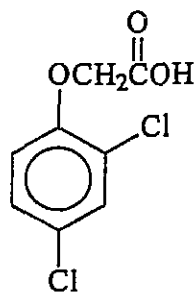## REFERENCE SUBSTANCES

The following analytical reference substances were used in this study.

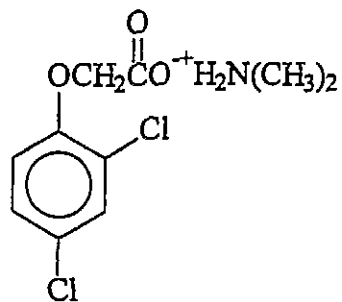---

Chemical Name: 2,4-Dichlorophenoxyacetic acid
Common Name: 2,4-D
Test Substance Number: AGR275828
Expiration Date: 2/12/99
Purity: 99.5%
Storage Conditions: Ambient
Structure:

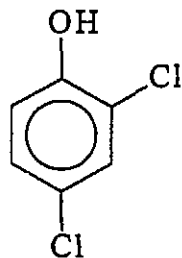---

Chemical Name: 2,4-Dichlorophenoxyacetic acid methyl ester
Common Name: 2,4-D ME
Test Substance Number: AGR235582
Expiration Date: 10/19/96
Purity: 99.7%
Storage Conditions: Ambient
Structure:
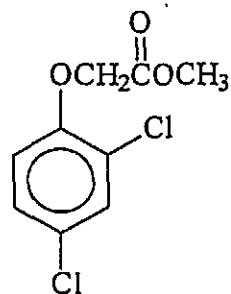
Chemical Name: Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid
Common Name: 2,4-D DMAS
Test Substance Number: TSN100485
Expiration Date: 6/29/96
Purity: >99%
Storage Conditions: Ambient
Structure:

Chemical Name: 2,4-Dichlorophenol
Common Name: 2,4-DCP
Test Substance Number: AGR182992
Expiration Date: 8/31/95
Purity: 99.6%
Storage Conditions: Ambient
Structure:

Chemical Name: 2,4-Dichloroanisole
Common Name: 2,4-DCA
Test Substance Number: TSN100134
Expiration Date: 9/16/96
Purity: >98%
Storage Conditions: Ambient
Structure:
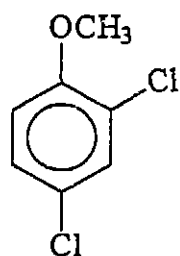
Chemical Name: 4-Chlorophenoxyacetic acid methyl ester
Common Name: 4-CPA ME
Test Substance Number: TSN100161
Expiration Date: 3/8/96
Purity: 99%
Storage Conditions: Refrigeration
Structure:

Chemical Name: 4-Chlorophenoxyacetic acid
Common Name: 4-CPA
Test Substance Number: TSN100096
Expiration Date: 9/15/96
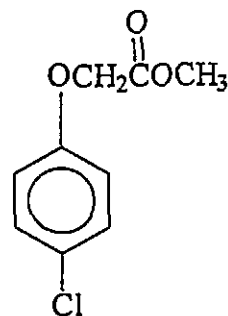Purity: >98%
Storage Conditions: Ambient
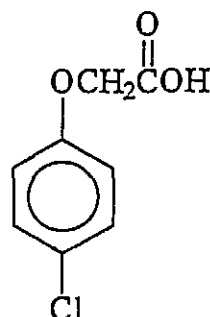Structure:

Chemical Name: 4-Chlorophenol
Common Name: 4-CP
Test Substance Number: TSN100174
Expiration Date: 9/15/96
Purity: >99%
Storage Conditions: Ambient
Structure:
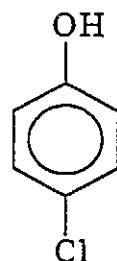
The Sponsor was responsible for the information concerning solubility, identity, uniformity, stability, and composition of the analytical reference substances. The Sponsor was responsible for reserve samples of the analytical reference substances.

PROCEDURES

Sample Identification

The sample numbers provided by the Sponsor were used to identify and track aquatic test-site samples. Only quality control samples (recoveries and reagent blanks) were identified by AGVISE. These samples were identified by the use of the sample number pertaining to the control matrix used for fortification in conjunction with the set number assigned.

Sample Tracking

Aquatic test-site samples and field spikes were received from the sample processing facility. Aquatic test-site samples were checked against a sample inventory list prepared from information provided by the Sponsor. The date of receipt and condition of the samples were recorded on the sample shipping forms (chain of custody documentation) which accompanied each box transferred to AGVISE's residue laboratory. These forms were returned to the originating facility. Internal freezer log sheets were used to record when samples were removed from freezers for analysis and when they were returned to storage.

Sample Storage

Storage conditions for aquatic test-site samples and field spikes were frozen (-10°C to -30°C).

Interval Coding Scheme

The code consists of two parts. The first part tells how many days after application the sample was taken from the aquatic test-site. The second part tells which application is referenced.

Code: nDAmA

> nDA = Scheduled days after application
> mA = application number

Examples

Code	Meaning
-1 DA1A	Immediately before first application
0 DA2A	Immediately after second application
1 DA1A	First day after first application
3 DA1A	Three days after first application
3 DA2A	Three days after second application

WATER METHOD SUMMARY

To analyze an aquatic sample for 2,4-D, 4-CPA, 2,4-DCA, 2,4-DCP, and 4-CP, residues of these analytes were extracted by a C18 solid phase extraction (SPE) cartridge. The eluants were separated into two fractions using two solvent systems. The first fraction, containing 2,4-DCA, 2,4-DCP, and 4-CP, was not derivitized. The second fraction, containing 2,4-D, and 4-CPA, was methylated using BF_3/methanol. The second fraction was then partitioned into hexane. The first fraction and the hexane solution were combined and analyzed by gas chromatography with mass selective detection. The method used has a limit of quantification of 0.001 ppm.

WATER METHOD VALIDATION

General Procedures

On-going validation of the method was performed to ensure quality results for all analysis sets. To validate each set, analytical reference substance was added in duplicate to control matrix in known concentrations and then analyzed. A control sample and reagent blank were also analyzed with each set. The method was considered acceptable if recoveries were within 70% to 120% and control values were less than 20% of the limit of quantification.

WATER ANALYSIS

2,4-DMAS, 4-CPA, 2,4-DCA, 2,4-DCP, and 4-CP were determined in 100 mL aliquots of 500 mL total volume aquatic samples in triplicate. Sampling continued through 180 days after the second application.

Samples were analyzed in sets of replicates with multiple sample points included in each set. Each set included one reagent blank, one control, and duplicate fortified controls. The

number of samples never exceeded sixteen such that a minimum of 10% of the samples in any set was made up of quality control fortifications.

Field spike samples were analyzed for 2,4-DMAS, 4-CPA, 2,4-DCA, 2,4-DCP, and 4-CP. Statistical treatment of the data was not performed.

WATER CALCULATIONS

Terminology:

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

Term	Symbol	Definition
ple ID	-	Code used to identify the sample
Sample Type	-	Whether the sample is a control, recovery or residue sample
Interval	-	When the sample was collected
Final Volume	V_f	Final volume of the sample extract (mL)
Sample Volume	V_s	Volume of sample (mL)
Dilution Factor	d	Ratio of final volume of dilution to volume of aliquot
Peak Area	A	Area of the analyte signal peak for the sample
µg/mL Found	F	Concentration of the analyte in the sample extract (µg/mL)
ppm Added	C_a	Theoretical concentration of the analyte in the fortified control (ppm)
ppm	C_{sa}	Concentration of the analyte in the aquatic sample (ppm)
Percent Recovery	R	Ratio of measured to theoretical concentration of the analyte in the fortified control (%)

Samples Used for Example Calculations

Example calculations are from Set 24D77 (-1, 0, and 1 DA1A, 14, 21, and 30 DA2A) and pertain to the 2,4-D analyte results only. The following samples were used.

Sample ID	Type of Sample	Example No.
AA271448	Residue	1
AA271047-F2	Recovery	2

Formulas and Example Calculations

The following calculations were performed using a combination of Fisons MassLab (version 1.12) and Crystal Reports (version 3.0.0.3) software.

µg/mL Found:

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

Where: F = µg/mL Found
           A = Peak area
           b = Intercept of the linear regression equation
           m = Slope of the linear regression equation

Examples

1. $A = 397364$
     $b = 14964.4137$
     $m = 1122002.0328$

     $$F = \frac{397364 - 14964.4137}{1122002.0328}$$

     $F = 0.34082$

2. $A = 243094$
     $b = 14964.4137$
     $m = 1122002.0328$

     $$F = \frac{243094 - 14964.4137}{1122002.0328}$$

     $F = 0.20332$

ppm Added:

$$C_a = \frac{V_a \, x \, C_s}{V_s}$$

Where: C_a = ppm Added
                      V_a = Volume of standard added (mL)
                      C_s = Concentration of standard (μg/mL)
                      V_s = Volume of sample (mL)

Examples

1.   Not applicable   2. V_a = 1.00
                                                                   C_s = 100
                                                                   V_s = 100

$$C_a = \frac{1.00 \, x \, 100}{100}$$

C_a = 1.0

ppm

$$C_{sa} = \frac{F \, x \, V_f}{V_s}$$

Where: C_{sa} = ppm
                      F = μg/mL found
                      V_f = Final volume (mL)
                      V_s = Sample volume (mL)

Examples

1.   F = 0.34082   2.   F = 0.20332
       V_f = 1000 V_r = 500
       V_s = 100 V_s = 100

$$C_{sa} = \frac{0.34082 \, x \, 1000}{100}$$ $$C_{sa} = \frac{0.20332 \, x \, 500}{100}$$

C_{sa} = 3.41 C_{sa} = 1.02

Percent Recovery:

$$R = \frac{C_{sa}}{C_a} x\,100$$

Where: R = Percent recovery
           C_{sa}= ppm
           C_a= ppm added

Examples

1.   Not applicable   2. C_{sa} = 1.02
                                                      C_a = 1.0

$$R = \frac{1.02}{1.0}\,x\,100$$

$$R = 102$$