I. OBJECTIVE / PURPOSE OF THE STUDY

The purpose of this study is to describe an analytical method for the analysis of dichlobenil and its metabolite 2,6-dichlorobenzamide in water.

II. SUMMARY / PRINCIPLE OF THE METHOD

A one liter water sample is passed through a divinylbenzene (DVB) Speedisk™ solid phase extraction disk under vacuum. The dichlobenil and its metabolite, 2,6-dichlorobenzamide (BAM), which are retained on the disk, are eluted with methylene chloride and gently concentrated under nitrogen with 1-heptanol as a keeper. For quantitation purposes, an internal standard, 2,4,6-trichlorobenzonitrile, is added to the concentrated sample. Analysis is by GC/MS in the selected ion monitoring (SIM) mode. The limit of quantitation (LOQ) for the method is 0.100 µg/L (ppb). A schematic summary of the analytical method is shown in Figure 1.

III. MATERIALS

A. EQUIPMENT

1. Analytical Evaporator, Model 111 "N-Evap", Organomation Associates, Inc. The evaporator is connected to a house nitrogen line fitted with a filter and a pressure regulator, Balston Filter Products.
2. Balance, analytical, Model 1602MP, Sartorius
3. Vortex mixer, Fisher Scientific
4. Vacuum manifold, Visiprep™ -DL with disposable liners, Supelco
5. Adjustable vacuum line
6. Water purification system, Model Milli-Q™, Millipore
7. Refrigerator / freezer, Model GE TDX 15 SNSBRAD, Fisher Scientific

Note: Equivalent equipment from other sources can be employed.

B. SUPPLIES

1. 1 mL and 2 mL graduated serological pipettes, Fisher Scientific
2. Customary analytical laboratory glassware and supplies:

100 mL beakers, 10 mL and 1.0 L volumetric flasks, 25 mL graduated cylinders, 9" Pasteur pipettes, 100 µL graduated syringes, 15 mL graduated glass centrifuge tubes, 1 mL and 10 mL grade A pipettes.

3. Vacuum flasks, 1.0 L, with rubber adapters, Fisher Scientific
4. 1.5 mL autosampler vials with septum caps, Fisher Scientific
5. DVB, 50 mm, solid phase extraction disks and reservoirs, Bakerbond Speedisk™, J.T. Baker

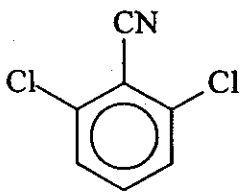
Note: Equivalent supplies from other sources can be employed. It is recommended, however, that SPE disks from J.T. Baker be used.

C. SOLVENTS AND REAGENTS

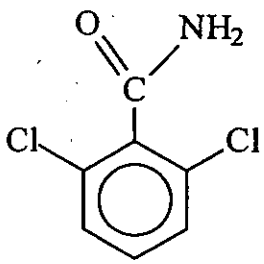
1. Methanol, Optima™ residue grade, Fisher Scientific
2. Acetone, Optima™ residue grade, Fisher Scientific
3. Methylene chloride, Optima™ residue grade, Fisher Scientific
4. n-Propanol, HPLC grade, Baxter Scientific
5. 1-heptanol, 98 % purity, Aldrich
6. 2,4,6-Trichlorobenzonitrile, 97 % purity, Lancaster Synthesis
7. Water, processed through Milli-Q™ purification system

Note: Solvents and reagents of equivalent purity from other sources can be used.

D. TEST SUBSTANCES



Chemical name: 2,6-dichlorobenzonitrile
Common name: Dichlobenil or Casoron
CAS number: 1194-65-6
Uniroyal Chemical Company Inc. Analytical Standard Code: C-01
Uniroyal Chemical Company Inc. Analytical Standard Lot No.: ARS-9108BA
Chemical Purity: 100 %
Storage Conditions: Freezer
Re-evaluation Date (for stability): 3/2001



Chemical Name: 2,6-dichlorobenzamide
Common Name: None (referred to as BAM in this report)
CAS number: 2008-58-4
Uniroyal Chemical Company Inc. Analytical Standard Code: C-03
Uniroyal Chemical Company Inc. Analytical Standard Lot No.: ARS-81C25N
Chemical Purity: 99.7%
Storage Conditions: Freezer
Re-evaluation Date (for stability): 3/2001

Test substances were stable over the duration of this study as evidenced by the consistency of analytical results during the course of investigation. Standards are archived by the Analytical Services Group of the Crop Protection Division, Uniroyal Chemical Company, Inc., Middlebury, CT.

E. GC/MS INSTRUMENTATION

Gas chromatograph: Model HP 5890A equipped with a HP Model 5971A mass selective detector and an autosampler-injector, Model HP 6890. Instrument parameters and data capture are controlled by HP Chemstation Software G-1701AA, Rev.A.03.00.

Column: RTX-200, 30 m X 0.25 mm X 0.5 µm film, Restek Corporation
Carrier Gas: Helium

F. SAFETY AND HEALTH

This method should be performed by trained chemical personnel only. Hazards associated with the solvents and reagents used are found in their respective Material Safety Data Sheets which can be obtained from the supplier of the chemical, and should be consulted prior to performing any analytical procedures. Material Safety Data Sheets for the test substances are provided in Appendix A.

IV. ANALYTICAL METHOD

A. WATER SOURCE AND CHARACTERIZATION

This method is expected to be applicable for water from various sources (surface, ground, and drinking waters). A 40 L grab sample of surface water from Preston Hill Pond, Middlebury, CT was collected by bucket from the edge of the pond for use in this study. The water sample was identified with the test system, study number, unique sample identification name, nature of the study, date of collection, and initials of the researcher.

Chemical properties of the pond water were determined by Agvise Laboratories, Inc. Northwood, ND from a 1 L subsample of the grab sample described above. Total organic carbon was 5.3 mg/L, total suspended solids was 2 mg/L, and total dissolved solids was 240 mg/L; these and other data are shown in Appendix B.

B. PREPARATION OF THE STANDARD SOLUTIONS

Note: All stock solutions should be stored in a freezer when not in use.

B-1a. Dichlobenil + BAM Stock Solution

On an analytical balance, accurately weigh 10.0 mg of dichlobenil standard and 10.0 mg of BAM standard into a single 100 mL volumetric flask, and dilute to volume with methanol. Make sure the standards are completely dissolved. The concentration of the stock solution is 100 mg/L each of dichlobenil and BAM. If necessary, correct the concentrations for the purity of the standards: mg/L X (percent purity/100).

B-1b. 2,4,6-Trichlorobenzonitrile Stock Solution

On an analytical balance, accurately weigh 10.0 mg of 2,4,6-trichlorobenzonitrile standard into a 100 mL volumetric flask, and dilute to volume with methanol. Make sure the standard is completely dissolved. The concentration of the stock solution is 100 mg/L. If necessary, correct the concentration for the purity of the standard: mg/L X (percent purity/100).

B-2a. Dichlobenil + BAM Working Solution, 10.0 µg/mL

Pipette 10 mL of dichlobenil + BAM stock solution B-1a into a 100 mL volumetric flask and dilute to volume with methanol. The concentration of the solution is 10.0 µg/mL.

B-2b. Dichlobenil + BAM Working Solution, 1.00 µg/mL

Pipette 10 mL of dichlobenil + BAM working solution B-2a into a 100 mL volumetric flask and dilute to volume with methanol. The concentration of the solution is 1.00 µg/mL.

B-3a. 2,4,6-Trichlorobenzonitrile Working Solution, 10.0 µg/mL

Pipette 10 mL of 2,4,6-trichlorobenzonitrile stock solution B-1b into a 100 mL volumetric flask and dilute to volume with methanol. The concentration of the solution is 10.0 µg/mL.

B-3b. 2,4,6-Trichlorobenzonitrile Working Solution, 1.00 µg/mL

Pipette 10 mL of 2,4,6-trichlorobenzonitrile working solution B-3a into a 100 mL volumetric flask and dilute to volume with methanol. The concentration of the solution is 1.00 µg/mL.

B-4a. Calibration Plot for Dichlobenil and BAM in the 1.00 ppb Range

Into five 10 mL volumetric flasks, pipette 1.50, 1.25, 1.00, 0.75, and 0.50 mL of 10.0 µg/mL dichlobenil + BAM working solution B-2a. Into each of the same five flasks, pipette 1.00 mL of 10.0 µg/mL 2,4,6-trichlorobenzonitrile working solution B-3a. Dilute to volume with methylene choride. The concentrations of the resulting standard solutions are 1.50, 1.25, 1.00, 0.75, and 0.50 µg/mL for dichlobenil and BAM, and 1.00 µg/mL for 2,4,6-trichlorobenzonitrile.

B-4b. Calibration Plot for Dichlobenil and BAM in the 0.100 ppb Range

Into five 10 mL volumetric flasks, pipette 1.50, 1.25, 1.00, 0.75, and 0.50 mL of 1.00 µg/mL dichlobenil + BAM working solution B-2b. Into each of the same five flasks, pipette 1.00 mL of 1 µg/mL 2,4,6-trichlorobenzonitrile working solution B-3b. Dilute to volume with methylene choride. The concentrations of the resulting standard solutions are 0.150, 0.125, 0.100, 0.075, and 0.050 µg/mL for dichlobenil and BAM, and 0.100 µg/mL for 2,4,6-trichlorobenzonitrile.

C. FORTIFICATION OF WATER SAMPLES

C-1 Fortification with Dichlobenil and BAM at the 1.00 ppb Level

Measure one liter of pond water using a 1 L graduated cylinder. Using a 100 µL syringe, add 100 µL of 10.0 µg/mL dichlobenil + BAM working solution B-2a. Cap the cylinder and mix well by shaking.

C-2 Fortification with Dichlobenil and BAM at the 0.100 ppb Level

Measure one liter of pond water using a 1 L graduated cylinder. Using a 100 µL syringe, add 100 µL of 1.00 µg/mL dichlobenil + BAM working solution B-2b. Cap the cylinder and mix well by shaking.

D. SAMPLE EXTRACTION PROCEDURE

1. Place a DVB Speedisk™ with reservoir onto a vacuum manifold attached to an adjustable vacuum source. Place a beaker, or other waste solvent receptacle, under the disk. Pass 15 mL of acetone through the disk under vacuum and continue to pull the vacuum until the disk is dry, repeat with 15 mL of n-propanol followed by 15 mL of methanol.
2. Condition the disk with 10 mL of methanol. Allow the methanol to flow through slowly by gravity. Do not allow the disk to dry.
3. When 3-5 mL of methanol remains on the disk, add 15 mL of Milli-Q™ water. Allow the water to flow through slowly by gravity. Do not allow the disk to

dry. When 3-5 mL of water remains on the disk, add an additional 10 mL of water and allow to flow through by gravity until about 5 mL of water remains.

4. Transfer the disk (with water still covering it) to a 1 L vacuum flask fitted with a rubber adapter and attached to an adjustable vacuum source. Without letting the disk go dry, begin adding the 1 L water sample, and adjust vacuum to achieve a flow rate of about 80 mL/minute. Process the entire water sample without letting the disk go dry.
5. After the sample has passed through the disk, remove the reservoir and leave the disk on full vacuum for about 3 minutes in order to completely dry the disk. Then release the vacuum.
6. Transfer the disk to a vacuum manifold attached to an adjustable vacuum source. Place a 15 mL graduated glass centrifuge tube under the port. Add a total of 15 mL methylene chloride to the disk in three aliquots of about 5 mL each. Allow the methylene chloride to elute by gravity. Apply slight vacuum to elute any remaining analyte.

Note: Multiple disks and samples can be processed simultaneously, but care should be taken to assure that conditioned disks do not go dry at any point during processing.

E. SAMPLE CONCENTRATION PROCEDURE

1. Add 160 µL of 1-heptanol as a keeper solvent to the top of each sample. Gently swirl the samples to get some mixing of the keeper with sample.
2. Concentrate the samples under a very gentle stream of nitrogen in a room temperature water bath. Dichlobenil is quite volatile, so care must be taken to avoid excessive agitation of the sample by the nitrogen stream; if a depression is observed on the surface of the sample, the flow of nitrogen is too high.
3. Concentrate the samples to 1300-1400 µL each. For samples in the 1 ppb range, add exactly 100 µL of 10.0 µg/mL 2,4,6-trichlorobenzonitrile working solution B-3a. For samples in the 0.1 ppb range, add exactly 100 µL of 1.00 µg/mL 2,4,6-trichlorobenzonitrile working solution B-3b as an internal quantitation standard.
4. Vortex each sample to obtain a homogeneous mixture, and transfer with a Pasteur pipette to an autosampler vial for GC/MS analysis.

F. GC/MS ANALYSIS PROCEDURE

F-1 Method of Analysis

Identification and quantitation of dichlobenil and BAM are by electron impact GC/MS in the selected ion monitoring mode. Identification is by comparison of the GC retention times and mass spectra (ratio of ions monitored) of the peaks in sample extracts with those of authentic dichlobenil and BAM standards. Quantitation is done by comparison of signal intensity (area counts) for the analyte quantitation ion against signal intensity (area counts) for the internal

standard quantitation ion. The ratio of analyte area counts to internal standard area counts is calculated for each sample, and this ratio is compared to the analogous ratio on a five-point calibration curve to determine the analyte concentration.

F-2 Calibration Procedure

To generate a calibration curve, ratios of analyte/internal standard area counts are determined in a set of five calibration solutions covering a range of analyte concentrations with internal standard concentrations remaining constant (see sections IV B-4a and 4b for directions on making calibration solutions). These ratios are plotted as a function of analyte concentration to produce the curve, and the analogous ratio obtained from a sample is read off the curve to obtain the amount of analyte in the sample (see section IV I for calculation methods, and Appendix C for calibration and data analysis spreadsheets). The same set of five calibration solutions is run at the beginning and end of a data set in order to correct for any instrumental drift during analysis. The two sets of calibration data are then combined to generate a single calibration curve used for the data set. A new calibration curve is generated for each data set. The calibration curve must bracket the concentrations of analyte observed in the data set; if a sample is outside the calibration range, it must be diluted and analyzed again.

F-3 GC/MS Operating Conditions

Column head pressure:	10 psi
Injector Temperature:	250°C
Injection Volume:	1.0 µL
Oven Temperature Program:	
Initial Temperature:	70°C
Initial Time:	1.0 min.

Ramp: (°C/min.)	Final Temp.(°C)	Final Time(min.)
10.0	270	1.0

Run Time:	22.0 min.
Equilibration Time:	3.0 min.
MSD Operation Parameters:	
Detector and Transfer Line:	280°C
Electron Multiplier (EM) Range:	~2500 V
EM offset (V above Autotune):	200 V
SIM Settings:	
Dichlobenil ions	m/z 171 (quantitation); m/z 173 (confirmation)
BAM ions	m/z 173 (quantitation); m/z 175, 189 (confirmation)
Internal standard ions	m/z 205 (quantitation); m/z 207 (confirmation)
Resolution:	Low
Dwell Time:	50 msec
Solvent Delay:	13.0 min

G. TIME REQUIRED FOR ANALYSIS

A sample set for method validation consists of a reagent blank, two control pond water samples, and five fortified pond water samples. It is suggested that a solvent blank be run between the initial set of calibration solutions and the sample set to verify the absence of carryover. The time required to complete the extraction and concentration of a mixture of dichlobenil and BAM from a sample set is about eight hours. The GC/MS analysis is carried out overnight using an autosampler injector.

H. POTENTIAL INTERFERENCES AND DIFFICULTIES

Overall, this analytical method is quite rugged and specific. No interferences were observed during method development and validation. Any potentially interfering compounds that may be present in a given sample would need to have the same GC retention time and mass spectrum as the analytes of interest. Interfering compounds could easily be detected by examination of the ions monitored for each analyte (i.e., the relative abundances of the quantitation and confirmation ions in the sample should be about the same as those in the test substance standards).

Any difficulties that may arise during sample preparation will likely be related to the volatility of dichlobenil. Dichlobenil volatilizes quite easily from sample extracts during concentration. Thus, care should be taken to avoid conditions favoring volatilization (elevated temperatures, sample agitation from nitrogen stream, concentration to very low volumes). The use of 1-heptanol as a keeper solvent reduces problems with dichlobenil volatility. However, care should be taken to avoid the addition of excess amounts of keeper. Because the volatilities (thus, GC retention times) of dichlobenil and the keeper are similar, large amounts of keeper can adversely affect the chromatography of dichlobenil by temporarily overloading the GC column.

Difficulties may also arise during sample analysis, especially at very low analyte concentrations. Any difficulties that may arise during BAM quantitation will likely be related to instrumental drift. Dichlobenil and the internal quantitation standard (2,4,6-trichlorobenzonitrile) do not produce appreciable amounts of fragment ions during electron impact ionization (Figures 2 and 3, respectively), therefore the ratio of dichlobenil/internal standard area counts are not particularly sensitive to minor fluctuations in instrumental conditions. BAM, however, does produce an abundant fragment ion that is used for quantitation (Figure 4). Thus, minor instrumental fluctuations can lead to fluctuations in the ratio of BAM/internal standard area counts, which can adversely affect BAM quantitation at low levels. The potential problem of instrumental drift is reduced by running calibration solutions at the beginning and end of each sample set, and using both sets of calibration data to generate the calibration curve.

I. CALCULATIONS

All calculations and statistics in this report were generated using Microsoft Excel 97 SR-2 software.

I-1 Calibration Plot

Ratios of analyte/internal standard peak areas of the standards (dichlobenil or BAM) are the dependent variables and the concentrations of the standard solutions, expressed as µg/mL, are the independent variables. They are used to generate calibration curves with a linear regression equation to determine the intercept, slope, and linearity of the detector response (coefficient of determination, R^2) for each analyte.

$$Ratio = Intercept + Slope\ X\ (\mu g / mL)_{Std.}$$

I-2 Calculation of the Amount of Dichlobenil or BAM in the Sample Extract

Using the ratio of analyte/internal standard found in the extract, determine the amount of analyte using the following equation:

$$dichlobenil(\mu g) = \frac{(Ratio_{sample} - Intercept)}{Slope}$$

I-3 Calculation of % Recovery of Dichlobenil and BAM from Fortified Pond Water

Divide the value calculated in equation I-2, which is the amount of analyte observed in the sample extract, by the amount of analyte spiked into the fortified pond water, and multiply this value by 100.

$$\%\ recovery = \frac{\mu g\ observed}{\mu g\ spiked} X 100$$

Example of the calculation method:

A water sample was fortified at the 0.100 ppb level with dichlobenil and BAM, and analyzed on 2/4/00, 0.100 µg/L fortification set A rep 3, sample ID DC020412 (Appendix C).

0.100 µg of dichlobenil and 0.100 µg BAM were added to a 1 L sample. sample DC020412 dichlobenil/internal standard ratio was: 1.11863515.

From the dichlobenil linear regression analysis: Intercept = -0.032682 and Slope = 12.30997.

The µg dichlobenil in the sample extract was calculated using the equation:

$$dichlobenil(\mu g) = \frac{(Ratio - Intercept)}{Slope}$$

{1.1186-(-0.032682)}/12.30997 = 0.093527 µg

0.100 µg of dichlobenil was spiked into the pond water sample, therefore the expected amount is 0.100 µg.

% Recovery:

$$\frac{\mu g\ observed}{\mu g\ spiked} X\ 100 = \%\,\mathrm{Rec.}$$

(0.093527/0.100) X 100 = 93.5 % Recovery

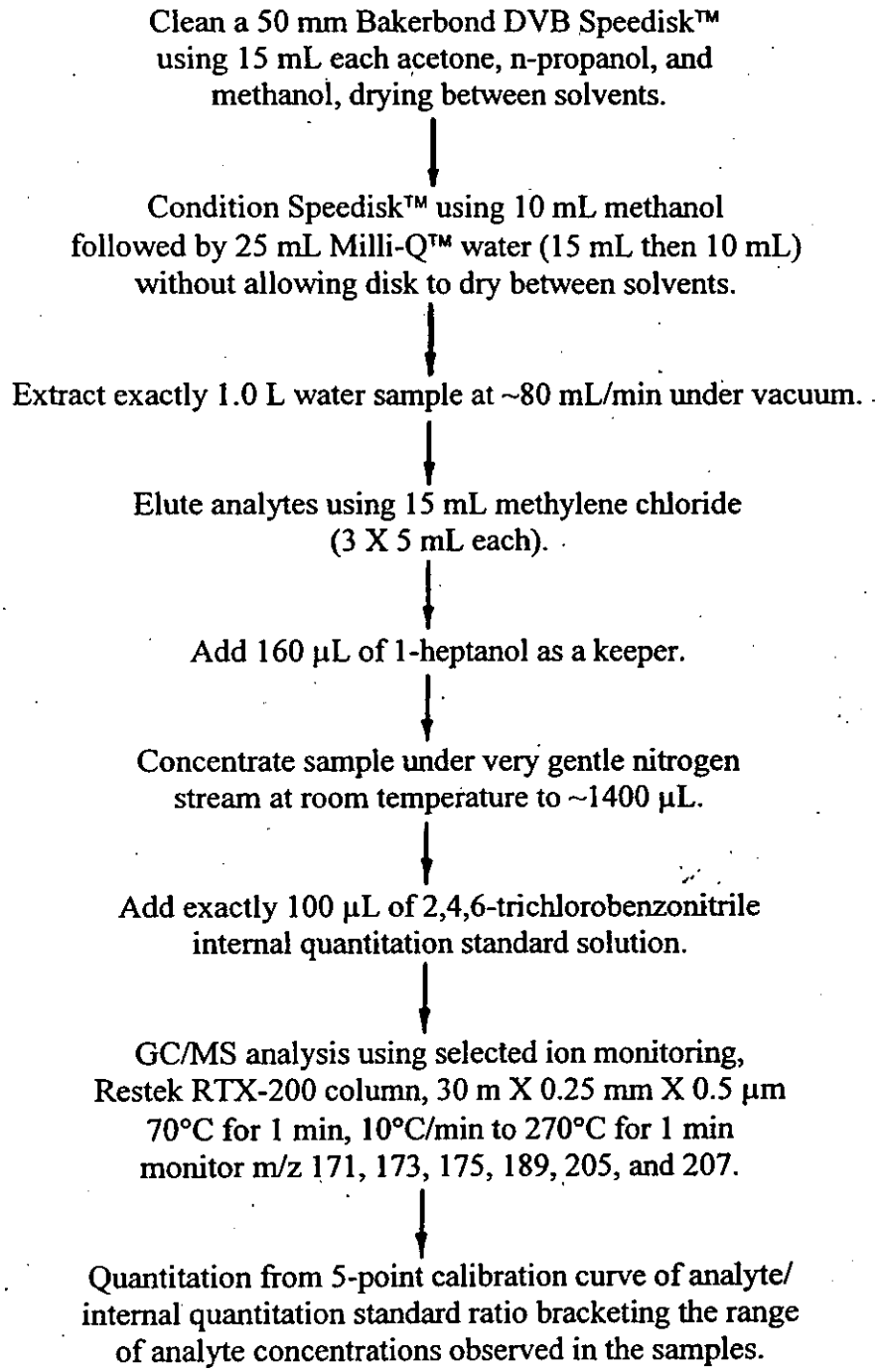


Figure 1. Schematic of Analytical Method for Analysis of Dichlobenil and 2,6-Dichlorobenzamide in Water