

## OBJECTIVE

The purpose of this study was to analyze field samples of soil/sediment for the presence of the test substance and specified metabolites. Soil/sediment samples from a small pond were analyzed for 2,4-D, 2,4-DCP, 2,4-DCA, 4-CP and 4-CPA.

## INTRODUCTION

This analytical report is organized into a main body and an appendix. The main body describes general information and procedures. Details on methodology, procedures, calculations, results and example chromatography are contained in the appendix.

## REFERENCE SUBSTANCES

The following analytical reference substances were used in this study.

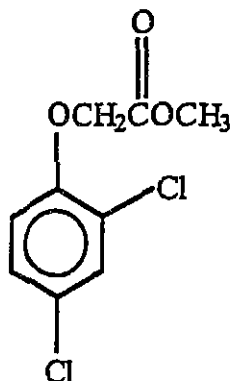
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Chemical Name:	2,4-Dichlorophenoxyacetic acid
Common Name:	2,4-D
Lot No.:	MORRIS/1710
Test Substance No.:	AGR275828
Expiration date:	2/12/99
Purity:	99.5%
Storage conditions:	Room temperature
Structure:	



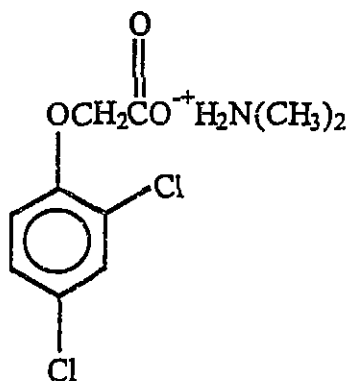
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Chemical Name: 2,4-Dichlorophenoxyacetic acid methyl ester  
Common Name: 2,4-D ME  
Lot No.: ACPR-24-143  
Test Substance No.: AGR235582  
Expiration date: 10/19/96  
Purity: 99.7%  
Storage conditions: Room temperature  
Structure:



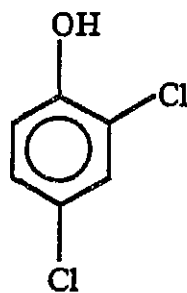
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Chemical Name: Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid  
Common Name: 2,4-D DMAS  
Lot No.: GHD2474-18  
Test Substance No.: AGR276899  
Expiration date: 5/2/97  
Purity: 100%  
Storage conditions: Room temperature  
Structure:



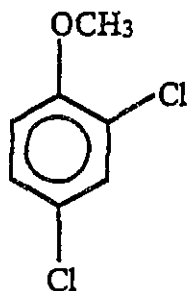
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Chemical Name: 2,4-Dichlorophenol  
Common Name: 2,4-DCP  
Lot No.: OCR 696-132-1  
Test Substance No.: AGR182992  
Expiration date: 8/31/95  
Purity: 99.6%  
Storage conditions: Room temperature  
Structure:



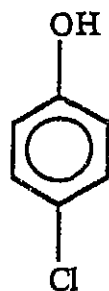
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Chemical Name: 2,4-Dichloroanisole  
Common Name: 2,4-DCA  
Lot No.: 37-46  
Test Substance No.: TSN100134  
Expiration date: 9/16/96  
Purity: >98%  
Storage conditions: Room temperature  
Structure:



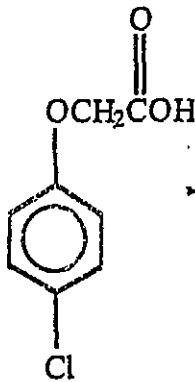
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Chemical Name: 4-Chlorophenol  
Common Name: 4-CP  
Lot No.: HZ 03530CX  
Test Substance No.: TSN100174  
Expiration date: 9/15/96  
Purity: >99%  
Storage conditions: Room temperature  
Structure:



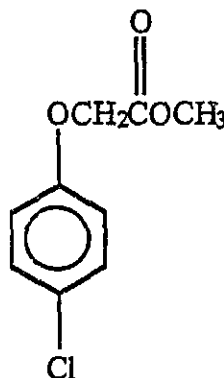
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Chemical Name: 4-Chlorophenoxyacetic acid  
Common Name: 4-CPA  
Lot No.: 089F002125  
Test Substance No.: TSN100163  
Expiration date: 9/15/96  
Purity: >99%  
Storage conditions: Room temperature  
Structure:



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Chemical Name: 4-Chlorophenoxyacetic acid methyl ester  
Common Name: 4-CPA ME  
Lot No.: 021H3778  
Test Substance No.: TSN100161  
Expiration date: 3/8/96  
Purity: 99%  
Storage conditions: Room temperature  
Structure:



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The Sponsor was responsible for the information concerning solubility, identity, uniformity, stability, and composition of the analytical reference substances. The Sponsor was responsible for reserve samples of the analytical reference substances.

## PROCEDURES

### Sample Identification

The sample numbers provided by the Sponsor were used to identify and track field samples. Only quality control samples (recoveries and reagent blanks) were assigned Hazleton numbers. These numbers were assigned sequentially. The prefix HAZ was used to clearly distinguish quality control samples from field samples.

### Sample Tracking

Soil/sediment field samples were received from the soil/sediment sample processing facility. Field samples were checked against a sample inventory list prepared from information provided by the Sponsor. Samples were grouped into boxes and assigned a unique box code. The box code, storage location, date received and date inventoried were recorded on the sample inventory list. The date of receipt and condition of the samples were recorded on the sample shipping forms (chain of custody documentation) which accompanied each box shipped to Hazleton. A box

transfer log was used to track when boxes were removed from their storage location for analysis and when they were returned to storage.

### Sample Storage

Storage conditions for field samples were as follows.

Matrix	Storage Condition
Soil/sediment	Frozen (-10° to -30°C)

The following deviations from the nominal storage conditions occurred during the storage period (receipt date of first sample to date of last injection). The storage period for this study was from 11/16/94 to 6/23/95. The duration is the maximum possible time samples were stored out of range. The temperature is the maximum deviation sustained on the given day.

<u>Probe</u>	<u>Date</u>	<u>Temperature (°C)</u>	<u>Duration (hr)</u>
124 (Freezer)	1/25/95	-9.0	4
	1/30/95	-3.9	16
	1/31/95	-3.5	16.5

### Interval Coding Scheme

The code consists of two parts. The first part tells how many days after application the sample was taken from the field. The second part tells which application is referred to.

Code: *nDAmA*

*nDA* = days after scheduled application  
*mA* = application number

#### *Examples*

<u>Code</u>	<u>Meaning</u>
-1DA1A	Within 24 hours prior to first application
0 DA2A	Within 24 hours following second application
1 DA1A	First day after first application
3 DA2A	Three days after second application

### Field Spiking Solutions

Preparation. Spiking solutions of 2,4-D DMAS, 2,4-DCP, 2,4-DCA, 4-CP and 4-CPA were prepared in methanol at an approximate concentration of 2 µg/mL. A calibrated Eppendorf

Repeat Pipetter (Model No. EPI) was used to deliver 1 mL of the appropriate spiking solution into 2 mL autosampling vials. The vials were capped with black phenolic Teflon® lined screw caps and stored under refrigeration (2° to 8°C) until shipped to the field. The caps were color coded to indicate which solution they were filled with. The following codes were used.

<u>Analyte</u>	<u>Color Code</u>	<u>Concentration (µg/mL)</u>
2,4-D DMAS (a)	Red	2.00
2,4-DCP	Black	1.99
2,4-DCA	White	1.98
4-CP	Yellow	2.00
4-CPA	Gray	2.00

(a) As 2,4-D acid equivalent.

**Verification.** The bulk spiking solutions were analyzed by gas chromatography with mass selective detection (GC/MSD) to verify the concentration of 2,4-D DMAS, 2,4-DCP, 2,4-DCA, 4-CP and 4-CPA. Results were as follows.

<u>Analyte</u>	<u>Percent Recovery</u>
2,4-D DMAS (a)	89.9
2,4-DCP	89.9
2,4-DCA	89.9
4-CP	101 (b)
4-CPA	90.0 (b)

(a) As 2,4-D acid equivalent.

(b) Mean of 3 analyses.

## SOIL/SEDIMENT METHOD SUMMARY

A 10 g sample of soil/sediment was vortexed and sonicated in 20 mL of 5% acetic acid in methanol, 20 mL of 50:50 5% acetic acid in methanol:5% acetic acid in deionized water and 20 mL of 5% acetic acid in deionized water for 20 minutes. The extracts were decanted, filtered after each extraction, combined, and brought to a known volume. An aliquot of extract (FRACTION A) was combined with hexane,  $\text{Na}_2\text{SO}_3$ , NaCl, 0.5 N NaOH (in that order exclusively) and 2,4-DCA partitioned to hexane (FRACTION B). FRACTION B was concentrated to 5 mL.

FRACTION A was acidified, and 2,4-D, 4-CPA, 2,4-DCP and 4-CP were partitioned to DCM (FRACTION C). The 2,4-D and 4-CPA were back-partitioned from FRACTION C to 0.25 N  $\text{NaHCO}_3$  (FRACTION D) and the 2,4-DCP and 4-CP were back partitioned from FRACTION C to 1.0 N NaOH (FRACTION E).

FRACTION D was acidified, saturated with salt, and 2,4-D and 4-CPA were partitioned twice to ether. The ether was evaporated to incipient dryness and the 2,4-D and 4-CPA derivatized with  $\text{BF}_3$  to 2,4-D ME and 4-CPA ME, respectively. The reactants were swamped with water and combined with FRACTION B. The aqueous layer was discarded. The hexane layer was concentrated to 1 mL.

FRACTION E was acidified, saturated with salt, and 2,4-DCP and 4-CP were partitioned twice to ether. The ether was concentrated to 5 mL combined with FRACTION D, and concentrated to 1 mL. A 2- $\mu\text{L}$  aliquot was injected on a gas chromatograph for quantitation by mass selective detection (MSD). The limit of quantitation for all analytes in this method was 0.01 ppm.



## SOIL/SEDIMENT ANALYSIS

2,4-D, 2,4-DCP, 2,4-DCA, 4-CP and 4-CPA were determined in soil/sediment samples from the 00-05 cm, 05-10 cm, 10-15 cm and 15-20 cm depths. All samples out to 180 DA2A were analyzed. Moisture content was determined on each soil/sediment assayed. Moisture was determined using Hazleton Standard Operating Procedure OP-AGC.9.

Samples were analyzed in sets of field samples, including one untreated control and two fortified controls. The recovery samples were randomly fortified at various residue levels. Results in ppm were reported as found on a wet weight basis, and calculated on a dry-weight basis based on the moisture value for each soil/sediment sample.

Field spike samples were analyzed for 2,4-D, 2,4-DCP, 2,4-DCA, 4-CP and 4-CPA. Moisture was not determined for soil/sediment from field spiked samples or recovery samples.

## SOIL/SEDIMENT CALCULATIONS

Terminology

The following is a definition of the terms used in the calculations and spreadsheets.

Term	Symbol	Definition
Sample ID	-	Code used to identify the sample
Sample Type	-	Whether the sample is an untreated control, recovery or residue sample
Interval	-	When the sample was collected
Depth	-	Soil/sediment core section from which the sample originated (cm or in.)
Final Volume	$V_f$	Final volume of the sample extract (mL)
Dilution Factor	$d$	Ratio of final volume of dilution to volume of aliquot
Sample Wet Weight	$W_w$	Weight of the soil/sediment sample before it was dried (g)
Percent Moisture	$M$	Percentage of water in the soil/sediment sample
Sample Dry Weight	$W_d$	Weight of the soil/sediment sample after it was dried (g)
Peak Area	$A$	Area of the analyte signal peak for the sample
$\mu\text{g/mL}$ Found	$F$	Concentration of the analyte in the sample extract ( $\mu\text{g/mL}$ )

ppm Added	$C_a$	Theoretical concentration of the analyte in the fortified control (ppm)
ppm Wet Weight	$C_w$	Concentration of the analyte in the wet soil/sediment sample (ppm)
ppm Dry Weight	$C_d$	Concentration of the analyte in the dry soil/sediment sample (ppm)
Percent Recovery	R	Ratio of measured to theoretical concentration of the analyte in the fortified control (%)
Flag	-	A flag that indicates when a dilution is required

#### Samples Used for Example Calculations

Example calculations are for 2,4-D. The following samples were used.

Sample ID	Set No.	Sample Type	Example No.
AA270351	8	Residue	1
HAZ021	8	Recovery	2

#### Formulas and Example Calculations

The following calculations were performed using Excel spreadsheet software. All calculations are rounded to the same number of figures used in the spreadsheets.

##### Percent Moisture.

$$M = \frac{C - D}{C - B} \times 100$$

Where:

- M = Percent moisture
- C = Container plus wet sample weight (g)
- B = Container weight (g)
- D = Container plus dry sample weight (g)

##### *Examples*

- |    |           |    |                |
|----|-----------|----|----------------|
| 1. | C = 12.26 | 2. | Not applicable |
|    | B = 2.26  |    |                |
|    | D = 7.31  |    |                |

$$M = \frac{12.26 - 7.31}{12.26 - 2.26} \times 100$$

$$M = 49.5$$

**Sample Dry Weight.**

$$W_d = \frac{100 - M}{100} \times W_w$$

Where:  $W_d$  = Sample dry weight (g)  
 $W_w$  = Sample wet weight (g)  
 $M$  = Percent moisture

*Examples*

- |    |                            |    |                |
|----|----------------------------|----|----------------|
| 1. | $W_w = 10.0$<br>$M = 49.5$ | 2. | Not applicable |
|----|----------------------------|----|----------------|

$$W_d = \frac{100 - 49.5}{100} \times 10.0$$

$$W_d = 5.05$$

 **$\mu\text{g/mL}$  Found.**

$$F = \frac{A - b}{m}$$

Where:  $F$  =  $\mu\text{g/mL}$  Found  
 $A$  = Peak area  
 $b$  = Intercept of the linear regression equation  
 $m$  = Slope of the linear regression equation

*Examples*

- |    |  |    |   |
|----|--|----|---|
| 1. | $A = 439500$<br>$b = -7090$<br>$m = 2370979$ | 2. | $A = 1046633$<br>$b = -7090$<br>$m = 2370979$ |
|----|--|----|---|

$$F = \frac{439500 - (-7090)}{2370979}$$

$$F = 0.1884$$

$$F = \frac{1046633 - (-7090)}{2370979}$$

$$F = 0.4444$$

## ppm Added.

$$C_a = \frac{V_s \times C_s}{W_w}$$

Where:  $C_s$  = ppm added  
 $V_s$  = Volume of standard added (mL)  
 $C_s$  = Concentration of standard ( $\mu\text{g/mL}$ )  
 $W_w$  = Wet sample weight (g)

## Examples

1. Not applicable

2.

$V_s = 1.00$

$C_s = 1.00$

$W_w = 10.0$

$$C_a = \frac{1.00 \times 1.00}{10.0}$$

$C_a = 0.100$

## ppm Wet Weight.

$$C_w = \frac{F \times V_f}{W_w} \times d \times m$$

Where:  $C_w$  = ppm wet weight  
 $F$  =  $\mu\text{g/mL}$  found  
 $V_f$  = Final volume (mL)  
 $W_w$  = Sample wet weight (g)  
 $d$  = Dilution factor  
 $m$  = Method factor

## Examples

1.  $F = 0.1884$ 

$V_f = 1.00$

$W_w = 10.0$

$d = 1$

$m = 2.000$

2.

$F = 0.4444$

$V_f = 1.00$

$W_w = 10.0$

$d = 1$

$m = 2.000$

$$C_v = \frac{0.1884 \times 1.00}{10.0} \times 1 \times 2.000$$

$$C_w = 0.038$$

$$C_w = \frac{0.4444 \times 1.00}{10.0} \times 1 \times 2.000$$

$$C_w = 0.089$$

**ppm Dry Weight.**

$$C_d = \frac{F \times V_f}{W_d} \times d \times m$$

Where:  $C_d$  = ppm dry weight  
 $F$  =  $\mu\text{g/mL}$  found  
 $V_f$  = Final volume (mL)  
 $W_d$  = Sample dry weight (g)  
 $d$  = Dilution factor  
 $m$  = Method factor

*Examples*

1.  $F = 0.1884$   
 $V_f = 1.00$   
 $W_d = 5.05$   
 $d = 1$   
 $m = 2.000$

2. Not applicable

$$C_d = \frac{0.1884 \times 1.00}{5.05} \times 1 \times 2.000$$

$$C_d = 0.075$$

**Percent Recovery.**

$$R = \frac{C_w}{C_a} \times 100$$

Where:  $R$  = Percent recovery  
 $C_w$  = ppm wet weight  
 $C_a$  = ppm added

*Examples*

1. Not applicable

2.  $C_w = 0.089$   
 $C_a = 0.100$

$$R = \frac{0.089}{0.100} \times 100$$

$$R = 89.0$$

**Corrected Recovery.**

In cases where an interference was found in the control, the recoveries for the set were corrected for the amount found in the control. This was done as follows.

$$R_c = \frac{C_{wr} - C_{wc}}{C_a} \times 100$$

Where:  $R_c$  = Corrected recovery (%)  
 $C_{wr}$  = ppm wet weight found in recovery  
 $C_{wc}$  = ppm wet weight found in control  
 $C_a$  = ppm added

*Example:*

Set 20, 20A, Sample HAZ057 (2,4-DCP).

$$C_{wr} = 0.354$$

$$C_{wc} = 0.012$$

$$C_a = 0.500$$

$$R_c = \frac{0.354 - 0.012}{0.500} \times 100$$

$$R_c = 68.4$$

**Dilution Factor.**

If a sample requires dilution because its peak area falls outside of the standard curve, the dilution factor is used to measure the amount of dilution. This factor is used subsequently in calculating the concentration of the analyte in the sample. If a dilution is not required, this factor is always 1. The dilution factor is defined as follows.

$$d = \frac{V_d}{V_a}$$

Where:  $d$  = Dilution factor  
 $V_d$  = Final volume of the dilution (mL)  
 $V_a$  = Volume of the aliquot used to make the dilution (mL)

Example: Set 4B, Sample AA270354, 2,4-D

$$V_d = 2.0$$

$$V_s = 0.2$$

$$d = \frac{2.0}{0.2}$$

$$d = 10$$