DATA EVALUATION RECORD

STUDY 6

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CAS No. 119446-68-3
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 42245132
Spare, W. C. 1987. Soil metabolism of CGA-169374 under aerobic, aerobic/anaerobic and sterile conditions. Laboratory Project No.: 1239. Unpublished study performed by Agrisearch Incorporated, Frederick, MD; and submitted by CIBA-GEIGY Corporation, Greensboro, NC.

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CONCLUSIONS

Metabolism - Aerobic and Anaerobic Soil

1. Both the aerobic and anaerobic soil metabolism studies are scientifically valid and provide useful information on the soil metabolism of difenoconozole in loam soil. However, data are of questionable accuracy because both the parent concentration data and the material balances were variable over time throughout the aerobic study and the aerobic phase of the anaerobic study. Because the parent was relatively stable, an additional study may not provide new useful information.

2. The studies do not meet Subdivision N Guidelines for the fulfillment of EPA data requirements on aerobic and anaerobic soil metabolism for the following reasons:

   (i) the test water was not characterized (anaerobic study);

   (ii) redox potential, pH, and dissolved oxygen data were not reported (anaerobic study); and

   (iii) material balances were outside the acceptable range of 90-110% (both studies).

3. In the aerobic soil metabolism study, radiolabeled [Δ-^{14}C]difenoconozole, at a nominal application rate of 10 ppm, was relatively stable in aerobic loam soil that was incubated in darkness at 25 ± 1 °C for up to 12 months. However, data were variable over time. Data reported percentages of the applied radioactivity represent percentages of the nominal application. Concentration data (in ppm) were reviewer-calculated based on the percentage of the applied radioactivity and the nominal application rate. The parent compound was initially present in the soil at 91.4% (9.1 ppm) of the applied radioactivity and was variable at 62.0-99.7% (6.2-10.0 ppm) at 1-365 days posttreatment. No major degradates were detected; one unidentified minor degradate was detected. Nonextractable [^{14}C]residues were initially (time 0) 2.3% (0.23 ppm) of the applied radioactivity, increased to 18.7% (1.9 ppm) by 3 months, and were 15.5% (1.6 ppm) at 12 months posttreatment (reviewer-calculated means). Evolved $^{14}CO_2$ and [^{14}C]organic volatiles were not detected.

   In the sterile control study, radiolabeled [Δ-^{14}C]difenoconozole, at a nominal application rate of 10 ppm, was stable in sterilized, aerobic loam soil that was incubated in darkness at 25 ± 1 °C for up to 6 months. Data reported as percentages of the applied radioactivity represent percentages of the nominal application. All data are reviewer-calculated means of two replicates which were each analyzed by two different TLC systems. Concentration data (in ppm) were reviewer-calculated based on the percentage of the applied radioactivity and the nominal application rate. The parent compound was present at 97.6-98.6% (9.8-9.9 ppm) of the recovered radioactivity at 1-6 months posttreatment. No

major degradates were detected; one unidentified minor degradate was detected. Nonextractable [^{14}C]residues were 5.6% (0.56 ppm) and 10.0% (1.0 ppm) of the applied radioactivity at 1 and 6 months posttreatment, respectively (Table 3, p. 20). Evolved $^{14}CO_2$ and [^{14}C]organic volatiles were not detected.

In the anaerobic soil metabolism study, radiolabeled [Δ-^{14}C]difenoconozole, at a nominal application rate of 10 ppm, was stable in flooded loam soil that was incubated anaerobically (nitrogen) in darkness at 25 ± 1 °C for up to 61 days following a 30-day aerobic incubation period. However, data were variable throughout the 30-day aerobic incubation, and only two samples were taken after anaerobic conditions were induced. Data reported as percentages of the applied radioactivity represent percentages of the nominal application. Data were not reported in units of concentration. Time 0 data were determined prior to flooding (following 30 days of aerobic incubation). Sampling intervals are reported as days following the initiation of the anaerobic phase of the study. Total system data were not reported. The parent compound was initially present in the soil phase at 87.1% of the applied radioactivity and was 83.2-83.3% at 28-61 days. No major degradates were detected; one unidentified minor degradate was detected. Nonextractable [^{14}C]residues were initially (time 0) 8.9% of the applied radioactivity and were 21.0-21.6% at 28-61 days following the initiation of anaerobic conditions (reviewer-calculated mean). Evolved $^{14}CO_2$ and [^{14}C]organic volatiles were not measured. [^{14}C]Residues in the water phase (≤2.1% of the applied radioactivity) were not characterized.

METHODOLOGY

A sample (325 g) of sieved (2 mm) loam soil (from Texas; 32.4% sand, 42.0% silt, 25.6% clay, 2.2% organic matter, pH 6.5, CEC 16.3 meq/100 g; p. 9) was weighed into an amber borosilicate glass bottle and treated with radiolabeled [Δ-^{14}C]difenoconozole {CGA-169374; 1-[2-(4-(4-chlorophenoxy)-2-chlorophenyl-(4-methyl-1,3-dioxolan-2-yl)-methyl)]-1H-1,2,4-triazole; radiochemical purity 96.8%, specific activity 19.7 μCi/mg; p. 10; Appendix A, Figure 1, p. 41; Appendix B, p. 50; see Comment #7}, dissolved in acetone, at a nominal application rate of 10 ppm (pp. 10, 11). The bottles were rotated for one hour prior to incubation to evenly distribute the compound. Subsamples (25 g) of the treated, mixed soil were weighed into Erlenmeyer flasks wrapped in aluminum foil, adjusted to 75% of 0.33 bar moisture content, and aerobically incubated in darkness at 25 ± 1 °C for up to 12 months; the flasks were sealed with polyurethane foam plugs. Sterilized soil samples (autoclaved) were prepared and incubated under similar conditions (p. 10). The soil moisture content was monitored weekly. Moist, CO_2-free air was pumped through the samples (see Comment #10). To capture volatiles, selected samples (12 months; sterilized 6 months) were connected to an ethylene glycol trap, a sulfuric acid trap, and two NaOH traps in series (Figure 2, p. 27). Duplicate soil samples were removed for analysis at 0, 1, 3, 7, 14 and 30 days; and at 3, 6, 9 and 12 months

posttreatment during the aerobic phase (p. 12; Appendix A, p. 35; see Comment #11). Duplicate sterilized soil samples were removed for analysis at 1 and 6 months posttreatment. Volatile trapping solutions were replaced at each sampling interval (p. 13).

Following 30 days of aerobic incubation, anaerobic conditions were induced in four samples by flooding with distilled water and pumping nitrogen gas through the flask (p. 11); the flasks were sealed with teflon-coated rubber stoppers. The samples were anaerobically incubated in darkness at 25 ± 1 °C for up to 61 days (p. 12); dextrose (0.25 g) was added to each flask after two weeks of anaerobic incubation. Duplicate samples were removed for analysis at 28 and 61 days after anaerobic conditions were induced (p. 12).

At each sampling interval, duplicate subsamples from each soil sample were analyzed for total radioactivity by LSC following combustion (p. 13). Soil samples were extracted by sonication with methanol:water (90:10, v:v), and the extracts were filtered; samples collected at 6, 9, and 12 months posttreatment were extracted again using the same method. The extracts were analyzed for total radioactivity by LSC (p. 12). The extracts were also analyzed by TLC on silica gel plates developed two times with acetonitrile OR developed with toluene:acetonitrile (75:25, v:v) followed by acetonitrile (p. 14). Samples were co-chromatographed with a nonradiolabeled reference standard of the parent which was visualized with UV (254 nm) light. Areas of radioactivity on the plates were quantitated using radioimage scanning. Duplicate subsamples of the post-extracted soil were analyzed by LSC following combustion (p. 13).

At each sampling interval, aliquots of the water layer from the anaerobic phase were analyzed for total radioactivity by LSC (p. 13). [^{14}C]Residues in the water were not characterized (see Comment #6).

At each sampling interval, aliquots of the volatile trapping solutions were analyzed for total radioactivity by LSC (p. 13). The presence of $^{14}CO_2$ in the NaOH traps was not confirmed.

To determine soil viability prior to treatment, at 12 months posttreatment, and at 60 days following the induction of anaerobic conditions, soil samples were analyzed by plating on plate count (bacteria), rose bengal (fungi), actinomycete isolation (actinomycetes), and thioglycollate (anaerobes) agars (p. 15; Table 1, p. 18); results indicated that the soils were viable.

DATA SUMMARY

Aerobic Soil Metabolism

Radiolabeled [Δ-^{14}C]difenoconozole (radiochemical purity 96.8%), at a nominal application rate of 10 ppm, was relatively stable in aerobic loam soil that was incubated in darkness at 25 ± 1 °C for up to 12 months (Table 7, p. 24; Figure 3, p. 28; see Comment #5). However, data were variable over time (see Comment #1). Data reported as percentages of the applied radioactivity represent percentages of the nominal application; data reported as percentages of the recovered radioactivity represent percentages of the radioactivity recovered following TLC analyses. All data are reviewer-calculated means of two replicates which were each analyzed by two different TLC systems, unless otherwise noted. Concentration data (in ppm) were reviewer-calculated based on the percentage of the applied radioactivity and the nominal application rate.

The parent compound was initially present in the soil at 91.4% (9.1 ppm) of the applied radioactivity and was variable at 62.0-99.7% (6.2-10.0 ppm) of the applied at 1-365 days posttreatment (registrant-calculated; Table 7, p. 24). An unidentified minor degradate (designated as "unknown") was ≤5.3% of the recovered radioactivity throughout the incubation period (Tables 4, 5, pp. 21, 22). Uncharacterized origin material was ≤4.7% of the recovered radioactivity throughout the incubation period. Uncharacterized radioactivity (designated as "remainder") was detected at ≤2.2% of the recovered radioactivity throughout the incubation period. Nonextractable [^{14}C]residues were initially (time 0) 2.3% (0.23 ppm) of the applied radioactivity, increased to 18.7% (1.9 ppm) of the applied by 3 months posttreatment, and were 15.5% (1.6 ppm) of the applied at 12 months posttreatment (Table 2, p. 19). Evolved $^{14}CO_2$ and [^{14}C]organic volatiles were not detected.

Material balances (based on LSC analysis of individual replicates) were 85.8-128.6% of the applied radioactivity throughout the aerobic incubation period, with no observed pattern of decline (Table 2, p. 19; see Comments #1, 4).

Aerobic Sterile Control Samples

Radiolabeled [Δ-^{14}C]difenoconozole (radiochemical purity 96.8%), at a nominal application rate of 10 ppm, was stable in sterilized, aerobic loam soil that was incubated in darkness at 25 ± 1 °C for up to 6 months (Tables 4, 5, pp. 21, 22). Data designated as percentages of the applied radioactivity represent percentages of the nominal application; data reported as percentages of the recovered radioactivity represent percentages of the radioactivity recovered following TLC analyses. All data are reviewer-calculated means of two replicates which were each analyzed by two different TLC systems. Concentration data (in ppm) were reviewer-calculated based on the percentage of the applied radioactivity and the nominal application rate.

The parent compound was present at 97.6-98.6% (9.8-9.9 ppm) of the recovered radioactivity at 1-6 months posttreatment (Tables 4, 5, pp. 21, 22). An unidentified minor degradate (designated as "unknown") was ≤1.6% of the recovered radioactivity throughout the incubation period (single replicate, each TLC system). Uncharacterized origin material was ≤1.9% of the recovered radioactivity throughout the incubation period. Uncharacterized radioactivity (designated as "remainder") was detected at ≤1.4% of the recovered radioactivity throughout the incubation period (single replicate, each TLC system). Nonextractable [^{14}C]residues were 5.6% (0.56 ppm) and 10.0% (1.0 ppm) of the applied radioactivity at 1 and 6 months posttreatment, respectively (Table 3, p. 20). Evolved $^{14}CO_2$ and [^{14}C]organic volatiles were not detected.

Material balances for the sterilized samples (based on LSC analysis of individual replicates) were 103.9-116.5% of the applied radioactivity (Table 3, p. 20; see Comment #4).

Anaerobic Soil Metabolism

Radiolabeled [Δ-^{14}C]difenoconozole (radiochemical purity 96.8%), at a nominal application rate of 10 ppm, was stable in flooded loam soil that was incubated anaerobically (nitrogen) in darkness at 25 ± 1 °C for up to 61 days following a 30-day aerobic incubation period (Table 8, p. 25; Figure 3, p. 28; see Comment #5). However, data were variable throughout the 30-day aerobic incubation, and only two samples were taken after anaerobic conditions were induced (Tables 4-6; pp. 21-23). Data reported as percentages of the applied radioactivity represent percentages of the nominal application; data designated as percentages of the recovered radioactivity represent percentages of the radioactivity recovered following TLC analyses. All data are reviewer-calculated means of two replicates each analyzed by two different TLC systems, unless otherwise noted. Data were not reported in units of concentration. Time-0 data were determined prior to flooding (following 30 days of aerobic incubation). Sampling intervals are reported as days following the initiation of the anaerobic phase of the study. Total system data were not reported.

The parent compound was initially present in the soil phase at 87.1% of the applied radioactivity and was 83.2-83.3% of the applied at 28-61 days (registrant-calculated; Table 8, p. 25). An unidentified minor degradate (designated as "unknown") was initially (time 0) 5.3% of the recovered radioactivity and was 1.2% of the recovered at 28 days following the initiation of anaerobic conditions (single replicate, each TLC system; Tables 4-6, pp. 21-23). Uncharacterized origin material was initially (time 0) 2.1% of the recovered radioactivity and was 2.3% of the recovered at 28 days following the initiation of anaerobic conditions (three of four replicates). Uncharacterized radioactivity (designated as "remainder") was initially (time 0) 2.2% of the recovered radioactivity and was 2.4% of the recovered at 28 days following the initiation of anaerobic conditions (three of four replicates). Nonextractable [^{14}C]residues were initially (time 0) 8.9% of the

applied radioactivity and were 21.0-21.6% of the applied at 28-61 days following the initiation of anaerobic conditions (Tables 2, 3, pp. 19, 20). Evolved $^{14}CO_2$ and [^{14}C]organic volatiles were not measured. [^{14}C]Residues in the water phase (≤2.1% of the applied radioactivity) were not characterized (p. 16).

Material balances (based on LSC analysis of individual replicates) were 89.7-112.8% and 101.9-113.4% of the applied radioactivity throughout the aerobic and anaerobic incubation periods, respectively; a pattern of loss was not observed (Tables 2, 3, pp. 19, 20; see Comment #4).

COMMENTS

1. The data were variable over time for both the parent compound and the material balances throughout the aerobic phase of the study. The parent compound was highly variable over time, with the maximum concentration (99.7% of the applied radioactivity) occurring at 92 days posttreatment (rather than at the initial sampling interval), and the minimum concentration (63.0% of the applied) occurring at 183 days posttreatment (rather than the final sampling interval, 365 days posttreatment; Table 7, p. 24). Similar variability was observed in the material balances (Table 2, p. 19). The study author did not discuss the variability. However, the parent compound appeared to be relatively stable under both aerobic and anaerobic conditions (also see Comment #5); therefore, it is unlikely that a new study would provide additional useful information.

2. In the anaerobic soil metabolism study, the test water used to induce anaerobic conditions at 30 days posttreatment was not characterized as required by Subdivision N Guidelines. The study author stated that the test water was distilled (p. 11), but data on the pH and dissolved oxygen content were not provided. The characteristics of the test water are important to define the conditions under which the study was conducted.

3. Redox potential, pH, and dissolved oxygen content data were not reported in the anaerobic metabolism study. Subdivision N Guidelines require that such data be reported to confirm the presence of anaerobic conditions. The reviewer noted that anaerobic conditions in the test systems were induced by flooding with water and pumping nitrogen gas through the samples. Generally, this is sufficient to produce anaerobic conditions.

4. In the aerobic study, the anaerobic study, and the sterile control study, material balances were not within the acceptable range of 90-110% of the applied radioactivity, as required by Subdivision N Guidelines. In the aerobic study, the material balances were outside the acceptable range in 7 of the 20 replicate samples (Table 2, p. 19). In the anaerobic study, the material balances were outside the acceptable range in two of the four replicate samples (Table 3, p. 20). In the sterile control study, the material balances for three of four replicate samples were outside the acceptable range. In each study, a pattern of

decline was not observed. The reviewer notes that material balance problems were also observed in the submitted 163-1 studies. In one (MRID 42245136) of the 163-1 studies, the study author reported using borosilicate glass tubes due to possible adsorption to the glass; however, adsorption to glass was not tested in either of the 163-1 studies. In the current study, it is possible that adsorption to glass occurred, thereby effectively removing the parent compound from the test systems and rendering it unavailable for microbial degradation.

5. The reviewer noted that the parent compound was relatively stable in both aerobic and anaerobic loam soil. The registrant-calculated half-lives for the parent in aerobic and anaerobic loam soil systems were 1600 days (Table 7, p. 24) and 947 days (Table 8, p. 25), respectively. At 365 days posttreatment (aerobic phase), the parent compound was still present at 79.2% of the applied radioactivity. Following 61 days of anaerobic incubation, the parent was still present at 83.2% of the applied radioactivity. The half-life of the parent compound was estimated assuming the continuation of the apparent degradation pattern beyond the scope of the observed data. However, data which appear linear may become curvilinear with time, and half-life estimations based on extrapolated data may be inaccurate. In addition, only three data points were used to calculated the half-life of the parent under anaerobic conditions. Generally, a minimum of four data points are necessary for the valid calculation of a half-life.

6. Radioactivity associated with the water phase of the anaerobic study was ≤2.1% of the applied radioactivity (Table 3, p. 20); however, [^{14}C]residues in the water phase were not characterized. On page 13, the study author stated that aliquots of the water layer from the anaerobic phase of the study were analyzed by TLC. However, [^{14}C]residues in the water were not characterized (p. 16). Thus, the study author did not report data on the decline of the parent in the whole system (soil plus water phases), and the half-life of the parent in the anaerobic phase was calculated by the registrant using only the data for the parent in the soil phase (Table 8, p. 25). The EPA prefers the use of data for the whole system in half-life calculations.

7. The position of the radiolabel on the parent compound structure was not reported in the main part of the report. The structure and chemical name of the parent were omitted from Figure 1 (p. 26). The reviewer reported the radiolabel position and chemical name provided in Appendix A (Figure 1, p. 41; p. 44).

8. Degradate data were only reported in the form of percentages of the recovered radioactivity. All degradate data were percentages of radioactivity recovered from the TLC analyses. In future studies submitted to the EPA, all data should be reported as percentages of the applied radioactivity.

9. Residue data were only reported in the form of percentages of the applied radioactivity for the parent compound and the nonextractable residues. Concentration data (in ppm)

were reviewer-calculated based on the percentage of the applied radioactivity remaining and the nominal application rate. In future studies submitted to the EPA, it is necessary that all residue data also be reported in units of concentration, such as ppm.

10. It is unclear to the reviewer whether moist, CO_2-free air was pumped through all samples during the aerobic phase of the study or just the samples (12 months; sterilized 6 months) used to capture volatiles (p. 10). The reviewer notes that without air circulation, samples can become anaerobic, which would preclude a valid determination of the rate of aerobic metabolism. Clarification by the registrant is necessary.

11. The reviewer is unclear whether duplicate samples were prepared for each sampling interval. A 325-g sample of loam soil was treated with radiolabeled [^{14}C]difenoconozole, and a 25-g subsample of the treated soil was weighed into each sample flask (p. 10). Based on the reported methodology, a maximum of 13 sample flasks were prepared; however, in order to remove duplicate samples at each sampling interval, twenty sample flasks were required for the aerobic phase of the study and four sample flasks were prepared for the anaerobic phase (for a total of 24 flasks; reviewer-calculated based the number of sampling intervals and replicate data results; pp. 11-12; Tables 2, 3, pp. 19-20). Thus, the treated 325-g sample would not have provided a sufficient amount of test soil to prepare duplicate samples for each sampling interval. Clarification by the registrant is necessary.

12. In the anaerobic metabolism study, nonextractable [^{14}C]residues were a maximum of 18.7% of the applied radioactivity at 3 months posttreatment (aerobic phase; Table 2, p. 19) and were present at 21.0-21.6% of the applied radioactivity at 28-61 days following the initiation of anaerobic conditions (reviewer-calculated means; Table 3, p. 20). Organic matter fractionation was not performed to determine the radioactivity associated with the humic acid, fulvic acid, and humin soil organic matter fractions; generally, such data are reported for soil metabolism studies.

13. Method detection limits were not reported. 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 test compound and its degradates.

14. The aqueous solubility of the parent was reported as 20 ppm at 20°C (unspecified pH; p. 10; Appendix A, p. 51).

15. It was unclear whether the loam soil was representative of the intended use area of difenoconozole. It is preferred that the soil used in aerobic and anaerobic soil metabolism studies be either a sandy loam or silt loam or representative of the intended use area. In addition, a soil series name was not reported.

RIN-0509-04

EFED review for MRID # 422451-32

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