I. Study Type: Anaerobic Aquatic Metabolism

II. Citation:

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V. Conclusions:
The study provides acceptable data on anaerobic aquatic metabolism of 2,4 dichlorophenoxyacetic acid (2,4-D). The data fulfill the Anaerobic Aquatic Metabolism (162-3) or Anaerobic Soil Metabolism (162-2) data requirements. Additional data are not needed at this time.

Radiolabeled 2,4-D, 4.9 µg/ml, had a first-order anaerobic aquatic half-life of 333 days. The major degrade in soil and water samples was identified as 2,4-dichlorophenol (2,4-DCP) (10.6 to 32% applied at 30 day posttreatment). Unidentified radiolabeled residues in sediment and water samples were also detected (7.3 to 7.9% of applied at 35 days). The unidentified residues were composed of several HPLC peaks. The major volatile degrade in KOH gas traps was tentatively identified as CO$_2$ (30% of applied). Organic volatiles were identified as 4 chlorophenol (4-CPA) and 2,4-DCA (<1.9% of applied). Radiolabeled residues were also detected (40.8% of applied at 240 days posttreatment) in non-labile organic matter fractions. Radiolabeled residue in fulvic was identified as 2,4-D.

The reported data indicate that 2,4-D should be stable in anaerobic aquatic environments.

VI. Materials and Methods:
Sediment and water were taken from a pond in Henry County, Illinois. Physicochemical properties of the pond water and sediment are shown in Table II. Microbiological viability of sediment and water were analyzed immediately before the study and 110 and 365 days posttreatment of $[^{14}C]$-2,4-D.
Microbial populations were quantified using a dilution plate method on an anaerobic agar at 35°C and devoid of oxygen for 3 days. Sediment and water samples were stored in an incubator at 25°C.

Preliminary Studies

1. Sediment and water samples were amended with isotopically diluted 2,4-D (SA=22.3 mCi/mmol; radiopurity=98.3%; isotopic dilution ratio=4/9) to yield nominal concentrations of 1 μg/ml. Each amended sample was placed into a 500 ml erlenmeyer flask equipped with a flow-through air exchange system connected to a series of polyurethane foam plugs, ethylene glycol, and 10% KOH gas traps. Two static biometry flasks were connected to KOH gas traps.

2. Sediment and water samples were amended with isotopically diluted 2,4-D (SA=22.3 mCi/mmol; radiopurity=98.3%; isotopic dilution ratio=4/9) to yield nominal concentrations of 5 μg/ml. Each amended sample was placed into a 500 ml erlenmeyer flask equipped with a flow-through air exchange system connected to a series of charcoal, Supelco OKBO-32 Waters Sep-Pak cartridge, ORBO-32 XAD-25 Resin, ethylene glycol, and KOH gas traps.

Definitive Study

Sediment (20 g oven dry weight) and pond water (80 ml) samples were placed into placed into each of 28 sterile, biometry flasks. Each biometry flask was connected to a reservoir with KOH solution and a foam plug. Twenty-six of the samples were amended with alfalfa (0.372 g) to enhance microbial activity. Two samples were amended with glucose (0.37 g). The samples were incubated under a static N₂ atmosphere for 29 days.

After the anaerobic preincubation period, each sample was amended with isotopically diluted [¹⁴C]-2,4-D (SA=22.3 mCi/mmol; radiopurity =98.3%; isotopic dilution ratio=4/9) to yield a nominal concentration of 4.9 μg/L. The flasks were stoppered and then placed into an incubator at 25°C in the dark. Duplicate samples were taken immediately posttreatment, 2, 13, 20, 26, 30, 35, 42, 56, 110, 121, 240, and 365 days posttreatment.

Analytical

A redox indicator (resazurin) was added immediately before sampling each sample. In addition, the headspace of each flask was evacuated with oxygen and analyzed for [¹⁴C]-CO₂. Each sample was acidified with 6N HCl and headspace gas were reanalyzed for [¹⁴C]-CO₂. The acidified samples were centrifuged to separate sediment and water phases. The water phase was decanted and stored for chemical analysis. The sediment color was described using a Munsell Color Chart. The sediment was extracted 3X with acetone:water:acetic acid 90:5:5 (v:v:v). The extracted sediment of the
Day 240 samples were further extracted with 0.5 N NaOH. The NaOH extracts were acidified (pH=1) to precipitate humic acid fraction. Acid soluble residues were designated as fulvic acid fraction.

Soluble residues in soil extracts and gas traps were separated by HPLC equipped with an Ompak Pax-500 column with a linear gradient solvent system of acetonitrile and 0.05% trifluoracetic acid. Residues in dichloromethane extracts of foam gas traps were separated by HPLC equipped with a Microsorb C-18 column with a solvent system of acetonitrile and 1% acetic acid. Separated residues were detected using a UV/VIS (254 and 280 nm) and radioisotope detectors. Residues were also separated with 1 and 2-dimensional TLC using toluene/ethyl acetate/acetic acid 10:10:1 (v:v:v) and hexane/2-propanol 1:1 (v:v) with 5% acetic acid solvent systems. Separated residues were identified using co-chromatography with known standards. The 14C content in soil and water extracts and gas traps was determined by LSC. The 14C content in extracted sediment samples was determined by combustion-LSC. Analytical detection limits were 0.001 µg/g (2X background) and 0.009 µg/g for combustion-LSC and HPLC-LSC, respectively.

VII. Author's Results and Conclusions:

A. The material balance of radiolabeled residues accounted for 92 to 115.6% of applied [14C]-2,4-D (Table VI). Radiolabeled residues were detected in water (76 to 85% of applied immediately posttreatment), sediment (59% of applied at 35 days posttreatment), foam plug gas traps (1.1 to 3.3% of applied at 240 days posttreatment), and KOH gas traps (12.6 to 30.8% of applied at 365 days posttreatment).

B. The first-order degradation half-life of 2,4-D in an anaerobic sediment/water system was 333 days (R²=0.473) or 312 days (R²=0.690; excluding Day 42 sample) (Figure 27).

C. The major degrade of [14C]-2,4-D was 2,4-DCP (10.6 to 32% applied at 30 day posttreatment to 1.5 to 6.8% of applied at 365 days posttreatment). Unidentified radiolabeled residues in the sediment/water were also detected (7.3 to 7.9% of applied at 35 days posttreatment to 3.9 to 7.1% of applied at 365 days posttreatment). (Table VII and Table VIII). The registrant stated the unidentified residues were composed of several HPLC peaks. The maximum concentration of any single peak would be equivalent to 1.5% of the applied dose.

D. The major volatile degrade in KOH gas traps was tentatively identified as CO₂ (30% of applied at 42 and 365 days posttreatment). The degraders 4-CPA and 2,4-DCP were detected (0.7% and 1.9% of applied, respectively at 365 days posttreatment) in the polyurethane foam plug extracts (Figures 23 and 24).

E. Sediment bound radiolabeled residues were detected at a
maximum concentration of 40.8% of applied at 240 days posttreatment. The registrant stated the bound radioactivity in the Day 240 sample was detected in humic acid fraction (2.4% of applied) and fulvic acid fraction (14.9% of applied). Radiolabeled residue in the fulvic acid was identified as 2,4-D (Figure 20).

F. Radioactivity in glucose amended samples at 121 days posttreatment was distributed in water (64% of applied), sediment (19.4% of applied), and sediment bound (9.8% of applied) (Table IX). The radioactivity in sediment/water was identified as 2,4-D (73.5% of applied) and 2,4-DP (11.9% of applied).

VII. Reviewer's Comments

A. The sediment/water system was incubated under static airflow conditions. This type of incubation should enhance anaerobic conditions because of poor air-exchange. The reported Munsell color of sediment samples 5Y 3/2 (dark olive gray) may be indicative of reduced environments. The redox potentials (pE+pH) indicate the sediment/water system was anoxic (pE+pH=4.47 to 10.35) (Sposito, G. 1989. The Chemistry of Soils). However, the dissolved oxygen concentrations (8-16 ppm) indicate the test water was oxic. EFGWB agrees with the registrant's conclusion that oxygen concentrations were high because of oxygen flushing procedures to remove headspace gases. EFGWB believes the study provides acceptable data on anaerobic aquatic metabolism of 2,4-D. No additional data are needed at this time.
The material not included contains the following type of information:

____ Identity of product inert ingredients.
____ Identity of product impurities.
____ Description of the product manufacturing process.
____ Description of quality control procedures.
____ Identity of the source of product ingredients.
____ Sales or other commercial/financial information.
____ A draft product label.
____ The product confidential statement of formula.
____ Information about a pending registration action.
_____ FIFRA registration data.
____ The document is a duplicate of page(s) ______.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.