

**SUMMARY**

Dichlobenil and its degradate, 2,6-dichlorobenzamide (BAM), were solvent extracted from surface water and analyzed separately. The extracts were each analyzed by GC using an electron capture detector.

**A) MATERIALS**

**A.1 Equipment**

Bond Elute C-18 SPE cartridge, Analytichem International

Baker-10 SPE extraction system

rotary vacuum evaporator

flatbottom flask, 250 mL

Erlenmeyer flask, 250 mL

microsyringe, 10  $\mu$ L

chromatography column, 70 mm X 10 mm

graduated cylinders, 100 mL, 250 mL

pipets, 2 mL, 10 mL, 25 mL

Pasteur pipet

graduated culture tube, 10 mL

separatory funnel, 500 mL

powder funnel

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### A.2 Reagents

alumina, 2 % deactivated	J. T. Baker
acetone, HPLC grade ✓	J. T. Baker
petroleum ether, 40 – 65 °C ✓	spectrograde
ethyl acetate ✓	J. T. Baker
ethanol, PHOTREX	J. T. Baker
deionized water	
sodium sulfate, anhydrous	AR

### A.3 Analytical Standards

Standard reference materials, dichlobenil and 2,6-dichlorobenzamide listed below, can be obtained from Uniroyal Chemical Company, Inc. Standards should be stored at -5 °C to -25 °C until use.

<u>Compound</u>	<u>Lot No.</u>	<u>Purity</u>
dichlobenil (2,6- dichlorobenzonitrile)	ARS82I01N	>99.5 %
2,6-dichlorobenzamide (BAM)	ARS81C25N	>99.0 %

MSDS sheets for the above standards and reagents are found in Appendix I.

## B. SAFETY AND HEALTH

This method should be performed by trained chemical personnel. Hazards associated with the chemicals used in this analytical method are shown in the MSDS sheets in Appendix I.

**C. ANALYTICAL METHOD**

**C.1a Principle of the Method – Dichlobenil**

A 10 mL water sample is passed through a C-18 solid phase extraction (SPE) cartridge, and eluted with acetone. The acetone extract is diluted with petroleum ether, and the water layer removed. The extract is analyzed for dichlobenil using packed column gas chromatography (GC) with electron capture detection.

**C.1b Principle of the Method – 2,6-Dichlorobenzamide**

A 100 mL water sample is solvent extracted with ethyl acetate. The ethyl acetate extract is dried over anhydrous sodium sulfate, and solvent exchanged into acetone / petroleum ether. The solvent exchanged extract is cleaned up over an alumina column, and eluted with ethanol / petroleum ether. The eluate is then solvent exchanged back into ethyl acetate, and analyzed for 2,6-dichlorobenzamide using packed column gas chromatography (GC) with electron capture detection.

**C.2 Types of Water Samples**

This method is predicted to be applicable to most waters. In Uniroyal Chemical Company, Inc. project 96150, pond water from a Florida location was used. The chemical characteristics of the water were as follows:

alkalinity.....	112 ppm CaCO <sub>3</sub>
total hardness .....	112 ppm CaCO <sub>3</sub>
calcium hardness.....	108 ppm CaCO <sub>3</sub>

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turbidity..... 2.75 ft optical resolution  
conductivity..... 125  $\mu$ Mhos  
pH .....8.2

This method was also successfully applied (Uniroyal Chemical Company, Inc. project 96151) to pond water from an Oregon location. The chemical characteristics of this water were as follows:

alkalinity..... 10.2 ppm  $\text{CaCO}_3$   
total hardness ..... 22.0 ppm  $\text{CaCO}_3$   
specific conductance ..... 3.8  $\mu$ Mhos/cm  
pH .....7.1  
turbidity.....3.0 NTU

### C.3 Sample Processing

No processing is required for water samples. Frozen samples are thawed at room temperature, and a representative subsample is removed for analysis.

#### C.4a Extraction Method - Dichlobenil

An Analytichem International Bond Elute C-18 cartridge was prepared by washing successively with 5 mL of acetone and 5 mL of deionized water utilizing a Baker Extraction System. A thoroughly mixed 10 mL subsample of the water to be analyzed was pipeted onto the prepared cartridge. Vacuum was applied to the system, and the sample was drained through the cartridge.

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Dichlobenil residues were eluted with 2 mL of acetone into a 10-mL graduated culture tube containing approximately 7 mL of petroleum ether. The culture tube was capped, shaken vigorously for about 30 seconds and allowed to settle. The aqueous layer (approximately 0.5 mL) was removed with a pasteur pipet and discarded, and the ether layer was brought to a 10 mL volume with additional petroleum ether. The sample was diluted if necessary and submitted for GC analysis.

#### **C.4b Extraction Method – 2,6-Dichlorobenzamide**

A representative sample was mixed thoroughly, and a 100 mL subsample for 2,6-dichlorobenzamide determination was measured using a 100-mL graduated cylinder. The sample was transferred to a 500-mL separatory funnel, and extracted with 100 mL of ethyl acetate. The layers were allowed to separate, and the aqueous layer was drained into an Erlenmeyer flask. The ethyl acetate layer was filtered through a powder funnel lined with fluted filter paper containing anhydrous sodium sulfate into a 250-mL graduated mixing cylinder. The aqueous layer was transferred back into the separatory funnel and extracted twice with 50 mL of ethyl acetate. After each partitioning, the ethyl acetate layer was filtered through the sodium sulfate and combined with the previous ethyl acetate extracts. The final volume of the combined extract was brought to 250 mL with additional ethyl acetate. The extract is "Solution A".

The 2 % (w/w) deactivated alumina necessary for the clean up of "Solution A" is prepared as follows: heat the alumina in an oven at 500 °C for 5 hours, allow to cool in a desiccator, pour into a bottle, add 2 % w/w of deionized water using a pipet, shake vigorously and then allow to stand for 24 hours. Keep the hygroscopic powder in the closed bottle.

A 70 mm X 10 mm column was prepared to contain 6 cm of 2 % (w/w) deactivated alumina topped with one-half inch of anhydrous sodium sulfate. The column was washed with 100 mL of petroleum ether, which was drained to the top of the sodium sulfate. Twenty-five milliliters of "Solution A" was pipetted into a 250 mL flatbottom flask and rotary vacuum evaporated (room temperature water bath) to dryness. The dried residue was dissolved in 2 mL of acetone, and 18 mL of petroleum ether was added to the sample flask. The sample was transferred to the prepared column, and drained to the top of the sodium sulfate. The column was washed with an additional 15 mL of acetone/petroleum ether (1 + 9) followed by 8 mL of ethanol/petroleum ether (1 + 19), both of which were drained to the sodium sulfate layer and discarded. The 2,6-dichlorobenzamide residues were eluted from the column with 30 mL of ethanol/petroleum ether (1 + 19) and collected in a 250 mL flatbottom flask. The sample was rotary vacuum evaporated (room temperature water bath) to dryness, quantitatively transferred to a calibrated culture tube using ethyl acetate and brought to a volume of 5 to 10 mL. The sample was diluted if necessary and submitted for GC analysis.

#### **C.5a Gas Chromatography Method - Dichlobenil**

The type of column packing used in the GC determination of dichlobenil is a 3 % OV-225 on 80/100 mesh Chromosorb W-HP, 3' X 2 mm ID. The samples (2 – 6 µL) are delivered to the column by direct injection. The injector temperature is 220 °C. The column temperature is isothermal at 140 °C. The detector temperature is 330 °C. The nitrogen carrier gas flow rate is 14 mL/min, and the nitrogen makeup gas flow rate is 30 mL/min.

The GC analysis of dichlobenil begins with the injection of a 2  $\mu$ L aliquot of each of six single-component dichlobenil calibration standards at concentrations of 1 ng/mL, 3 ng/mL, 5 ng/mL, 10 ng/mL, 30 ng/mL, and 50 ng/mL. The resulting standard peak heights are plotted versus concentration (ng/mL) of the corresponding standard to obtain a standard calibration curve by linear regression. Standard curves are generated for each analysis day, using all six standards.

Control water samples fortified to levels of 0.01 ppm and 0.10 ppm (by the addition of 40  $\mu$ L of 25  $\mu$ g/mL and 100  $\mu$ L of 100  $\mu$ g/mL fortification standards, respectively) are prepared immediately prior to extraction and run concurrently with the field samples to monitor overall method accuracy (recovery) and precision (standard deviation).

A 2-6  $\mu$ L aliquot of the prepared control or field sample is injected into the GC. If the compound peak height in the field sample is greater than the peak height of the highest standard, the sample extract is diluted with petroleum ether until the signal response falls within the standard curve range. The peak heights of the compounds are recorded and the concentration of each compound is determined relative to the standard curve generated for that day using the equation detailed in section I of this report.

#### **C.5b Gas Chromatography Method – 2,6-Dichlorobenzamide**

The type of column packing used in the GC determination of 2,6-dichlorobenzamide is a 3 % OV-225 on 80/100 mesh Chromosorb W-HP, 3' X 2 mm ID. The samples (2 – 6  $\mu$ L) are delivered to the column by direct injection. The injector temperature is 270 °C. The column temperature is ramped from 190

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to 200 °C for 7 minutes, then to 240 °C for 5 minutes. The detector temperature is 330 °C. The nitrogen carrier gas flow rate is 10 to 14 mL/min, and the nitrogen makeup gas flow rate is 11 to 26 mL/min.

The GC analysis of 2,6-dichlorobenzamide begins with the injection of a 2 µL aliquot of each of six single-component 2,6-dichlorobenzamide calibration standards at concentrations of 1 ng/mL, 3 ng/mL, 5 ng/mL, 10 ng/mL, 30 ng/mL, and 50 ng/mL. The resulting standard peak heights are plotted versus concentration (ng/mL) of the corresponding standard to obtain a standard calibration curve by linear regression. Standard curves are generated for each analysis day, using all six standards.

Control water samples (100 mL) fortified to levels of 0.01 ppm and 0.10 ppm (by the addition of 40 µL of 25 µg/mL and 100 µL of 100 µg/mL fortification standards, respectively) are prepared immediately prior to extraction and run concurrently with the field samples to monitor overall method accuracy (recovery) and precision (standard deviation).

A 2-6 µL aliquot of the prepared control or field sample is injected into the GC. If the compound peak height in the field sample is greater than the peak height of the highest standard, the sample extract is diluted with ethyl acetate until the signal response falls within the standard curve range. The peak heights of the compounds are recorded and the concentration of each compound is determined relative to the standard curve generated for that day using the equation detailed in section I of this report.



### C.6 Preparation of Spiking and Standard Solutions

Analytical standards of dichlobenil and 2,6-dichlorobenzamide are used to prepare individual compound stock solutions from which calibration standards and fortification standards are prepared. Stock solutions of each compound, at a concentration of 1.0 mg/mL, are made by weighing out 50 mg of the analytical standard on an analytical balance and dissolving it in 50 mL of petroleum ether (for dichlobenil) or ethyl acetate (for 2,6-dichlorobenzamide). Serial dilutions of the stock solutions (in the same solvents) are made by diluting 10 mL of the stock into 100 mL of solvent to give 100  $\mu\text{L/mL}$ , diluting 1.0 mL of the 100  $\mu\text{L/mL}$  solution into 100 mL of solvent to give 1.0  $\mu\text{L/mL}$ , and diluting 10 mL of the 1.0  $\mu\text{L/mL}$  solution into 100 mL of solvent to give 0.1  $\mu\text{L/mL}$ . These stock solutions and dilutions of stock solutions are used to make calibration standards and fortification standards as follows.

Individual calibration standards of dichlobenil (at concentrations of 10 ng/mL, 30 ng/mL, and 50 ng/mL) are made by diluting 1.0 mL, 3.0 mL, and 5.0 mL each of the 1.0  $\mu\text{L/mL}$  dichlobenil solution above into 100 mL of petroleum ether to give the 10 ng/mL, 30 ng/mL, and 50 ng/mL calibration standards, respectively. The 1.0 ng/mL, 3.0 ng/mL, and 5.0 ng/mL dichlobenil calibration standards are made by diluting 1.0 mL, 3.0 mL, and 5.0 mL each of the 0.1  $\mu\text{L/mL}$  dichlobenil solution above into 100 mL of petroleum ether to give the 1.0 ng/mL, 3.0 ng/mL, and 5.0 ng/mL calibration standards, respectively. An analogous series of calibration standards for 2,6-dichlorobenzamide are made by making the above dilutions in ethyl acetate rather than petroleum ether.

Individual fortification standards of dichlobenil and 2,6-dichlorobenzamide are made from the stock solutions described above. A 100  $\mu\text{g/mL}$  fortification

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standard of dichlobenil is made by diluting 10 mL of the dichlobenil stock solution at 1.0 mg/mL into 100 mL of acetone. A 25 µg/mL fortification standard of dichlobenil is made by diluting 2.5 mL of the dichlobenil stock solution at 1.0 mg/mL into 100 mL of acetone. An analogous set of fortification standards for 2,6-dichlorobenzamide are made by making the above dilutions in ethyl acetate rather than acetone.

### C.7 Field Spike Stability

One-hundred milliliter samples of bulk control water were fortified at the test location on March 21, 1989. Fortification levels were 0.05 ppm for both dichlobenil and 2,6-dichlorobenzamide. Results for ppm found and spike percent recovery are shown in Tables 47 and 48 of Uniroyal Project 96150 (Tables I and II of this report) for dichlobenil and 2,6-dichlorobenzamide, respectively. Analysis of triplicate dichlobenil field spikes resulted in an average spike recovery of 97 % and a corrected ppm level of 0.049 (Table I), and analysis of triplicate 2,6-dichlorobenzamide field spikes resulted in an average spike recovery of 88 % and a corrected ppm level of 0.051 (Table II). These results indicate that the analytes did not undergo significant breakdown under the conditions of handling and shipping. Examples of chromatograms and data calculation spreadsheets for the field spikes are presented in Appendix II.

### C.8 Fortifications

Recoveries of fortified water samples analyzed concurrently with field samples for dichlobenil and 2,6-dichlorobenzamide are reported in Tables 12 and 13 of Uniroyal Project 96150 (Tables III and IV of this report), respectively. The overall average percent recovery and the standard deviation for each fortification level are also included. Control water samples were fortified to levels of 0.01

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ppm, 0.05 ppm, and 0.10 ppm for both dichlobenil and 2,6-dichlorobenzamide.

Overall average percent recovery for dichlobenil was 97.0 %  $\pm$  8.0 % while for 2,6-dichlorobenzamide the overall average percent recovery was 87.0 %  $\pm$  11.0 %.

The laboratory spike results indicate that the analytical methodology provided reliable results during the course of study 96150. An example of chromatograms of control samples and spiked control samples are shown in Appendix II (taken from study 96150).

#### D. Instrumentation

Sample analyses were done on a Varian 3700 gas chromatograph equipped with an electron capture detector. Data for residue quantitation was acquired via a Beckman interfaced to a Hewlett-Packard 1000 mini-computer. Other parameters were as follows:

##### for dichlobenil analysis

Column:	3 % OV-225 on 80/100 mesh Chromosorb W-HP, 3' x 2 mm ID
Nitrogen, Carrier Gas:	14 mL/minute
Nitrogen, Makeup Gas:	30 mL/minute
Detector Temperature:	330 °C
Injector Temperature:	220 °C
Column Temperature:	140 °C isothermal
Electrometer Range:	10 <sup>-11</sup>

##### for 2,6-dichlorobenzamide analysis

Column:	3 % OV-225 on 80/100 mesh Chromosorb W-HP, 3' x 2 mm ID
Nitrogen, Carrier Gas:	10 - 14 mL/minute
Nitrogen, Makeup Gas:	11 - 26 mL/minute

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Detector Temperature: 330 °C  
Injector Temperature: 270 °C  
Column Temperature: 190 – 200 °C for 7 min., ramp to 240 °C for 5 min.  
Electrometer Range:  $10^{-11}$  to  $10^{-12}$

**E. Sample Bracketing**

Calibration was done by standard bracketing. A typical run involved analyses of two calibration standards (1 ng/mL and 3 ng/mL, respectively), followed by a control water and two control spikes (at 0.01 ppm 0.10 ppm, respectively), then a 5 ng/mL calibration standard, three samples, a 10 ng/mL calibration standard, three more samples, a 30 ng/mL calibration standard, two more samples, and two final calibration standards (50 ng/mL and 10 ng/mL, respectively). Data from a typical run are shown in Appendix II.

**F. Potential Interferences**

This method could have interferences from compounds that elute with similar retention times as the analytes and are capable of efficiently capturing electrons (e.g. halogenated compounds). One should consider the sample history in this respect, and a confirmatory technique such as GC/MS should be used if a problem is suspected.

**G. Time Required for Analysis**

The extraction of eight water samples, two spiked control waters, and one unspiked control water takes one (for dichlobenil analysis) to two (for 2,6-dichlorobenzamide analysis) days. The chromatography to develop the daily standard curve and run the eight samples, two control spikes, and one unspiked control can be done in 24 hours.

**H. Modification or Potential Problems**

None.

**I. Data Acquisition and Calculations**

The Computer Automated Laboratory System (CALC) allows for data acquisition, data analysis, results reporting, and information management. Peak heights were measured using the CALC system. Concentrations of each compound were then calculated using the CALC system pre-programmed with a standard curve of nanograms/milliliter (ng/mL) of each compound versus peak height.

The ng/mL values were then converted to parts per billion (ppb) in the CALC program by entering the equivalent sample weight in grams loaded onto the cleanup column (C-18 and alumina) and the final dilution volume in milliliters.

$$\text{found ppb residue} = \frac{\text{ng/mL detected} \times \text{final volume (mL)}}{\text{equivalent sample weight (g)}}$$

final volume = dilution volume of the equivalent weight sample

Concentrations of the individual residues, expressed in ppm, were then entered into LOTUS spreadsheets.

Recoveries from fortified samples were determined by the formula:

$$\% \text{ recovery} = \frac{\text{ppm residue} - \text{ppm residue in the control}}{\text{ppm residue added}} \times 100$$

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Residues found in the treated water samples were corrected for procedural recoveries by the following calculation:

$$\text{Corrected ppm} = \frac{A}{B}$$

Where A = found ppm residue

B = average procedural recovery for the QC fortifications performed during the authentic sample analysis for the compound of interest

Uncorrected ("raw") residue values measuring less than the minimum quantifiable limit (0.01 ppm) were not corrected for procedural recovery, these residue values were recorded as "< 0.01 ppm" on the LOTUS spreadsheets.