from North Carolina; 78% sand, 10% silt, 12% clay, pH 6.6, 1.4% organic matter, CEC 5.5 meq/100 g; Table 3, p. 61) were weighed into aluminum trays, adjusted to 75% of the moisture content at 0.33 bar, and pre-incubated in darkness for 7 days at approximately 25 °C (pp. 27, 28). Following pre-incubation, soil subsamples were transferred to silanized amber glass bottles and treated by syringe with uniformly phenyl ring-labeled [^{14}C]trifloxystrobin {methyl (E,E)-alpha-(methoxyimino)-2-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]

amino]oxy]methyl]benzeneacetate; radiochemical purity 98.7%, specific activity 55.8 μCi/mg; p. 25; Figure 1, p. 91}, dissolved in acetonitrile, at a nominal application rate of 0.5 mg/kg. The acetonitrile was evaporated and each soil sample was mixed. Treated soil samples were incubated in darkness at 25 ± 1 °C (range of 22.0-27.0 °C; p. 44) for up to 365 days (p. 29); the moisture level was monitored bi-weekly and adjusted as necessary throughout the incubation period (p. 45). Moist CO_2-free air was pumped through the incubation bottles and into sequential organic volatile (toluene, polyurethane foam plugs, and ethylene glycol), CO_2 (two of 10% KOH, w:v), solvent vapor (water), and organic volatile (Tenax® charcoal) traps (p. 30; Figure 5, p. 98). Duplicate samples were removed for analysis at 0 and 6 hours; and at 1, 7, 14, 28, 43, 90, 150, 210, 270 and 365 days posttreatment. Additional duplicate samples were removed for analysis at each sampling interval to measure pH, dissolved oxygen, redox potential and temperature. An additional set of duplicate samples was treated at an exaggerated rate of 3.0 mg/kg and incubated in the same manner as the 0.5 mg/kg treated samples. Samples were removed for analysis at 0, 43, 150 (single sample) and 365 days posttreatment. All samples were extracted and analyzed within 30 days of collection (p. 32).

Soil samples were extracted twice by shaking with acetonitrile:water (8:2, v:v) followed by centrifugation; the extracts were combined, and triplicate aliquots were analyzed for total radioactivity by LSC (pp. 22, 23; Figure 6, p. 99). Aliquots of extracts were concentrated by rotary evaporation, and transferred to a graduated cylinder by rinsing; the rinsate plus extract were concentrated a second time under nitrogen, diluted with acetonitrile, and centrifuged. Extracts were analyzed by HPLC (Hibar Lichrosorb RP-18 column) using a mobile phase gradient of aqueous 0.1% formic acid:acetonitrile (100:0 to 35:65 to 0:100, v:v) with UV (250 nm) and radioactive flow detection (pp. 33, 34); samples were co-chromatographed with nonradiolabeled reference standards. The limit of quantitation was 0.008-0.016 μg/mL (pp. 37, 38). Eluent fractions were collected at one-minute intervals and analyzed by LSC; the limit of quantitation was 0.002-0.004 μg/mL. Post-extracted soils were re-extracted by Soxhlet extraction with acetonitrile:water (9:1, v:v) for two hours; triplicate aliquots of the extracts were analyzed for total radioactivity by LSC. Aliquots of Soxhlet extracts were concentrated by rotary evaporation, partitioned three times with ethyl acetate, and the ethyl acetate partitions were combined; triplicate aliquots were analyzed for total radioactivity by LSC. The remaining Soxhlet extract was dried through Na_2SO_4, concentrated, and analyzed by HPLC as described above. A second Soxhlet extraction was performed with methanol for four hours; triplicate aliquots of the methanol extract were analyzed for total radioactivity by LSC. The methanol Soxhlet extracts for samples collected beyond 14 days posttreatment were not analyzed by HPLC; LSC analysis indicated that total radioactivity was <5% in these extracts (p. 32). Triplicate subsamples of post-extracted soil were analyzed for total radioactivity by LSC following combustion; the limit of detection was 0.2-0.3% of the applied radioactivity.

To confirm compound identities, selected extracts were analyzed by two-dimensional

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TLC on Kieselgel or silica gel plates developed perpendicularly with toluene:chloroform:ethyl ether:88% formic acid (60:34:5:1, v:v:v:v) followed by toluene:ethyl acetate:acetic acid (70:30:1.5, v:v:v; pp. 34, 35). Radiolabeled analytes were detected with a radioimaging scanner; samples were co-chromatographed with nonradiolabeled reference standards which were visualized by UV light (254 nm). To quantify parent and degradates, radiolabeled areas were scraped into methanol and quantified by LSC. Selected samples (150 days posttreatment, 3.0 mg/kg treatment) of the acetonitrile:water 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 followed by MS performed in the positive/negative selective ion monitoring modes (pp. 35, 36); limits of detection were 0.001 μg/mL (trifloxystrobin, CGA-357261, CGA-357276, and CGA-357262) and 0.005 μg/mL (CGA-321113, CGA-373465, and CGA-373466; p. 38). Following LC/MS analysis, column eluent was collected in 0.5 minute intervals and analyzed for total radioactivity by LSC.

In an attempt to remove bound residues, selected post-extracted soil samples (day 365) were extracted by shaking with 0.1 *N* HCl (p. 32). The sample was centrifuged and triplicate aliquots of the extract were analyzed by LSC. To determine the remaining radioactivity associated with the humic acid, fulvic acid and humin fractions of the soil organic matter, subsamples (1 g) of post-extracted soil were extracted by shaking overnight with 3 *N* NaOH (10 mL) plus $CaCl_2$ (0.10 g); samples were centrifuged and the extract decanted (p. 32; Figure 7, p. 100). The extract was acidified (pH 1) with concentrated HCl to allow humic acids to precipitate. Following centrifugation and filtration, the supernatant containing fulvic acids was analyzed for total radioactivity by LSC; the precipitate (containing humic acids) was analyzed for total radioactivity by LSC following combustion. Following NaOH extraction, subsamples of soil were analyzed for total radioactivity by LSC following combustion to determine the humin-bound fraction.

Volatile traps were sampled, but were not replaced at 0 and 6 hours posttreatment; following 2 days of incubation, toluene traps were replaced twice weekly and the ethylene glycol and KOH traps were replaced biweekly (p. 30). The Tenax®/charcoal traps were removed simultaneously with soil samples at each sampling interval. The polyurethane foam plugs were extracted with methanol, and triplicate aliquots analyzed for total radioactivity by LSC (p. 31). The Tenax®/charcoal traps were extracted with carbon disulfide:propanol (95:5, v:v), and triplicate aliquots of the extracts were analyzed for total radioactivity by LSC. The ethylene glycol, KOH, and water trapping solutions were each adjusted to a volume of 15 mL with reagent-grade water and triplicate aliquots of each were analyzed for total radioactivity by LSC. To confirm the presence of $^{14}CO_2$ in selected KOH traps, trap solutions were precipitated with $BaCl_2$, centrifuged and the supernatant was analyzed for total radioactivity by LSC. Following $BaCl_2$ precipitation, KOH traps were attached to 2-3 phenethylamine traps, concentrated HCl was added to the KOH, and the system was attached to a vacuum and incubated overnight; aliquots of the phenethylamine traps were analyzed for total radioactivity by LSC.

To confirm the viability of the soil, microbial populations were determined prior to treatment and at 1, 47 and 365 days posttreatment (0.5 mg/kg treatment), or at 365 days posttreatment (3.0 mg/kg treatment; pp. 27, 44). Total microbial populations were determined by substrate-induced respiration and by plate counts on peptone/dextrose, malt, actinomycete, and NA plus soil extract agars; data indicated that soil samples were viable throughout the incubations (p. 44; Table 4, p. 62).

DATA SUMMARY

Uniformly phenyl ring-labeled [^{14}C]trifloxystrobin (radiochemical purity 98.7%), at a nominal application rate of 0.5 mg/kg, degraded with a registrant-calculated half-life of 2.4 days ($r^2 = 0.82$) in sandy loam soil maintained at 75% of 0.33 bar moisture content and incubated in darkness at 25 ± 1 °C for up to 365 days (Table 14, p. 83; Figure 9, p. 103). 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. The parent compound was initially present at 98.2% (491 ppb) of the applied radioactivity, decreased to 53.8% (269 ppb) of the applied by 6 hours posttreatment and 24.9% (125 ppb) of the applied by 1 day, and was 0.7-1.8% (3.3-9.0 ppb) at 210-365 days posttreatment (Table 13, pp. 80-82). The major degradate

CGA-321113 (E,E isomer; chemical structure presented in Table 2, p. 56)

was initially detected at 3.7% (18.7 ppb) of the applied radioactivity at time 0, increased to a maximum of 85.2% (426 ppb) by 7 days posttreatment, and was 43.4% (217 ppb) at 365 days posttreatment. The minor degradate CGA-357276 (E isomer; chemical structure presented in Table 2, p. 58) was detected sporadically through 43 days posttreatment, then increased to a maximum of 4.9% (24.5 ppb) of the applied radioactivity by 270 days posttreatment and was 3.4% (17.1 ppb) at 365 days. The minor degradates CGA-373466 (Z,E isomer; chemical structure presented in Table 2, p. 59) and CGA-357262 (Z,Z isomer; chemical structure presented in Table 2, p. 58), and an unidentified degradate were each present at ≤4.1% (20.6 ppb) of the applied radioactivity from 90 to 365 days posttreatment; data for 0 to 43 days posttreatment were not reported.

Nonextractable [^{14}C]residues were initially detected at 0.9% of the applied radioactivity at day 1 and increased to a maximum of 22.4% of the applied radioactivity by 365 days posttreatment (Table 10, p. 74). Radioactivity (following and additional acid extraction) associated with the fulvic acid, humic acid, and humin fractions from 365 days posttreatment were 0.3%, <0.1%, and 0.2% of the applied radioactivity, respectively (Table 15, p. 84). Radiolabeled [^{14}C]volatiles were 1% of the applied radioactivity at 14 days posttreatment, and increased to 8.7% by 150 days and to a maximum of 19.5% of the applied radioactivity by 365 days posttreatment. Most of the [^{14}C]volatiles were

identified as $^{14}CO_2$ (p. 46).

Material balances were 93.2-104.3% of the applied radioactivity throughout the incubation and did not show a clear pattern of loss with time (Table 10, p. 74).

Additional data (for only four sampling intervals) were reported for a separate set of samples treated at a nominal application rate of 3.0 mg/kg (see Comment #3).

COMMENTS

1. The registrant-calculated half-life of 2.4 days was greater than the apparent half-life of <1 day. By 1 day posttreatment, only 24.9% of the applied radioactivity was present as parent compound (Table 13, p. 80).

2. The study authors stated that the test samples were placed in an environmental chamber set to maintain the temperature at 25 ± 1 °C (p. 29). However, temperatures were not held constant at ± 1 °C, but ranged from 22.0 °C to 27.0 °C (p. 44). The study author stated that the temperature excursions beyond 25 ± 1 °C were short-lived and occurred well past the half-life of the parent.

3. A second study was conducted at an application rate of 3.0 mg/kg and utilized only four sampling intervals [0, 43, 150 (single replicate only) and 365 days]; these data were not reported by the reviewer. The reviewer notes that the percentages of applied radioactivity present respectively as parent compound, CGA-321113 and CGA-357276 at 365 days posttreatment were similar between the two studies (Table 16, pp. 85-86).

4. The study author stated that the 0.5 mg/kg treatment rate was equivalent to a maximum single application of 0.5 lb a.i./A/season (p. 39). 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. Limits of detection OR quantitation were reported for each analysis (pp. 37, 38). Both limits of detection and quantitation should be reported for each analysis to allow the reviewer to evaluate the adequacy of the method for the determination of the test compound and its degradates.

6. The study was conducted using uniformly phenyl ring-labeled [^{14}C]trifloxystrobin labeled on the β-ring. Two additional studies were done using uniformly phenyl ring-labeled [^{14}C]trifloxystrobin labeled on the α-ring (MRIDs 44496731 and 44496732).

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