Subject: Data Evaluation Record (DER) for Aerobic Aquatic Metabolism in a Water/Sediment System Submitted in Support of 2,4-dichlorophenoxyacetic acid (2,4-D)

To: Mark Seaton, Chemical Review Manager
Reregistration Branch II
Special Review and Reregistration Division (7508C)

From: Mark Corbin, Environmental Scientist
Environmental Risk Branch I
Environmental Fate and Effects Division (7507C)

Date: December 10, 2003
Chemical: 2,4-D
PC Code: 030001
DP Barcode: Not assigned

Attached is the final Data Evaluation Record (DER) for the Aerobic Aquatic Metabolism study submitted in support of the re-registration of 2,4-D (PC Code 030001). The study has been deemed supplemental because the study was terminated at 30 days before a half life could be estimated.
Data Evaluation Report on the aerobic biotransformation of 2,4-D in a water-sediment system

PMRA Submission Number {......} EPA MRID Number 44188601

Data Requirement: PMRA Data Code:
EPA DP Barcode: Not assigned as of review data.
OECD Data Point:
EPA Guideline: 162-4

Test material:
Common name: 2,4-D.
Chemical name
   IUPAC: 2,4-Dichlorophenoxyacetic acid.
   CAS name: 2,4-Dichlorophenoxyacetic acid.
   CAS No: 94-75-7.
   Synonyms: None.
SMILES string:

Primary Reviewer: Joan Gaidos
Dynamaec Corporation

QC Reviewer: Kathleen Ferguson
Dynamaec Corporation

Secondary Reviewer: Mark Corbin
EPA

Company Code: [for PMRA]
Active Code: [for PMRA]
Use Site Category: [for PMRA]
EPA PC Code: 030001

Signature: [Signature]
Date: [Date]

Data Evaluation Report on the aerobic biotransformation of 2,4-D in a water-sediment system

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  Synonyms: None.
SMILES string:

Primary Reviewer: Joan Gaidos
Dynamac Corporation

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Company Code: [for PMRA]
Active Code: [for PMRA]
Use Site Category: [for PMRA]
EPA PC Code: 030001

EXECUTIVE SUMMARY

The biotransformation of [phenyl-U-\(^{14}\)C] 2,4-dichlorophenoxyacetic acid (2,4-D) was studied in a lake water-sand sediment system (water pH 8.46; sediment pH 8.8, organic matter 0.4%) from Madison, Wisconsin for 30 days under aerobic conditions in darkness at 25 ± 1°C. \(^{14}\)C\(2,4\)-D was applied at the rate of ca. 11 mg a.i./L water. The water:sediment ratio was 10:1 (30 g water:3 g dry wt. sediment). This experiment was conducted in accordance with USEPA Subdivision N Guideline §162-4 and USEPA Good Laboratory Practice Standards (40 CFR 160). The test system consisted of glass containers with the treated water-sediment connected to a continuous flow through volatile trapping system. Duplicate samples were collected after 0, 1, 3, 8, 16, 23 and 30 days of incubation. Water and sediment were separated. Water samples were analyzed without manipulation. Sediment samples were extracted twice with acetic acid in methanol and once with aqueous acetic acid. Water, sediment extracts, extracted sediment and trapping materials were analyzed for total radioactivity using LSC. Water and sediment extracts were analyzed using HPLC. 2,4-D was identified by comparison to a reference standard that was cochromatographed with the samples; the identification was confirmed using 2-dimensional TLC.

Test conditions were measured for the entire system rather than separately for the water and sediment. The pH increased from 7.77-7.90 at day 0 to 8.90-9.06 at day 30. Redox potentials decreased from 350-367 mV at day 0 to 79.8-92.5 mV at day 30. Dissolved oxygen content was variable; values were 5.0-5.4 mg/L at day 0, 2.6-9.7 mg/L at days 3 through 23 with no discernable pattern, and 2.1-3.1 mg/L at day 30. It was reported that temperatures were maintained at 25 ± 1°C during the study; however, no supporting records were provided.

Overall recovery of radiolabeled material averaged 99.5 ± 2.2% (range 95.2-103.5%, n = 14) of the applied. There was no apparent loss of radioactivity with time.

In the total system, \(^{14}\)C\(2,4\)-D averaged 103.0% of the applied on day 0 and ranged from an average 96.9 to 100.7% of the applied at 1 through 30 days with no discernable pattern of decline. At all intervals, >95% of the applied \(^{14}\)C\(2,4\)-D was associated the water layer; the maximum average concentration in the sediment was 3.1% of the applied at 23 days posttreatment. There were no major transformation products detected in the water or sediment, and no minor transformation products were identified. In one of the two samples from 30 days posttreatment, an unidentified region of radioactivity (Region 1) was isolated from the sediment at 0.1% of the applied. Extractable \(^{14}\)C residues in the sediment increased from an average of 1.6% of the applied at day 0 to a maximum of 3.1% at 23 days 23, and were 2.5% at 30 days. Nonextractable \(^{14}\)C residues were detected in the sediment only at 23 and 30 days at average concentrations of 0.1% and 0.05% of the applied, respectively. At study termination (30 days posttreatment), \(^{14}\)CO\(_2\) totaled approximately 0.28% of the applied. Organic volatiles were not detected.

Based on first order linear regression analysis, 2,4-D degraded from the total system with a half-life of >4 years and from the water with a half-life of >2 years. These half-lives are of uncertain value.
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because they are extrapolated well beyond the 30-day duration of the study; the correlation coefficients are only 0.07 and 0.13, respectively. The sediment data could not be used to estimate a half-life.

No transformation pathway was proposed by the study author. No transformation products were identified, other than very minor amounts of CO₂.

Results Synopsis:

Test system: Water-sand sediment (10:1, v:w) from a lake in Wisconsin.
Half-life values:
  - Entire system: 1,386.3 days \( (r^2 = 0.0741) \).
  - Water layer: 866.4 days \( (r^2 = 0.1318) \).
  - Sediment: Unable to calculate due to insufficient sampling intervals.
Major transformation product:
  - None.
Minor identified transformation products:
  - CO₂.

Study Acceptability: This study is classified supplemental. It is scientifically valid, but does not fulfill the requirements for an aerobic aquatic metabolism study because the study duration was insufficient to calculate an accurate half-life for degradation of 2,4-D. In addition, the water:sediment ratio was 10:1 (v:w), therefore, there was too little sediment in the system to accurately assess the partitioning of 2,4-D between the water and sediment fractions. Also, this is a significantly higher ratio of water to soil than is typically used in aerobic aquatic metabolism studies, so that the results of this study are not comparable to the results of aerobic aquatic metabolism studies performed for other pesticides.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with USEPA Subdivision N Guideline §162-4 (pp. 10, 12). The only significant deviation from Subdivision N guidelines was:

The study design was not typical of an aerobic aquatic metabolism experiment conducted under Subdivision N guidelines. The system contained only 3 g of sediment compared to 30 mL of water, so that the partitioning of 2,4-D between the water and sediment fractions could not be accurately assessed. This does not affect the validity of the study.

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COMPLIANCE: This study was conducted in compliance with USEPA Good Laboratory Practice Standards (40 CFR 160; p. 3). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A Certification of Authenticity was not provided.

A. MATERIALS:

1. Test Materials: [Phenyl-U-14C]2,4-D (p. 13).

Chemical Structure: See Attachment 2.

Description: Not reported.

Purity:

Labeled Radiochemical purity: 100.0% (p. 13).
Lot No.: None, was prepared from the ester in the testing laboratory.
Specific activity: 106.8 mCi/mMol; 0.483 mCi/mg.
Label position: Uniformly on phenyl ring.

Unlabeled Analytical purity: 99.5% (p. 13).
Lab Inventory No.: 6166; Lot No.: AGR 275828.

The test substance was prepared by cutting the labeled material with unlabeled 2,4-D at a ratio of 195:7500 (w:w; p. 16). The specific activity of the mixture was 27, 126 dpm/μg.

Storage conditions of test chemicals: Not reported.
Table 1: Physico-chemical properties of 2,4-D.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>221 g/mol</td>
<td></td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C₆H₅Cl₂O₃</td>
<td></td>
</tr>
<tr>
<td>Water solubility</td>
<td>Not reported.</td>
<td></td>
</tr>
<tr>
<td>Vapor pressure/volatility</td>
<td>Not reported.</td>
<td></td>
</tr>
<tr>
<td>UV absorption</td>
<td>Not reported.</td>
<td></td>
</tr>
<tr>
<td>pKₐ</td>
<td>Not reported.</td>
<td></td>
</tr>
<tr>
<td>Kₐw/log Kₐw</td>
<td>Not reported.</td>
<td></td>
</tr>
<tr>
<td>Dissociation constant</td>
<td>Not reported.</td>
<td></td>
</tr>
<tr>
<td>Hydrolytic stability</td>
<td>Not reported.</td>
<td></td>
</tr>
<tr>
<td>Stability of compound at room temperature:</td>
<td>Stable.</td>
<td>(see Reviewers Comment # 3).</td>
</tr>
</tbody>
</table>

Data obtained from p. 13; Appendix B, pp. 57-58 of the study report.
2. Water-sediment collection, storage and properties:

Table 2: Description of water-sediment collection and storage.

<table>
<thead>
<tr>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographic locations:</td>
<td>Lake water and associated sediment was collected from Lake Mendota, Dane County, Madison, Wisconsin.</td>
</tr>
<tr>
<td>Pesticide use history at the collection site:</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Collection date:</td>
<td>Not reported. It was stated that a “fresh sample” of lake water and associated sediment was used.</td>
</tr>
<tr>
<td>Collection procedures:</td>
<td></td>
</tr>
<tr>
<td>water:</td>
<td>Not reported.</td>
</tr>
<tr>
<td>sediment:</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Sampling depth:</td>
<td></td>
</tr>
<tr>
<td>water:</td>
<td>Not reported.</td>
</tr>
<tr>
<td>sediment:</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Storage conditions:</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Storage length:</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Preparation:</td>
<td></td>
</tr>
<tr>
<td>water:</td>
<td>Not reported.</td>
</tr>
<tr>
<td>sediment:</td>
<td>Excess water was drained, and the sediment was air-dried and then sieved (2-mm).</td>
</tr>
</tbody>
</table>

Data obtained from p. 14 of the study report.
**Data Evaluation Report on the aerobic biotransformation of 2,4-D in a water-sediment system**

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### Table 3: Properties of the water.

<table>
<thead>
<tr>
<th>Property</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C):</td>
<td>18</td>
</tr>
<tr>
<td>pH</td>
<td>8.46</td>
</tr>
<tr>
<td>Redox potential (mV):</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Dissolved oxygen (ppm):</td>
<td>6.3</td>
</tr>
<tr>
<td>Alkalinity (mg of CaCO₃/L)</td>
<td>190</td>
</tr>
<tr>
<td>Suspended solids (ppm)</td>
<td>4</td>
</tr>
<tr>
<td>Total hardness (mg/L)</td>
<td>236</td>
</tr>
<tr>
<td>Dissolved organic carbon (mg/L):</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Total organic carbon (mg/L):</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Electrical conductivity (mMhos):</td>
<td>0.5</td>
</tr>
<tr>
<td>Biomass (mg microbial C/100 g, CFU or other):</td>
<td>Not reported.</td>
</tr>
</tbody>
</table>

Data obtained from Text Table 1, p. 14 of the study report.
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Table 4: Properties of the sediment.

<table>
<thead>
<tr>
<th>Property</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Textural classification:¹</td>
<td>Sand</td>
</tr>
<tr>
<td>% sand:</td>
<td>98</td>
</tr>
<tr>
<td>% silt:</td>
<td>2</td>
</tr>
<tr>
<td>% clay:</td>
<td>0</td>
</tr>
<tr>
<td>pH:</td>
<td>8.8</td>
</tr>
<tr>
<td>Moisture content (%):</td>
<td>0.0745 (after air-drying and sieving).</td>
</tr>
<tr>
<td>Organic matter (%):</td>
<td>0.4</td>
</tr>
<tr>
<td>Organic carbon (%):</td>
<td>Not reported.</td>
</tr>
<tr>
<td>CEC (meq/100 g):</td>
<td>8.4</td>
</tr>
<tr>
<td>Redox potential (mV):</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Bulk density (g/cm³):</td>
<td>Not reported.</td>
</tr>
<tr>
<td>Biomass:</td>
<td>Not reported.</td>
</tr>
</tbody>
</table>

Data obtained from Text Table I, p. 14 of the study report.

¹ The soil textural classification was not reported by the study author. Based on the USDA soil textural classification triangle, the sediment is a sand.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: The study author reported in the protocol that a preliminary study would be performed to define the experimental parameters of the definitive study (Appendix A, p. 46). Parameters to be measured included: the extractability of 2,4-D and ¹³C-transformation products from sediment, radioactivity in the trapping media, and chromatographic behavior of reference standards and transformation products, and the tentative degradation rate to determine sampling intervals. Sediment-water samples would be fortified with approximately 10 ppm of the test material and analyzed at selected intervals (e.g., 0, 1, 3, 5, and 7 days).

The study author reported that results would be discussed with the sponsor before initiation of the definitive study. Further specifics of this preliminary experiment were not provided and results were not presented.
2. Experimental conditions:

Table 5: Study design.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of the test:</td>
<td>30 days.</td>
</tr>
<tr>
<td>Water:</td>
<td></td>
</tr>
<tr>
<td>Filtered/unfiltered water:</td>
<td></td>
</tr>
<tr>
<td>Type and size of filter used:</td>
<td></td>
</tr>
<tr>
<td>Amount of sediment and water/treatment:</td>
<td></td>
</tr>
<tr>
<td>water:</td>
<td>30 mL.</td>
</tr>
<tr>
<td>sediment:</td>
<td>3 g dry wt.</td>
</tr>
<tr>
<td>Water:sediment ratio:</td>
<td>10:1 (mL:g.).</td>
</tr>
<tr>
<td>Application rate (mg a.i./L):</td>
<td>325 µL of a 1.0125 µg/µL solution added to 30 mL water;</td>
</tr>
<tr>
<td></td>
<td>equivalent to ca. 11 mg a.i./L water.</td>
</tr>
<tr>
<td>Control conditions, if used:</td>
<td>No controls were used.</td>
</tr>
<tr>
<td>No. of Replications:</td>
<td></td>
</tr>
<tr>
<td>Controls, if used:</td>
<td>No controls were used.</td>
</tr>
<tr>
<td>Treatments:</td>
<td>Duplicate samples removed at each collection interval.</td>
</tr>
<tr>
<td>Test apparatus (type/material/volume):</td>
<td>Glass containers (not further characterized) containing water (30 mL)</td>
</tr>
<tr>
<td></td>
<td>and sediment were treated with 325 µL of test solution, sealed with</td>
</tr>
<tr>
<td></td>
<td>a stopper containing inlet and outlet tubes, and gently shaken.</td>
</tr>
<tr>
<td></td>
<td>The containers were connected in sets to a flow through trapping</td>
</tr>
<tr>
<td></td>
<td>apparatus and maintained in the dark at 25 ± 1°C.</td>
</tr>
<tr>
<td></td>
<td>The test apparatus is illustrated in Figure 1, p. 29.</td>
</tr>
<tr>
<td>Details of traps for CO₂ and organic volatiles, if any:</td>
<td>Using vacuum, air was continuously drawn into the containers,</td>
</tr>
<tr>
<td></td>
<td>through the sample solution, and then sequentially through tubes of</td>
</tr>
<tr>
<td></td>
<td>ethylene glycol, 0.1N sulfuric acid and 2N KOH (two tubes).</td>
</tr>
<tr>
<td>If no traps were used, is the system closed/open?</td>
<td>Volatile traps were used.</td>
</tr>
<tr>
<td>Identity and concentration of co-solvent:</td>
<td>Acetonitrile, final concentration 1.1% by volume.</td>
</tr>
<tr>
<td>Test material application:</td>
<td></td>
</tr>
<tr>
<td>Vol. of test solution used/treatment:</td>
<td>325 µL/sample.</td>
</tr>
<tr>
<td>Application method:</td>
<td>Not reported. After dosing, sample containers were sealed and the</td>
</tr>
<tr>
<td></td>
<td>treated solution gently shaken.</td>
</tr>
<tr>
<td>Any indication of the test material adsorbing to the walls of the test apparatus?</td>
<td>Not reported.</td>
</tr>
</tbody>
</table>
Data Evaluation Report on the aerobic biotransformation of 2,4-D in a water-sediment system

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<table>
<thead>
<tr>
<th>Criteria</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass (CFU/mL of a sediment-water slurry):</td>
<td></td>
</tr>
<tr>
<td>Aerobic:</td>
<td>Initial¹:</td>
</tr>
<tr>
<td></td>
<td>Final:</td>
</tr>
<tr>
<td></td>
<td>1,250,000</td>
</tr>
<tr>
<td></td>
<td>12,200,000</td>
</tr>
<tr>
<td>Anaerobic:</td>
<td>Initial:</td>
</tr>
<tr>
<td></td>
<td>Final:</td>
</tr>
<tr>
<td></td>
<td>53,000</td>
</tr>
<tr>
<td></td>
<td>12,800</td>
</tr>
<tr>
<td>Experimental conditions:</td>
<td>Temperature (°C):</td>
</tr>
<tr>
<td></td>
<td>25 ± 1°C.</td>
</tr>
<tr>
<td>Continuous darkness:</td>
<td>Yes.</td>
</tr>
<tr>
<td>Other details, if any:</td>
<td>None.</td>
</tr>
</tbody>
</table>

Data obtained from pp. 15-17; Text Table II, p. 15; Text Table V, p. 17 of the study report.

¹Initial and Final intervals 0 and 30 days posttreatment, respectively.

3. **Aerobic conditions:** Air was continuously drawn through the water (p. 17). Dissolved oxygen saturation levels and redox potentials were measured at each sampling interval (Table 2, p. 24).

4. **Supplementary experiments:** No supplementary experiments were conducted.

5. **Sampling:**

**Table 6: Sampling details.**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling intervals:</td>
<td>0, 1, 3, 8, 16, 23, and 30 days.</td>
</tr>
<tr>
<td>Sampling method:</td>
<td>Duplicate samples (glass containers) were collected at each sampling interval.</td>
</tr>
<tr>
<td>Method of collection of CO₂ and volatile organic compounds:</td>
<td>At each sampling interval, the trapping solutions were collected and replaced with fresh media.</td>
</tr>
<tr>
<td>Sampling intervals/times for: Sterility check, if sterile controls are used: Redox potential/other:</td>
<td>Sterile controls were not used. Dissolved oxygen saturation levels, pH and redox potentials were measured at each sampling interval.</td>
</tr>
<tr>
<td>Sample storage before analysis:</td>
<td>Not specified. Samples were stored frozen after analysis.</td>
</tr>
<tr>
<td>Other observations, if any:</td>
<td>None.</td>
</tr>
</tbody>
</table>

Data obtained from pp. 17 and 19 of the study report.
C. ANALYTICAL METHODS:

Separation of water and sediment: The water layer and sediment were separated by centrifugation (no further details; p. 18).

Extraction/clean up/concentration methods: Water was analyzed directly using LSC and HPLC (p. 18). The sediment was sequentially extracted with 5% (v:v) acetic acid in methanol, 5% acetic acid: methanol (50:50, v:v); and 5% (v:v) aqueous acetic acid by vortexing (1 minute) and sonicating (10 minutes). After each extraction, the mixture was centrifuged and the supernatant removed by pipetting. The extracts were combined, brought to 18 mL with methanol, and analyzed using LSC. An aliquot (10 mL) of each sample extract was concentrated to dryness by rotary evaporation, and the resulting residues were diluted with methanol:water (1:1) and analyzed by HPLC.

Nonextractable residue determination: Extracted sediments were homogenized by stirring or grinding, and portions were analyzed using LSC following combustion (p. 19).

Volatile residue determination: The ethylene glycol, sulfuric acid and KOH solutions were analyzed directly for total radioactivity using LSC (p. 19). The presence of $^{14}$CO$_2$ in the KOH solutions was not confirmed.

Total $^{14}$C measurement: Total $^{14}$C residues were determined by summing the concentrations of [14C]residues measured in the water layers, sediment extracts, extracted sediment, and volatile trapping media (p. 20).

Derivatization method, if used: A derivatization method was not employed.

Identification and quantification of parent compound: Water and sediment extracts were analyzed using HPLC under the following conditions: Omnipak-Pak-500 (10-32) column (250 mm x 4.6 mm, Dionex), gradient mobile phase combining (A) 0.05% trifluoroacetic acid in water and (B) acetonitrile [percent A:B at 0 minutes 75:25; 10 minutes 60:40; 30 minutes 50:50; 35 minutes 10:90; 45-50 minutes 0:100; and 55-60 minutes 75:25], flow rate 1.0 mL/minute, and UV (254 nm) and radioactive flow detection (pp. 18, 19). Samples were cochromatographed with an unlabeled reference standard of 2,4-D. The total eluate was collected and analyzed using LSC to determine column recovery (not reported).

Aliquots of the 0-, 16- and 30-day water samples were also analyzed using two-dimensional TLC on plates (not described) developed in hexane:2-propanol:acetic acid (10:10:1, v:v:v) and toluene:ethyl acetate:acetic acid (10:10:1; v:v:v; p. 19). Radioactive regions were identified by comparison to the movement of an unlabeled reference standard that was cochromatographed with the sample.
Identification and quantification of transformation products: Transformation products were separated and quantified using the HPLC and TLC analyses as described for parent. It did not appear that any reference standards of possible transformation products were used.

Detection limits (LOD, LOQ) for the parent compound: The Limit of Detection for the LSC method was 68 dpm (twice background) and for HPLC was 130 dpm (p. 20). The LOD for TLC was not reported. Limits of Quantification were not reported.

Detection limits (LOD, LOQ) for the transformation products: The Limits of Detection and Quantification were the same as for the parent compound.

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS: Measurements were made on the entire water/sediment system; separate values were not determined for the water and sediment (Table 2, p. 24). The pH increased from 7.77-7.90 at day 0 to 8.90-9.06 at day 30. Redox potentials decreased from 350-367 mV at day 0 to 79.8-92.5 mV at day 30. Dissolved oxygen content was variable; values were 5.0-5.4 mg/L at day 0, 2.6-9.7 mg/L at days 3 through 23 with no discernable pattern, and 2.1-3.1 mg/L at day 30. It was reported that temperatures were maintained at 25 ± 1°C during the study (pp. 10,17); however, no supporting records were provided.

B. MATERIAL BALANCE: Overall recovery of radiolabeled material averaged 99.5 ± 2.2% (range 95.2-103.5%, n = 14) of the applied (Appendix C, p. 68; see DER Attachment 1). There was no apparent loss of material from the system.
Table 7: Biotransformation of [14C]2,4-D, expressed as percentage of applied radioactivity (mean ± standard deviation, n = 2), in a water-sediment system under aerobic conditions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sampling times (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2,4-D</td>
<td></td>
</tr>
<tr>
<td>water</td>
<td>101.4 ± 1.1</td>
</tr>
<tr>
<td>sediment</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>system</td>
<td>103.0 ± 0.8</td>
</tr>
<tr>
<td>Unidentified</td>
<td></td>
</tr>
<tr>
<td>(Region 1)</td>
<td></td>
</tr>
<tr>
<td>sediment</td>
<td>ND</td>
</tr>
<tr>
<td>Total extractable residues</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Nonextractable residues</td>
<td>0.000</td>
</tr>
<tr>
<td>Total CO₂ 1</td>
<td>NA</td>
</tr>
<tr>
<td>Total volatile organics</td>
<td>NA</td>
</tr>
<tr>
<td>Total % recovery</td>
<td></td>
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<tr>
<td>water</td>
<td>101.4 ± 1.1</td>
</tr>
<tr>
<td>sediment</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>system</td>
<td>103.0 ± 0.8</td>
</tr>
</tbody>
</table>

Means and SD calculated by the reviewer using data obtained from Tables 3-6, pp. 25-28; Appendix C, pp. 68, 74, 77, 79 of the study report (see Attachment 1).
1 Total CO₂ was calculated by the reviewer as the sum of the two KOH traps.
2 Total % recovery in sediment was calculated by the reviewer as the sum of extractable and nonextractable residues.
3 Unidentified radioactivity detected at 0.1 % of the applied in one of two samples.
NA = Not Applicable.
ND = Not Detected.
Data Evaluation Report on the aerobic biotransformation of 2,4-D in a water-sediment system

PMRA Submission Number {......} EPA MRID Number 44188601

C. TRANSFORMATION OF PARENT COMPOUND: In the total system, $[^{14}\text{C}]$-2,4-D averaged 103.0% of the applied on day 0 and ranged from an average 96.9 to 100.7% of the applied at 1 through 30 days with no discernable pattern of decline (Appendix C, p. 74). At all intervals, $>95\%$ of the applied $[^{14}\text{C}]$-2,4-D was associated the water layer; the maximum average concentration in the sediment was 3.1% of the applied at 23 days posttreatment (Appendix C, pp. 74, 77).

HALF-LIVES: Based on first order linear regression analysis, 2,4-D degraded from the total system with a half-life of $>4$ years and from the water with a half-life of $>2$ years (Appendix C, pp. 74, 77, 79; see DER Attachment 1). These half-lives are of uncertain value because they are extrapolated well beyond the 30-day duration of the study; the correlation coefficients are only 0.07 and 0.13, respectively. The sediment data could not be used to estimate a half-life. The study author concluded that 2,4-D was stable in this system and did not attempt to calculate half-lives (p. 22).

### Half-lives ($t_{1/2}$).

<table>
<thead>
<tr>
<th>System</th>
<th>First order Linear</th>
<th>DT50</th>
<th>DT90</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Half-life (days)</td>
<td>Regression equation</td>
<td>$r^2$</td>
</tr>
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<td>Water</td>
<td>866.4</td>
<td>$y = -0.0008x + 4.5829$</td>
<td>0.1318</td>
</tr>
<tr>
<td>Sediment</td>
<td>Insufficient data were available to permit calculation of a half-life.</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Entire System</td>
<td>1,386.3</td>
<td>$y = -0.0005x + 4.6047$</td>
<td>0.0741</td>
</tr>
</tbody>
</table>

Half-lives were calculated by the reviewer using Excel and data obtained from Appendix C, pp. 74, 77, 79 of the study report.

NA = Not applicable

TRANSFORMATION PRODUCTS: There were no major transformation products detected in the water/sediment system (pp. 21-22). No minor transformation products were identified. In one of the two samples from 30 days posttreatment, an unidentified region of radioactivity (Region 1) was isolated from the sediment at 0.1% of the applied (Appendix C, p. 77).

NONEXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable $[^{14}\text{C}]$residues in the sediment increased from an average of 1.6% of the applied at day 0 to a maximum of 3.1% at 23 days, and were 2.5% at 30 days (Table 3, p. 25). Nonextractable $[^{14}\text{C}]$residues were detected in the sediment only at 23 and 30 days at average concentrations of 0.1% and 0.05% of the applied, respectively.

VOLATILIZATION: At study termination (30 days posttreatment), $^{14}\text{CO}_2$ totaled approximately 0.28% of the applied. Organic volatiles were not detected (Table 3, p. 25; Appendix C, pp. 68).

TRANSFORMATION PATHWAY: No transformation pathway was proposed by the study author. No transformation products were identified, other than very minor amounts of $\text{CO}_2$. 

Page 14 of 17
Data Evaluation Report on the aerobic biotransformation of 2,4-D in a water-sediment system

PMRA Submission Number {......} 

EPA MRID Number 44188601

Table 9: Chemical names for transformation products of 2,4-D.

<table>
<thead>
<tr>
<th>Applicant's Code</th>
<th>CAS Number</th>
<th>Chemical Name</th>
<th>Chemical formula</th>
<th>Molecular weight (g/mol)</th>
<th>SMILES string</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No transformation products were identified.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: No supplementary experiments were conducted.

III. STUDY DEFICIENCIES:

1. There was only 3 g of sediment in the system compared to 30 mL of water, so the partitioning of 2,4-D between the water and sediment fractions was difficult to assess. Since the aerobic aquatic metabolism study is intended to approximate conditions in a field that is irrigation-flooded at the time of pesticide treatment, this study design does not adequately replicate the desired environmental conditions. Also, this is a significantly higher ratio than is typically used in aerobic aquatic metabolism studies, so that the results of this study are not comparable to the results of aerobic aquatic metabolism studies performed for other pesticides.

2. The sediment that was used in this study was almost pure sand (98%) with no clay and only 0.4% organic matter. This would not be typical of the sediments found in most locations where 2,4-D is used and is probably not typical of the soils/sediments used in other metabolism studies submitted for 2,4-D. However, it may represent a worst-case scenario in that 2,4-D would be less likely to adsorb to a low-organic sand than to a soil/sediment with a higher clay and organic content.

3. The study author did not state that the water-sediment system was treated and flooded in close succession (p. 16). The redox potential of the sediment was never measured separate from the water.

IV. REVIEWER'S COMMENTS:

1. The 2,4-D used in the definitive study was received on 6/14/96 as 2,4-D isopropyl ester and converted to 2,4-D by the following method (Appendix B, p. 57). Dichloromethane (DCM) was allowed to evaporate off of approximately 100 μL (526 μCi) of the isopropyl ester of [14C]2,4-D (Inventory No. 1857); 1N hydrochloric acid was added and stirred for approximately 4 hours at approximately 80°C (designated solution no. 1857-2). The solution was purified by extracting several times (not specified) with DCM. The DCM was evaporated off, the residue dissolved in acetonitrile (2 mL) and the extract identified by cochromatographed with an unlabeled reference
standard using one-dimensional TLC. The TLC plate was scraped and the silica gel extracted three time with acetonitrile:water (4:1, 10 mL); the extract was further extracted several time (not specified) with DCM, evaporated and redissolved in acetonitrile (designated solution no. 1857-2B). The extract was analyzed by HPLC, then further purified by collecting thirty-second HPLC fractions, and analyzing by LSC to determine fractions containing $[^1]^{14}$C2,4-D (fractions 27-48). The combined, repurified fractions were re-analyzed by HPLC and determined to be 100% $[^1]^{14}$C2,4-D (Figure B-1, p. 59). The test material was confirmed by LC/MS using negative ion detection Figure B-2, p. 60; Figure B-3, p. 61).

2. The stability of the radiolabeled test material was determined by comparing the radiopurity of the test material in the dosing solution at the time of dosing, with the radiopurity originally determined by the testing lab (p. 13; Appendix B, p. 58). The reviewer assumes that the sample from the lab (CHW) was measured on August 16, 1996, while the concentration of the test material in the dosing solution was collected and analyzed immediately before dosing, on August 19, 1996 (p. 13). The study author states that “...aliquots (100 μL) of the dosing solution were taken before and after dosing...” (p. 16). The time frame was not clearly specified, and the storage conditions and results of the stability assessment are not reported, however, the study author reports that the radiolabeled test material was considered stable. Clarification is needed.

The nonradiolabeled reference standard was considered stable in storage under freezer conditions (not specified) by comparing the initial chromatographs (presumably measured on August 19, 1996) with the chromatographs at experimental termination (November 1, 1996; p. 13; Appendix B, p. 58). Again, the time frame and storage conditions are not clearly specified and clarification is needed.

3. The information provided for the water-sediment collection and storage was incomplete (p. 14). The pesticide use history of the site, collection date, procedures, and sampling depth were not reported. Storage conditions and length of storage were also unreported, and the preparation of the water sample was not discussed. Assessing the integrity of the water-sediment sample prior to treatment is difficult in the absence of this information.

4. Microbial analysis was performed on a slurry of lake water and sediment (p. 14). Six serial dilutions (11 mL sediment in 99 mL phosphate buffer) were plated for aerobic and anaerobic visual count assays after incubation at 35 ± 2°C for 48 ± 3 hours. It was not stated whether the ratio of water to sediment was 10:1 as was used in the study.

5. The nominal application rate of 10 μg/g (10 ppm) was reported to be equivalent to the highest application rate used in the field (p. 15).

6. The efficiency of the oxidizer (Model OX-500, R.J. Harvey Instrument Corp.) was determined prior to each series of sediment combustions (p. 15). Sample oxidation efficiencies were >95% for all analyses, therefore oxidation values were not adjusted for efficiency.
7. An additional sample was collected on day 30 for microbial analysis, and three samples were collected on day 42, frozen, but were not analyzed (p. 17).

V. REFERENCES:


Attachment 1

Excel Spreadsheets
**Chemical Name:** 2,4-D  
**PC Code:** 030001  
**MRID:** 44188601  
**Guideline No.:** 162-4  

**Half-life (days):** Insufficient data

<table>
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<tr>
<th>Day</th>
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<th>Ln (% applied)</th>
</tr>
</thead>
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</tr>
<tr>
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</tr>
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</tr>
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<tr>
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<td>0.9933</td>
</tr>
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</table>

Data obtained from Appendix C, p. 68 of the study report.

**[\textsuperscript{14}C]2,4-D in sediment phase of water-sediment system**

![Graph showing the decay of [\textsuperscript{14}C]2,4-D in sediment phase over days posttreatment.]
Chemical Name: 2,4-D  
PC Code: 030001  
MRID 44188601  
Guideline No. 162-4

**Half-life (days):** 866.4

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</thead>
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<td>4.5633</td>
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</table>

Data obtained from Appendix C, p. 68 of the study report.

**[^14]C2,4-D in water phase of water-sediment system**

\[
y = -0.0008x + 4.5829 \\
r^2 = 0.1318
\]
Chemical Name: 2,4-D  
PC Code: 030001  
MRID 44188601  
Guideline No. 162-4

**Half-life (days):** 1386.3

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</tbody>
</table>

Data obtained from Appendix C, pp. 68, 79 of the study report.

![Graph](image)

\[ y = -0.0005x + 4.6047 \]

\[ r^2 = 0.0741 \]
### Material Balance

#### Mean % of Applied

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<th>Day</th>
<th>Water Layer Ave</th>
<th>Ave Sediment Extract</th>
<th>Ave Sediment St.dev</th>
<th>Extracted Sediment Ave</th>
<th>St.dev</th>
<th>Ethylene Glycol Trap Acid Trap</th>
<th>KOH 1</th>
<th>KOH 2</th>
<th>KOH Total</th>
<th>Material Balance Ave</th>
<th>St.dev</th>
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<tbody>
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</table>

Data obtained from Appendix C, p. 68 of the study report.

ND = Not detected
NA = Not analyzed

### Total Material Balance in Sediment

#### Mean % of Applied

<table>
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<th>Day</th>
<th>Sediment Extract</th>
<th>Sediment Total</th>
<th>Ave</th>
<th>St.dev</th>
</tr>
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<td>0.3</td>
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### [14C] Residues

#### Water:Sediment ratios

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Data obtained from Appendix C, p. 68 of the study report.
Chemical Name: 2,4-D  
PC Code: 030001  
MRID: 44188601  
Guideline No.: 162-4

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Data obtained from Appendix C, pp. 68, 79 of the study report.
Attachment 2

Structures of Parent and Transformation Products
2,4-D

IUPAC: 2,4-Dichlorophenoxyacetic acid
CAS name: 2,4-Dichlorophenoxyacetic acid
CAS No: 94-75-7

\[
\begin{align*}
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\text{Cl} & \quad \text{O} \quad \text{C} \quad \text{O} \\
\text{Cl} & \quad \text{O} \quad \text{H} \\
\end{align*}
\]

U\textsuperscript{14}C- Phenyl-labeled 2,4-D

\[
\begin{align*}
\text{Cl} & \quad \text{O} \quad \text{Cl} \\
\text{Cl} & \quad \text{O} \quad \text{C} \quad \text{O} \\
\text{Cl} & \quad \text{O} \quad \text{H} \\
\end{align*}
\]
Attachment 3

Illustration of Test System
Figure 1. Schematic Diagram of Aerobic Aquatic Incubation Apparatus.