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DATA EVALUATION RECORD

I. Study Type: Aerobic Soil Metabolism

II. Citation:

Concha, Marian, and Kathryn Shepler. 1994. Aerobic Soil Metabolism of [¹⁴C]-2,4-Dichlorophenoxyacetic Acid. Performed by PTRL-West, Inc. Richmond, CA. Submitted by Industry Task Force II on 2,4-D Research Data c/o DowElanco, Indianapolis, IN. MRID 43167501.

III. Reviewer:

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18 SEP 1995

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EFGWB/EFED/OPP

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V. Conclusions:

The study provides acceptable data on degradation of 2,4-dichlorophenoxyacetic acid (2,4-D) in aerobic mineral soil. This data in conjunction with the acceptable soil metabolism data (MRID 00116625) fulfills the Aerobic Soil Metabolism (162-1) data requirement. No additional data are needed at this time.

Radiolabeled 2,4-D, at 5 µg/g, in a Catlin silty clay loam had a first-order half-life of 1.7 days. Soil degradates were identified as 2,4-dichlorophenol (2,4-DCP) (3.5% of applied) and 2,4-DCA (2.5 to 2.8% of applied). Unidentified extractable residues (several HPLC peaks) were also detected (<0.8% of applied at Day 16). Radiolabeled residues were detected (45 to 60% of applied at Day 5) in non-labile soil organic matter. Radiolabeled residue in the fulvic acid fraction was identified as 2,4-D. Volatile degradates were identified as [¹⁴C]-CO₂ (50% of applied at Day 16) and 2,4-DCA (0.3% of applied at Day 16).

The reported data indicate that 2,4-D rapidly degrades in aerobic mineral soil.

VI. Materials and Methods:

Catlin silty clay loam (Typic Argiudolt) and Hanford sandy loam (Typic Xerorthent) soils were used in aerobic soil metabolism studies. Field moist soils were passed through a 2mm sieve, and then stored in air-tight plastic bags at room temperature in the dark.

(Reviewer Note: The registrant did not indicate soil moisture conditions during storage.) Microbial viability of test soils and the Catlin soil at Day 16 Rep A was quantified using a dilution plate method on soy agar (TSA), actinomycete isolation agar (AIA), and potato dextrose agar (PPA). Physicochemical and microbiological properties of test soils are shown in Table II and III.

Preliminary Studies

Five preliminary studies were conducted to evaluate testing procedures.

1. A subsample of each soil was amended with radiolabeled 2,4-D (radiopurity=98.1%; SA=22.3 mCi/mmol; phenyl ring labeled; isotopic dilution ratio=0.55) to yield a soil concentration of 1.9 $\mu\text{g/g}$ or 3.8 lbs ai/A at a 6 inch soil depth. Each treated soil sample was placed in an incubation flask equipped with a continuous flow through air system connected to sequential gas traps (polyurethane plugs, ethylene glycol, and 10% KOH). Samples were taken at immediately posttreatment, 7, 14, and 20 days posttreatment.

2. A study was conducted using a similar method as described in experiment 1. Volatile were sampled at 30 minute intervals for 5 days.

3. A subsample of each soil was amended with radiolabeled 2,4-D to yield a soil concentration of 5 $\mu\text{g/g}$ (10 lbs ai/A at a 6 inch soil depth). Each treated soil sample was placed in each of two biometer flasks equipped with a polyurethane plug and 10% KOH gas traps. The flasks were opened at 7 day intervals to facilitate air exchange. Samples were taken at 4 and 32 days posttreatment. Biometer flasks for the Day 32 samples were placed in freezer to promote condensation of volatile degradates prior to sampling.

4. A preliminary study was conducted to assess gas trapping efficiency in static and flow-through gas trapping systems. Subsamples of test soil were amended with radiolabeled 2,4-D to yield a nominal concentration of 5 $\mu\text{g/g}$. Treated soil samples were placed into biometer flasks equipped with polyurethane foam and 10% KOH gas traps and biometer flasks with flow-through charcoal and 10% KOH gas traps. Samples were taken at 5 days posttreatment.

5. Subsamples of Hanford sandy loam soil were amended radiolabeled 2,4-D (radiopurity=98.1%; SA=22.3 mCi/mmol; phenyl ring labeled; isotopic dilution ratio=0.55) to yield a nominal concentration of 5 $\mu\text{g/g}$. Treated soil samples were placed biometer flasks equipped with polyurethane foam and 10% KOH gas traps. Samples were taken at 20 days posttreatment.

Definitive Study

Subsamples of Catlin silty clay loam (50 g) were placed into each of 26 sterile, biometer flasks. Each soil sample was amended with isotopically diluted 2,4-D (radiopurity=98.1%; SA=22.3 mCi/mmol; phenyl ring labeled; isotopic dilution ratio=0.55) to yield a nominal soil concentration of 5 µg/g. This application rate is equivalent to 10 lbs ai/A at a 15 cm incorporation depth. Fourteen of the biometer flasks were further amended with an additional 9.3 g of soil to readjust the soil pesticide concentration to 5.1 µg/g². The biometer flasks were incubated at 25°C in the dark. [Reviewer Note: The biometer flasks were incubated under static conditions. The registrant did not indicate if biometer flasks were opened for air exchange.] Duplicate soil and gas trap samples were taken immediately posttreatment, 1,2,3,5,7,9,13 and 16 days posttreatment.

Analytical

Each biometer flask was placed in a freezer for 30 minutes prior to sampling. The walls of each flask were rinsed with 50 ml acetone:water:acetic acid to remove any volatile residues in the condensate. An additional 50 ml of extracting solution was used to transfer soil from the biometer flask into extraction flask.

Each soil sample was extracted 3X with ??????. The extracts for each soil sample were combined, evaporated to dryness with N₂, and the remaining residues were redissolved in acetonitrile. Extracts samples were taken before evaporation to ensure quantity and identity of residues in original extracts. Each extract sample was spiked with non-labeled 2,4-DCA, concentrated via evaporation with N₂, and remaining residue were redissolved in acetonitrile. Foam plugs gas traps were extracted with dichloromethane.

Extracted soil samples from Day 20, Pilot #1 were further extracted to assess the non-labile soil residues. Each sample was sequentially extracted with acetone:water:acetic acid 90:5:5 (v:v:v) at 50°C, acetone:1N HCl 90:10 (v:v), and 1 N NaOH. Extracted soil samples for Day 5 Rep A were also extracted with 0.5N NaOH. The NaOH extracts were acidified with HCl (pH=1) to precipitate humic acids. The fulvic acid fraction was considered as the soluble fraction in acidified samples of 0.5 M NaOH. Humic acid fractions were redissolved in 0.5 NaOH for LSC. Fulvic acid fractions in acidified NaOH extracts were analyzed using HPLC and LSC.

1-There was an error in dispensing the stock solution of isotopically diluted 2,4-D. Fourteen biometer flasks were amended with an additional 0.8 ml of 2,4-D stock solution. Therefore, the registrant added more soil (9.3 g) to the fourteen flasks to normalize soil pesticide concentrations at 5.1 µg/g.

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Extractable soil residues were separated using HPLC equipped with an OMIPAK PAX-500 and C-18 column and a linear gradient solvent system of acetonitrile/0.05% trifluoroacetic acid; and separated residues were detected with UV/VIS (254 nm) and flow-through radioisotope detectors. Extracted residues were also separated using 1 and 2-D TLC with toluene/ethyl acetate/acetic acid 10:10:1 (v:v:v) and hexane/2-propanol 1:1 (v:v) with 5% acetic acid. Separated residues were identified using co-chromatography with known standards. All extracts were analyzed within 48 hours of the sampling. The total ^{14}C content in soil was determined by combustion-LSC. The total ^{14}C content in soil extracts was determined by LSC. BaCO_3 precipitation was used to confirm the presence of CO_2 in 10% KOH gas traps. The detection limits were $0.001 \mu\text{g/g}$ and $0.0012 \mu\text{g/g}$ for combustion and HPLC, respectively.

Storage Stability

Soil extractions were completed on the day of sampling. Extracts were stored frozen ($<0^\circ\text{C}$) prior to chemical analysis. Reference standards were reanalyzed during the storage time. In addition, the extract of the Day 7 (Rep B) soil sample was reanalyzed at 80 days postextraction.

VII. Author's Results and Conclusions:

A. Low material balances were observed in preliminary studies using biometer flasks with flow-through air system (Appendix B). The registrant believes low material balances were attributed to evolution of CO_2 and volatile degradates of 2,4-D (possibly 2,4-DCP and/or 2,4-DCA). These results were used to justify the use of static incubation biometer flasks.

B. The material balance of radiolabeled residue ranged from 89 to 114% of the applied radiolabeled 2,4-D (Table V). The radioactive residues were distributed in soil extracts (88.6 to 91.5 % applied immediately posttreatment), nonextractable soil fraction (45 to 60% of applied at Day 5), KOH gas trap (52% of applied at Day 16), and the foam plug gas trap (0.3% of applied at Day 16) (Table V).

C. The first-order half-life of 2,4-D was 1.7 days in a Catlin silty clay loam (Fig 20).

D. Degradates of [^{14}C]-2,4-D were identified as 2,4-DCP (3.5% of applied at Day 2 to 0.4% at Day 16) and 2,4-DCA (2.5 to 2.8% of applied at Day 9 to 1.4 to 1.6% at Day 16) (Tables VI and VII). Unidentified extractable residues (several HPLC peaks) were also detected ($<0.8\%$ of applied at Day 16).

E. Radiolabeled residues were detected (45 to 60% of applied at Day 5) in non-labile soil organic matter (Table VII). Radiolabeled residue in the fulvic acid soil fraction was identified as 2,4-D (Figure 18). Caustic extracts of Day 20

soil samples of Hanford and Catlin soils indicate radiolabeled residue distributed in organic acid extracts (1.6 to 2% of applied), mineral acid extracts (6.7 to 8% of applied), and caustic extracts (10.5 to 12.1% of applied) (Appendix D) .

F. Volatile degradates were identified as [¹⁴C]-CO₂ (50% of applied at Day 16) and organic volatile in foam plug trap (0.3% of applied at Day 16). Radiolabeled residue in the foam plug was identified as 2,4-DCA.

G. The registrant proposed 2,4-D degradation was dependent on decarboxylation to form 2,4-DCP (Figure 19). The degradate 2,4-DCP is further mineralized to CO₂ or methylated to form 2,4-DCA. The 2,4-DCA is further mineralized to form CO₂.

H. The storage stability study indicates residue stability over an 80 day storage period at < 0° C (Appendix E) .

VIII. Reviewer's Comments

A. The study was conducted in a static biometer flask. As per Subdivision N guidelines, aerobic soil metabolism studies should be conducted using a flow-through air system. EFGWB believes the test system design does not jeopardize interpretation of data because aerobic conditions were maintained to facilitate oxidative mineralization of 2,4-D to CO₂.

2,4-D EFED Review

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