DATA EVALUATION RECORD

STUDY 5

CHEM 129112 Trifloxystrobin §162-1
CAS No. 141517-21-7
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44496732
Fathulla, R. N. 1997. Aerobic soil metabolism of (U)^{14}C-phenyl-glyoxylat-CGA-279202 in loamy sand soil. Laboratory Project ID: HWI 6117-268. Unpublished study performed by Hazleton Wisconsin, Inc., Madison, WI; and submitted by Novartis Crop Protection, Inc., Greensboro, NC.

DIRECT REVIEW TIME = 64 Hours

REVIEWED BY:	H. L. Evans, B.S.	Signature: Henry L. Evans
TITLE:	Scientist	Date: 3/23/99
EDITED BY:	P. C. DeLeo, Ph.D.	Signature: Paul C DeLeo
TITLE:	Senior Scientist	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 Soil

1. This study is scientifically valid and provides useful information on the aerobic soil metabolism of trifloxystrobin in loamy sand 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, at a nominal application rate of 3.0 ppm, degraded with a registrant-calculated half-life of 1.9 days ($r^2 = 0.89$; 0-7 days data) in loamy sand soil maintained at 75% of the moisture content at 0.33 bar 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. The reported residue concentrations (in parent equivalents) were calculated by the reviewer from the nominal application rate (3.0 ppm) and the percentages of recovered radioactivity. Based on HPLC analysis, the parent compound was initially present at 99.3% (3.0 ppm) of the applied radioactivity, decreased to 37.3% (1.1 ppm) by 1 day posttreatment and 20.3% (0.60 ppm) by 2 days, and was last detected at 271 days (2.7%). The major degradate CGA-321113 was a maximum of 93.2% (2.8 ppm) of the applied radioactivity at 14 days posttreatment and was 56.8% (1.7 ppm) at 365 days. Evolved $^{14}CO_2$ was 4.5-5.1% of the applied radioactivity at 271-365 days posttreatment; [^{14}C]organic volatiles were negligible.

METHODOLOGY

Subsamples of sieved (2 mm) loamy sand soil (85% sand, 9% silt, 6% clay, 0.7% organic matter, pH 7.1, CEC 4.7 meq/100 g; Table I, p. 21) adjusted to 75% of the moisture content at 0.33 bar were pre-incubated in darkness at approximately 25 °C for 24 hours, then were placed in glass containers and treated with uniformly phenyl ring-labeled [^{14}C]trifloxystrobin {CGA-279202; (E,E)-α-(methoxyimino)-2-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl]-benzeneacetic acid, methyl ester; radiochemical purity 99.1%, specific activity 53.2 μCi/mg; p. 16}, dissolved in acetonitrile (<1% by weight), at a nominal application rate of 3.0 ppm (p. 23). Samples were incubated in darkness at approximately 25 ± 1 °C for up to 365 days (p. 24). Moist air was pumped through the incubation chamber and into organic volatile (ethylene glycol) and CO_2 (two; 2-ethoxyethanol:ethanolamine; 1:1, v:v) traps in sequence (Figure 2, p. 65). Sample containers were weighed at approximately 15 and 30 days posttreatment and every 30 days thereafter to verify that the moisture content of the soil was maintained at approximately 75% of the moisture content at 0.33 bar; moisture was adjusted as necessary. Duplicate soil samples were removed for analysis at 0, 1, 2, 7, 14,

30, 90, 152, 210, 271 and 365 days posttreatment. Volatile traps were removed for analysis and replaced at each soil sampling interval or approximately every 30 days (whichever came first).

Following collection, samples were transferred to Nalgene containers, extracted three times by stirring with acetonitrile:aqueous acetic acid (pH 4; 8:2, v:v), and centrifuged (p. 25). Triplicate aliquots of the combined extracts were analyzed by LSC; the limit of quantitation was <0.01% of the applied radioactivity (p. 34). The extract was filtered (unspecified size) and analyzed by LSC. An aliquot of the filtered extract was concentrated and triplicate aliquots were analyzed by LSC. Samples collected at 90-365 days posttreatment were further extracted three times with acetone:2 M aqueous acetic acid (8:2, v:v) and twice with dimethylformamide:1 M aqueous acetic acid (1:1, v:v); sample extracts were analyzed by LSC as previously described. Aliquots of each extract were further analyzed by HPLC (Phenomenex RP18 column) using a mobile phase gradient of acetonitrile:0.1% aqueous formic acid (0:100 to 65:35 to 100:0, v:v) with UV (250 nm) and radioactive flow detection (p. 27). Extracts were co-chromatographed with nonradiolabeled reference standards. Eluent fractions were collected at one-minute intervals and analyzed by LSC. Radioactivity in each extract (0-271 days) was quantified by radioactive flow detection and fraction collection; samples collected at 365 days posttreatment were quantified by fraction collection only. The limits of quantitation were 0.2% (acetonitrile:acetic acid extract), 0.3% (acetone:acetic acid extract) and 0.2% (dimethyl formamide:acetic acid extract) of the applied radioactivity (p. 35). An aliquot of soil extract from each sampling interval was further analyzed using two-dimensional TLC on silica gel plates. Aliquots of the acetonitrile:acetic acid extract were developed with diethyl ether:toluene:chloroform:formic acid (5:70:24:1 or 5:60:34:1, v:v:v:v) followed by ethyl acetate:toluene:acetic acid (50:50:4.5, v:v:v; p. 28); the limits of quantitation were the same as those reported for HPLC analysis. Aliquots of the acetone:acetic acid and dimethyl formamide:acetic acid extracts were developed with chloroform: methanol:formic acid:water (80:20:4:2, v:v:v:v) followed by ethyl acetate:toluene:acetic acid (50:50:4.5, v:v:v). Samples were co-chromatographed with nonradiolabeled reference standards which were visualized under UV light (unspecified wavelength). Radiolabeled residues were visualized and quantified by an autoradiography system. Radioactive areas from selected plates were scraped off and analyzed by LSC. Triplicate subsamples of the post-extracted soil samples were analyzed for total radioactivity by LSC following combustion (p. 26); data were corrected for combustion efficiency (93.6-99.1%).

To confirm the identity of the parent compound and the degradate (E,E)-(methoxyimino)-[2-[1-(3-(trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl] acetic acid (CGA-321113), the parent and the degradate were separated in aliquots of selected extracts (1 day) by HPLC (YMC ODS preparative column; method 8A) using the mobile phase gradient previously described (p. 29). Eluent fractions corresponding to the parent compound and the degradate CGA-321113 were collected separately and concentrated by

rotary evaporation. Isolated fractions were analyzed by HPLC (Phenomenex RP18 column) as previously described; samples were co-chromatographed with nonradiolabeled reference standards. Isolated fractions were further analyzed by two-dimensional TLC as previously described and LC/MS with positive ion detection.

Selected extracts containing areas of unidentified radioactivity (designated as HPLC regions 2, 3 and 5) were isolated and analyzed by HPLC and TLC as previously described (p. 30). Isolated fractions were further analyzed by LC/MS or LC/MS/MS with positive ion detection. A selected extract containing an area of unidentified radioactivity (designated as region 7) was isolated by HPLC as described previously, and analyzed by HPLC (Waters column) using a mobile phase gradient of hexane:dichloro methane (95:5 to 0:100, v:v) with UV (250 nm) and radioactive flow detection (p. 31). Eluent fractions were collected at one-minute intervals and analyzed by LSC. The isolated fraction was further analyzed by LC/MS/MS with positive ion detection and two-dimensional TLC on silica gel plates developed with toluene:chloroform:diethyl 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. 32).

To determine radioactivity associated with the humic acid, fulvic acid and humin fractions of the soil organic matter, soil subsamples (one replicate) collected at 90-365 days posttreatment were extracted by stirring with 1 *M* NaOH for approximately 18 hours, and centrifuged (p. 32); a second extraction was performed for 30 minutes. The supernatants were combined and analyzed by LSC. The combined supernatants were acidified (HCl) to allow humic acid to precipitate. Following centrifugation, the precipitate (humic acid) was redissolved in 1 *M* NaOH and triplicate aliquots were analyzed by LSC. Triplicate aliquots of the acidic supernatant (fulvic acid) were analyzed by LSC. The remaining extracted soil was air-dried and analyzed by LSC for total radioactivity following combustion (humin).

Aliquots of the organic volatile (ethylene glycol) and CO_2 (two; 2-ethoxyethanol: ethanolamine; 1:1, v:v) traps were analyzed for total radioactivity by LSC (p. 26). To confirm the presence of $^{14}CO_2$ in the 2-ethoxyethanol:ethanolamine traps, samples collected at 210, 271 and 365 days posttreatment were acidified (H_2SO_4) and the released $^{14}CO_2$ was trapped in 10% KOH solution; the acidified trap and 10% KOH solutions were analyzed by LSC. The 10% KOH solution was precipitated with $BaCl_2$ and centrifuged; triplicate aliquots of the supernatant were analyzed by LSC.

The frozen storage stability of trifloxystrobin and its degradates in soil extracts was determined by analyzing aliquots of selected extracts; data from extracts stored for up to 852 days were compared over time (p. 26; see Comment #7).

To determine the viability of the soil, soil samples collected at 0, 180 and 365 days posttreatment were plated on nutrient media and analyzed visually for microbial growth (p. 21); separate counts of yeast, mold, aerobic and anaerobic microorganisms were

performed. Results indicated that the soil was viable throughout the incubation period, but with an altered microbial population composition observed over time (Table III, p. 22).

DATA SUMMARY

Uniformly phenyl ring-labeled [^{14}C]trifloxystrobin (radiochemical purity 99.1%), at a nominal application rate of 3.0 ppm, degraded with a registrant-calculated half-life of 1.9 days (r^2 = 0.89; 0-7 day data) in loamy sand soil maintained at 75% of the moisture content at 0.33 bar and incubated in darkness at 25 ± 1 °C for up to 365 days (Figure 24, p. 87). 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. The reported residue concentrations (in ppm; reported in parent equivalents) were calculated by the reviewer from the nominal application rate (3.0 ppm) and the percentages of recovered radioactivity. Based on HPLC analysis, the parent compound was initially present at 99.3% (3.0 ppm) of the applied radioactivity, decreased to 37.3% (1.1 ppm) by 1 day posttreatment and 20.3% (0.60 ppm) by 2 days posttreatment, and was last detected at 271 days (2.7%; Table 17, p. 59). The major degradate

(E,E)-(methoxyimino)-[2-[1-(3-(trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl] acetic acid (CGA-321113)

was initially detected at 1.2% (0.04 ppm; day 0) of the applied radioactivity, increased to 62.4% (1.9 ppm) by 1 day posttreatment and a maximum of 93.2% (2.8 ppm) by 14 days posttreatment and was 56.8% (1.7 ppm) at 365 days posttreatment. An unidentified area of radioactivity (designated as region 7), comprised of three compounds, was initially present at 2.4% (0.07 ppm) of the applied radioactivity at 152 days posttreatment, increased to a maximum of 12.2% (0.36 ppm) by 271 days posttreatment and was 8.7% (0.26 ppm) at 365 days posttreatment (see Comment #9). Six unidentified areas of radioactivity (designated as regions 2-6 and 8) were present at a total of ≤6.5% (0.19 ppm) of the applied radioactivity (see Comment #8). Nonextractable [^{14}C]residues comprised a maximum of 22.3% (reviewer-calculated mean; 0.66 ppm) of the applied radioactivity at 210 days posttreatment (Table 3, p. 45). Based on organic matter fractionation analysis, 3.1% of the applied radioactivity was associated with the humic acid fraction and 12.0% was associated with the fulvic acid fraction at 210 days posttreatment (Table 19, p. 61). Evolved $^{14}CO_2$ was 4.5-5.1% of the applied radioactivity at 271-365 posttreatment (Table 3, p. 45); [^{14}C]organic volatiles were negligible (<0.02%).

Material balances (for individual replicates) were 98.1-107.5% of the applied radioactivity throughout the incubation period (Table 3, p. 45).

COMMENTS

1. The registrant-calculated half-life of 1.9 days was greater than the apparent half-life of <1 day. By 1 day posttreatment, only 37.3% of the applied radioactivity was present as parent compound (Table 17, p. 59).

2. The study author stated that the nominal treatment rate (3.0 ppm) for trifloxystrobin was the approximate proposed maximum label rate for a single application (p. 22).

3. The incubation temperature was held constant (± 1 °C) most of the time throughout the incubation period with only a few deviations (pp. 204-213). The deviations ranged from 21.5 to 28.4 °C.

4. The reviewer noted that the limits of quantitation were reported (pp. 34, 35), but that the limits of detection were not. It is necessary that both limits of detection and quantitation be reported to allow the reviewer to evaluate the adequacy of the method for the parent compound and its degradates.

5. 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 44496731) and the β-ring (MRID 44496730).

6. The study author stated that all samples or subsamples were analyzed immediately or placed in frozen storage prior to analysis (p. 26). The length of the storage periods was not reported. Storage stability studies are required for samples stored for longer than 30 days.

7. The frozen storage stability of trifloxystrobin and its degradates in selected soil extracts was determined (pp. 26, 27). Chromatographs of the extracts stored for up to 852 days were compared over time (Appendix B, Figures B-5 to B-8, pp. 153-156). The study author stated that since no qualitative difference was noted between chromatographs, the extracts were assumed to be stable under frozen storage conditions (p. 27).

8. Three areas of unidentified radioactivity (designated as HPLC regions 2, 3 and 5) were isolated and further analyzed; however, complete chemical names were not reported. Instead, proposed compound structures and formation processes were reported based on MS analysis. The study author stated that analytical results suggested that region 2 was formed from the demethylation of the methoxyimino group of the acid (CGA-321113), region 3 was formed from the hydroxylation of the ethylidene group of the acid, and region 5 retained the methoxyimino benzeneacetic acid, methyl ester moiety of the parent compound (pp. 39, 40, 42).

6

9. The area of unidentified radioactivity (designated as region 7) exceeded 10% of the applied radioactivity at only one of the sampling intervals (12.2% at 271 days; Table 17, p. 59). Therefore, only the acetonitrile:acetic acid extract (10.3% of the applied) of the sample collected at 271 days was isolated and further analyzed (p. 31). It was determined that region 7 was comprised of three compounds (p. 40). Based on the results of the analysis, 7.4% of the applied was the minor degradate (E,Z)-2[[[[1-[3-(trifluoromethyl) phenyl]ethylidene]amino]oxy]methyl]-benzonitrile (CGA-357276), 2.0% was parent compound (CGA-279202), and 0.04% was an unidentified minor degradate (p. 40).

10. The reviewer could not confirm that the moist air pumped through the incubation chamber was CO_2-free.

7

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