DATA EVALUATION RECORD

I. Study Type: Aerobic Aquatic Metabolism

II. Citation:

Concha, Marian and Kathryn Shepler. 1993. Aerobic Aquatic Metabolism of [^{14}C] 2,4-D. 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 42979201.

III. Reviewer:

Name: James A. Hetrick, Ph.D. James A. Hetrick
Title: Soil Chemist 18 SEP 1995
Organization: Environmental Chemistry Review Section #1
EFGWB/EFED/OPP

IV. Approved by:

Name: Paul J. Mastradone, Ph.D. Paul J. Mastradone
Title: Section Chief 18 SEP 1995
Organization: Environmental Chemistry Review Section #1
EFGWB/EFED/OPP

V. Conclusions:

The study provides supplemental data on the degradation of 2,4-dichlorophenoxyacetic acid (2,4-D) in an anaerobic aquatic environments. The study cannot be used to fulfill the aerobic aquatic metabolism (162-4) because the sediment/water systems appeared to be anaerobic. (Please see Section VIII for more details.) A new study is needed to evaluate the behavior of 2,4-D in aerobic aquatic environments.

Radiolabeled 2,4-D, at 5 µg/ml, had a Monod-with-growth kinetic model half-life of 4.5 days in anaerobic sediment-water system. The DT_{50} of 2,4-D was estimated at 28.5 days. EFGWB notes the 50% dissipation time (DT_{50}) should be more representative of the persistence of 2,4-D because it accounts for a 25 day lag time in degradation. The degradates of [^{14}C]-2,4-D in soil and water were 2,4-dichlorophenol (2,4-DCP) [1.25% of applied] , 4-chlorophenoxyacetic acid (4-CPA)(1.61% of applied), and 4-chlorophenol (4-CPP) (1.13% of applied). An unknown degradate (separate HPLC peak) also was detected (1.58% of applied at Day 46) in water samples at 35 days posttreatment. Additional unknowns (several small HPLC peaks) were also detected (4.02% of applied immediately posttreatment). The major volatile degradate was tentatively identified as CO_2 (64% of applied at 46 days posttreatment). Unidentified sediment bound residues accounted for 17% of applied [^{14}C]-2,4-D at 46 days posttreatment.

The reported data suggest 2,4-D appears to be moderately persistent in anaerobic aquatic environments.

VI. Materials and Methods:

Preliminary Studies

The registrant performed some preliminary studies to assess trapping efficiencies of gas trap systems (charcoal/ethylene glycol/10% KOH or polyurethane foam/10% KOH). Sediment/water samples was amended with Radiolabeled 2,4-D ((dilution ratio ([^{14}C]-2,4-D:2,4-D)=0.55; SA 22.3 mCi/mmol; phenyl ring labeled; radiopurity =98.1%) to yield nominal concentrations of 2 and 7 µg/ml.

The sediment and water sample, at 2 µg/ml 2,4-D, was placed into a sterile,biometer flask equipped with sequential gas traps of charcoal, ethylene glycol, and 10% KOH. The sediment and water sample was incubated for 27 days. Gas trapping was intermittent during the incubation.

The sediment and water sample, at 7 µg/ml 2,4-D, was placed into a sterile, biometer flask equipped with polyurethane foam and 10% KOH. The sediment and water sample was incubated for 2 and 5 days. Gas trapping was continuous during the incubation.

Definitive Study

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. [Reviewer Note: No redox potentials were reported for sediment and water samples at sampling.] However, microbiological viability of the sediment and water were analyzed immediately before the study and 35 days posttreatment of [^{14}C]-2,4-D. Microbial (bacteria, actinomycete, and fungi) populations were quantified using a dilution plate method on soy agar (TSA), actinomycete isolation agar (AIA), and potato dextrose agar (PPA). Sediment and water samples were stored in an incubator at 25°C.

Sediment (20 g oven dry weight) and pond water (100 ml) were placed into each of 18 sterile, biometer flasks[1]. Each biometer flask was connected to a reservoir with KOH solution and a foam plug. Each sample was amended with

---

1-Sediment and water samples was placed into additional biometer flask to assess cumulative gas trapping efficiency during the study. A sediment and water sample was amended with isotopically diluted 2,4-D (isotopic dilution ratio = 0.22) to yield a concentration of 4.94 µg/ml. The sample was placed in a biometer flask equipped with side-arm with a polyurethane foam plug attached to a reservoir of 10% KOH. The biometer flask was incubated at 25°C for 39 days. Sediment and water samples were extracted and analyzed using procedures outlined in the Analytical Section.

isotopically diluted 2,4-D (dilution ratio ([^{14}C]-2,4-D:2,4-D)=0.55; SA 22.3 mCi/mmol; phenyl ring labeled; radiopurity=98.1%) to yield nominal concentrations of 5 µg/ml. The flasks were incubated at 25°C in the dark. Each flask was opened every 7 days and at every sampling time to facilitate air exchange during incubation. [**Note**: The incubation was conducted under a static air flow system.] Duplicate sediment and pond water samples were taken immediately posttreatment, 4,7,11,14,20,25,35,and 46 days posttreatment. Gas traps were sampled on each soil and water sampling date and at 7 day intervals.

Analytical

The pH, redox potential (Eh), and dissolved oxygen concentrations were measured in sediment/water samples. The color of the sediment also was determined using a Munsell color chart. Sediment samples were sequentially extracted using acetone:water:1M NH_4OH and acetone:water:acetic acid (95:5:5 v:v:v). Sediment extracts were concentrated under N_2, and remaining residues were redissolved in acetonitrile. Water samples were acidified with HCl prior to chemical analysis. The foam plug gas trap was extracted with dichloromethane.

Soluble radiolabeled residues in soil and water extracts were separated using the following 3 different HPLC systems: Omipak Pax-500 column with a linear gradient of acetonitrile and 0.05% trifluoroacetic acid solvent system; C-18 column with an acetonitrile and 0.05% trifluoroacetic acid; and Bio-Rad Aminex Ion Exclusion HPX-87H with an isocratic 0.01N H2SO4 solvent system. 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 a 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 ^{14}C content in soil and water extracts and gas traps was determined by LSC. The ^{14}C content in extracted sediment samples was determined by combustion-LSC. Analytical detection limits were 0.002 µg/g (2X background) and 0.0012 µg/g for combustion-LSC and HPLC-LSC, respectively.

Storage Stability

Samples were extracted on the day of sampling. The sample extracts were reanalyzed for day 25 Rep 1 samples. Water samples were stored at < 10°C. Sediment samples and standards were stored frozen (< 0°C).

Standards were analyzed during the course of the study to assess stability. In addition, sediment extracts for Day 25 Rep 1 and water extracts for Day 46 Rep 2 were reanalyzed after 2 months storage.

VII. Author's Results and Conclusions:

A. The material balance of radiolabeled residues accounted for 85 to 99% of applied [^{14}C]-2,4-D (Table VI). Radiolabeled residues were detected in water (80 % of applied immediately posttreatment), sediment (23 % of applied at 25 days posttreatment), and foam plug gas traps (0.08 % of applied at 39 days posttreatment), and KOH gas traps (64% of applied at 46 days posttreatment). [**Reviewer Note:** The material balance of radiolabeled residues dropped to 77% at 35 days posttreatment. The registrant attributed a low material balance to rapid and massive loss of ^{14}C-CO_2. The registrant conducted a companion study to assess the volatility of CO_2 and degradates of 2,4-D.]

B. The half-life of 2,4-D in aerobic sediment/water system was 4.5 days using a Monod-with-growth kinetic model. The DT_{50} of 2,4-D was estimated at 28.5 days. [**Reviewer Note:** No first-order degradation was estimated because there was an initial 25 day lag phase in 2,4-D degradation. This lag phase was attributed to the time required for microbial adaptation.)

C. The degradates of [^{14}C]-2,4-D in water and soil extracts were 2,4-DCP (1.25 % of applied at 35 days posttreatment to 0.09% of applied at 46 days postreatment), 4-CPA (1.61% of applied at 20 days posttreatment to non-detectable at 46 days posttreatment), and 4-CPP (1.13% of applied at 25 days posttreatment to non-detectable at 46 days posttreatment) (Table VII). A unidentified degradate also was detected (1.58% of applied) in water samples at 35 days posttreatment. Additional unknowns (several small HPLC peaks) were also detected (4.02% of applied immediately posttreatment) in soil water extracts. Unidentified sediment bound residues accounted for 17% of applied at 46 days posttreatment (Table VI).

D. The major volatile degradate was tentatively identified as CO_2 (64% of applied at 46 days posttreatment) (Table VI). Unidentified degradates also were detected (0.08% of applied at 39 days) in the foam plug gas trap.

E. The registrant proposed a degradation pathway of 2,4-D in aerobic aquatic environments (Figure 16). They proposed that 2,4-D degradation is dependent upon on dechlorination and oxidative cleavage of the acetic acid processes to form a common degradate 4-CPP. Intermediate degradates were identified as 4-CPA and 2,4-DCP. The degradate 4-CPP is further degraded through oxidative mineralization to CO_2.

VII. Reviewer's Comments

A. The sediment/water system was incubated under static air-flow conditions. This type of incubation may promote anaerobic conditions because of poor air-exchange. Redox measurements indicate the sediment/water system had an average pE+pH of 6.09. This redox potential is considered

anoxic (pE+pH) in soil environments (Sposito, 1989. The Chemistry of Soils). More importantly, the reported color of sediment samples (5Y 3/2-dark olive gray) commonly indicates reduced environments.

Because reported redox potentials and sediment color in the study were representative of anoxic (anaerobic) environments, EFGWB believes the study cannot be used to fulfill the aerobic aquatic metabolism (162-4) data requirement. However, the study provides supplemental data on 2,4-D degradation in anaerobic aquatic environments. A new study is needed to evaluate the behavior of 2,4-D in aerobic aquatic environments.

B. There was a low material balance (77% of applied) at 35 days posttreatment. The registrant attributed the low material balance to rapid and massive loss of ^{14}C. EFGWB notes similar observations were observed in a previous aquatic metabolism study (MRID 41557901). EFWGB believes the low material balance at 35 days posttreatment does not jeopardize acceptance of the study because it occurred at a single sampling time.

C. The degradation rate was not determined using a first-order degradation model because there was a significant lag time (25 days) in 2,4-D degradation (Figure 17). The registrant used a Monod growth kinetic model to estimate a 2,4-D half-life of 4.5 days. The DT_{50} of 2,4-D was 28.5 days. EFGWB believes the DT_{50} is more representative of the persistence of 2,4-D because it accounts for the 25 day lag time in 2,4-D degradation.

2,4-D EFED Review

Page _____ is not included in this copy.

Pages 6 through 18 are not included in this copy.

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.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

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.