3-11-2002

DATA EVALUATION RECORD

STUDY 4

CHEM 120603 Tetraconazole §162-1
CAS No. 112281-77-3
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44367005
Waller, R. L. 1991. Aerobic soil metabolism of the triazole fungicide M-14360. Laboratory Project ID: 89-0245. Unpublished study performed by Ricera, Inc., Painesville, OH; and submitted by Sostram Corporation, c/o Landis International, Inc., Valdosta, GA.

DIRECT REVIEW TIME = 89 Hours

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CONCLUSIONS

Metabolism - Aerobic Soil

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

2. Uniformly triazole ring-labeled and uniformly phenyl ring-labeled [^{14}C]tetraconazole, at a nominal application rate of 0.7 ppm (reviewer-calculated actual application rates of 0.97 ppm and 0.64 ppm, respectively), was stable in sandy loam soil adjusted to 75% of 0.33 bar moisture content and incubated in darkness at 25 ± 1°C for up to 52 weeks; the parent was 82-82.5% (both labels) of the applied radioactivity at 52 weeks posttreatment. In the triazole label experiment, the initial (0 day) extractible parent residues declined from 94% to 90% of the initial radioactivity after week 8 and 82% after week 52. The soil bound residues were initially (0-7 days) 6.0% and increased to a maximum of 15.5% at week 52. In the phenyl labeled study, the initial (0 day) extractible parent residues increased from 90% to 94% of the initial radioactivity at 7 days, and declined to 82.5 at week 52. The soil bound residues were initially (0 day) 10%. They decreased to 5.6% at day 28, and increased to a maximum of 16.1% at week 52. Radioactivity associated with the humic acid, fulvic acid, and humin fractions were not determined. Evolved $^{14}CO_2$ and [^{14}C]organic volatiles were negligible.

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

   A. time 0 soil samples were not analyzed until 65 hours posttreatment (see Comment #1);

   B. inadequate material balance data were reported (see Comment #2); and

   C. residue data for the degradates were reported as percentages of the total recovered radioactivity from each HPLC analysis rather than as percentages of the applied (see Comment #5).

4. The submitted data indicate that tetraconazole is microbialy stable in sandy loam soil under aerobic conditions. EFED does not require a new aerobic soil metabolism study for tetraconazole at this time because a new study would not provide substantially new information.

METHODOLOGY

Samples (400 g) of sieved (2 mm) sandy loam soil (collected from Donalsonville, GA; 77% sand,

14% silt, 9% clay, 2% organic matter, pH 6.9, CEC 2.4 meq/100 g; p. 21) were weighed into jars and treated with uniformly triazole ring-labeled [^{14}C]tetraconazole {[^{14}C-Tr]-M-14360; (±)-2-(2,4-dichlorophenyl)-3-(1,2,4-triazol-1-yl)-propyl-1,1,2,2-tetrafluoroethyl ether; radiochemical purity 96.3%, specific activity 42.2 mCi/mmole; pp. 17, 18} OR uniformly phenyl ring-labeled [^{14}C]tetraconazole {[^{14}C-Ar]-M-14360; radiochemical purity 98.4%, specific activity 39.2 mCi/mmole; pp. 18, 19}, dissolved in acetone, at a nominal concentration of 0.7 ppm (p. 26; reviewer-calculated actual application rates of 0.97 ppm and 0.64 ppm, respectively). The jars were capped and rotated for 65 hours prior to incubation to evenly distribute the test compound. When treated soil was determined to be homogenous equivalent aliquots (15 g) of the soil were weighed into Erlenmeyer flasks, adjusted to 75% of 0.33 bar moisture content, and incubated in desiccators in darkness at 25 ± 1°C for up to 52 weeks; control soils were prepared and incubated under similar conditions. Moist CO_2-free air was passed through the desiccator and into two organic volatile (Chromosorb®) traps followed by two $^{14}CO_2$ (1 *N* NaOH) traps (Figure 1, p. 70). Additional soil samples treated at a nominal concentration of 7 ppm were prepared for metabolite identification (p. 25). The temperature was monitored continuously using a temperature log (Omega OM-302) and a mercury thermometer located in the chamber (p. 23). The soil moisture level was monitored and adjusted as necessary throughout the incubation period (p. 29). Duplicate treated soil samples for each radiolabel and a single control sample were collected for analysis at (0), 7 and 14 days; and at 4, 8, 12, 17, 26, 39, and 52 weeks posttreatment (p. 28; see Comment #1); samples were stored frozen (-13 ± 2°C) prior to analysis (p. 29). Volatile traps were removed for analysis at each sampling interval.

At each sampling interval, triplicate subsamples from each soil sample were analyzed for total radioactivity by LSC following combustion (p. 29; Figure 2, p. 71). Soil samples were extracted five times with methanol:0.01 *N* HCl (90:10, v:v) and filtered (Whatman #1 filter paper; p. 31); soil samples from day 0 were extracted three times with acetone (see Comment #11). The extracts were combined and analyzed for total radioactivity by LSC. The combined extracts were adjusted to pH 5-9 with 1 *N* NaOH and concentrated by rotary evaporation. The concentrated extracts were extracted three times with methylene chloride and analyzed for total radioactivity by LSC. The methylene chloride extracts were concentrated to dryness by rotary evaporation, reconstituted with acetonitrile, filtered (0.2 micron), and analyzed for total radioactivity by LSC. Aliquots of the extracts were further analyzed by HPLC (Supelco Suplex pkb-100 column) using a mobile phase gradient of acetonitrile:water (30:70 to 50:50 to 70:30 to 80:20 to 100:0, v:v) with UV (250 nm) and radioactive flow detection (Table 1, p. 43); the limit of detection was 1×10^{-9} g (p. 34). Extracts were co-chromatographed with nonradiolabeled reference standards of the parent and potential degradates: 2-(2,4-dichlorophenyl)-3-(1,2,4-triazol-1-yl)-propanol and 1,2,4-triazole (pp. 30, 33). Triplicate subsamples of the post-extracted soil were analyzed for total radioactivity by LSC following combustion.

Triplicate aliquots of the organic volatile traps (Chromosorb®) were analyzed for total radioactivity by LSC following combustion (p. 29). Aliquots of the $^{14}CO_2$ (1 *N* NaOH) traps were analyzed for total radioactivity by LSC; the method used to confirm the presence of $^{14}CO_2$ was not reported.

Subsamples of the sieved soil were removed for microbial analysis (p. 23). Microbial activity was determined by total plate counts and substrate-induced respiration (Appendix C, p. 94); data were not reported. Total plate count results prior to sieving were reported along with the soil characterization data; results indicated that the soil was viable (p. 21).

DATA SUMMARY

Uniformly triazole ring-labeled AND uniformly phenyl ring-labeled [^{14}C]tetraconazole (radiochemical purity 96.3% and 98.4%, respectively), at a nominal application rate of 0.7 ppm (reviewer-calculated actual application rates of 0.97 ppm and 0.64 ppm, respectively), was relatively stable in sandy loam soil adjusted to 75% of 0.33 bar moisture content and incubated in darkness at 25 ± 1°C for up to 52 weeks (p. 41); the parent was 82-82.5% (both labels) of the applied radioactivity at 52 weeks posttreatment (Tables 25, 26, pp. 68, 69). Residue data for the degradates were reported as percentages of the total recovered radioactivity from each HPLC analysis rather than as percentages of the applied. Residue data (in ppm; based on parent equivalents) were calculated by the reviewer from percentages of the recovered radioactivity (degradate) or the applied radioactivity (parent) and the actual application rate (see Comment #6). The total mass balance ranged from 96.3 to 111.8% of the initial combustion, time zero, for both experiments.

Uniformly triazole ring-labeled [^{14}C]tetraconazole {[^{14}C-Tr]-M-14360}

The parent compound was initially present at 94% (0.91 ppm) of the applied radioactivity, was 90-93% (0.87-0.90 ppm) of the applied at 7 days through 12 weeks posttreatment, decreased to 86-87% (0.83-0.84 ppm) of the applied by 17-39 weeks, and was 82% (0.80 ppm) of the applied at 52 weeks posttreatment (Table 25, p. 68). An unidentified minor degradate was initially (day 7) present at 1.2% (0.012 ppm) of the recovered radioactivity, generally increased to a maximum of 2.9% (0.028 ppm) of the recovered by 12 weeks posttreatment, and was 2.2% (0.021 ppm) of the recovered at 52 weeks posttreatment (reviewer-calculated means; Tables 21, 22, pp. 64, 65). Nonextractable [^{14}C]residues were initially (0-7 days) 6.0% (0.058 ppm) of the applied radioactivity, generally increased to 10.8% (0.10 ppm) of the applied by 17 weeks posttreatment, were 8.0-9.8% (0.078-0.095 ppm) of the applied at 26-39 weeks posttreatment, and were a maximum of 15.5% (0.15 ppm) of the applied at 52 weeks posttreatment (Table 25, p. 68); radioactivity associated with the humic acid, fulvic acid, and humin fractions was not determined. Evolved $^{14}CO_2$ was ≤0.3% of the applied radioactivity throughout the incubation period (Table 9, p. 51); [^{14}C]organic volatiles were negligible.

Material balances (based on LSC analysis) were determined from volatiles data and initial combustion data prior to soil extraction (see Comment #3); recoveries were 96.3-111.8% of the applied radioactivity throughout the incubation period and, following week 17, generally exhibited an unexplained pattern of increase (Table 9, p. 51).

Uniformly phenyl ring-labeled [^{14}C]tetraconazole {[^{14}C-Ar]-M-14360}

The parent compound was initially present at 90% (0.58 ppm) of the applied radioactivity, was 94% (0.60 ppm) of the applied at 7 days through 4 weeks posttreatment, decreased to 89-93% (0.57-0.59 ppm) of the applied by 8-26 weeks posttreatment, and was 82.5% (0.53 ppm) of the applied at 52 weeks posttreatment (Table 26, p. 69). An unidentified minor degradate was ≤1.4% (0.0090 ppm; reviewer-calculated mean) of the recovered radioactivity at 26-52 weeks posttreatment (Tables 23, 24, pp. 66, 67). Nonextractable [^{14}C]residues were initially (day 0) 10.0% (0.064 ppm) of the applied radioactivity, decreased to 5.6% (0.036 ppm) of the applied by 4 weeks posttreatment, and increased with variability to a maximum of 16.1% (0.10 ppm) of the applied by 52 weeks posttreatment (Table 26, p. 69); radioactivity associated with the humic acid, fulvic acid, and humin fractions was not determined. Evolved $^{14}CO_2$ was ≤0.6% of the applied radioactivity throughout the incubation period (Table 10, p. 52); [^{14}C]organic volatiles were negligible.

Material balances (based on LSC analysis) were determined from volatiles data and initial combustion data prior to soil extraction (see Comment #3); recoveries were 100.0-111.7% of the applied radioactivity throughout the incubation period and generally exhibited an unexplained pattern of increase with time (Table 10, p. 52).

COMMENTS

1. The experimental method was questionable because time 0 soil samples were not analyzed until 65 hours posttreatment. Also, the samples prepared for each sampling interval were not placed in incubation until 65 hours posttreatment. The study author stated that following treatment, the samples were capped and rotated for 65 hours prior to incubation to evenly distribute radioactivity (p. 26). Generally, immediately following treatment, time 0 samples are analyzed and the samples prepared for subsequent sampling intervals are placed in incubation. However, because the parent compound was observed to be relatively stable throughout the 52-week study, an additional study may not provide new information.

2. The reported decline of the parent is questionable because it was determined using samples treated at an exaggerated rate. Tetraconazole was applied at a nominal exaggerated rate of 700 g/ha, which is equivalent to 0.70 ppm; the reviewer-calculated actual application rates were 0.97 ppm and 0.64 ppm. The maximum label rate for combined multiple applications of tetraconazole was reported as 300-500 g/ha (p. 21). The use of exaggerated dose rates may affect the degradation rate of the chemical relative to the degradation rate that would occur under normal use rates. While exaggerated dose rates may be used to facilitate residue identification, EPA requires that kinetics studies be performed using the proposed maximum application rate (US EPA. 1993. *Pesticide Reregistration Rejection Rate Analysis: Environmental Fate*. EPA 738-R-93-010, p. 67).

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3. Inadequate material balances were reported. Material balances were determined from volatiles data and initial combustion data prior to extraction (Tables 9-10, pp. 51-52). Material balances following extraction (including extraction data) need to be reported to allow the reviewer to account for all radioactivity throughout the study. The reviewer notes that extraction efficiency data were reported for each sampling interval (Tables 12-20; pp. 55-63)

4. Nonextractable [^{14}C]residues were maximums of 15.5% and 16.1% of the applied radioactivity in the triazole and phenyl label studies, respectively, at 52 weeks posttreatment (Tables 25, 26, pp. 68, 69). Organic matter fractionation was not performed to determine the radioactivity associated with the humic acid, fulvic acid and humin fractions; generally, such data are reported for aerobic soil metabolism studies.

5. Residue data were reported only as percentages of the applied radioactivity; concentration data were not reported. All concentration data (in ppm based on parent equivalents) were calculated by the reviewer from the reviewer-calculated actual application rates (0.97 ppm for the triazole label study; 0.64 ppm for the phenyl label study; also see Comment #6) and the reported percentages of the applied radioactivity (parent) or the recovered radioactivity (degradate). In future studies submitted to the EPA, it is necessary that data be reported as both percentages of the applied radioactivity and in units of concentration, such as ppm.

6. The reviewer calculated the actual application rate of the parent based on "total carbon-14 found on the soil (dpm/g)" data reported for time 0 in Tables 25 and 26 (pp. 68, 69). The reviewer used the equation reported by the study author in Footnote "(a)" (Tables 25, 26, pp. 68, 69) to calculated the equivalent actual rates by replacing "dpm found on soil residue/g soil" with "total carbon-14 found on the soil (dpm/g)." The reviewer-calculated actual application rates are 0.97 ppm and 0.64 ppm for the triazole and phenyl label studies, respectively.

7. The limit of detection was reported for HPLC (p. 34), but not LSC analysis. Both limits of detection and quantitation should be reported to allow the reviewer to evaluate the adequacy of the method for the determination of the parent compound and its degradates.

8. The registrant-calculated half-life for the parent was not determined since 50% of the compound did not degrade by 52 weeks posttreatment.

9. The soil series name was not reported.

10. Soil viability was confirmed prior to the incubation period (p. 21), but was not confirmed at the termination of the incubation period. Generally, metabolism studies include data demonstrating the viability of the soil microbial population at the initiation and termination of the study.

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11. The methodology initially provided for extraction of the soil samples with acetone. After extraction of the day 0 samples, it was determined that the acetone did not provide a complete extraction of the radioactivity (pp. 16, 31); therefore, methanol:0.1 *N* HCl (90:10, v:v) was used as the extracting solvent for the remainder of the study.

12. The study author stated (p. 21) that the soil characterization data presented in Appendix B were not obtained using Good Laboratories Practices (as required by FIFRA). The soil characterization data reported in this review were presented on page 21 of the main study text.

ATTACHMENT 1
Data Critical to the Study Interpretation

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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Page _____ is not included in this copy.

Pages 9 through 31 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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