FINAL REPORT

Crude MCHM

HAEI No. 97-0216
CAS No. Not Available

EAN 972790
PM No. 18717-00

AN ACUTE AQUATIC EFFECTS TEST WITH THE DAPHNID,
Daphnia magna

GUIDELINES

OECD 202 and EEC/Annex V C.2

AUTHOR

Gregory C. Light, B.S.

TESTING FACILITY

Environmental Sciences Section
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6278
USA

LABORATORY PROJECT ID

Study No. EN-431-972790-A

STUDY SPONSOR

Eastman Chemical Company
200 S. Wilcox Drive
Kingsport, Tennessee 37660

STUDY COMPLETION DATE

February 9, 1998
QUALITY ASSURANCE INSPECTION STATEMENT
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

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ACCESSION NUMBER: 972790

STUDY TYPE: ACUTE AQUATIC EFFECTS TEST (DAPHNID-FL.)

(AUDITOR, QUALITY ASSURANCE UNIT) DATE

1/21/98

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to:


United States Environmental Protection Agency, 40 CFR Part 792 (TSCA) as revised August 17, 1989.

Annex 2 of the Organisation for Economic Cooperation and Development Guidelines for Testing of Chemicals [C(81)30(Final)].

With the following exceptions:

The stability of the test substance was not determined prior to test start at the request of the sponsor.

The uniformity and concentration of the test substance in the exposure solutions were not confirmed periodically throughout the test by analytical methods at the request of the sponsor.

Study Director
Marianne P. Hirsch, Ph.D.

Sponsor's Representative
Karen R. Miller, Ph.D.

2/9/98
Month/Day/Year

2/5/98
Month/Day/Year
SIGNATURE PAGE

AN ACUTE AQUATIC EFFECTS TEST WITH THE DAPHNID,
Daphnia magna

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AN ACUTE AQUATIC EFFECTS TEST WITH THE DAPHNID, 
Daphnia magna

ABSTRACT

The acute toxicity of the test substance to the Daphnia magna was determined by a 48-hour, static, aquatic effects test. The test substance exposures were prepared to contain nominally 6.25, 12.5, 25.0, 50.0 and 100 mg/L by adding the appropriate amount of test substance directly to test vessels containing 20 liters of laboratory dilution water that were prepared for a concurrently run fathead minnow test. Aliquots (250-mL) of each exposure solution were transferred to the daphnid test vessels and served as the exposure solutions for this study. Test substance exposures and dilution water controls were prepared in replicates of two. The concentration of the test substance in the exposure solutions was not analytically verified because the test substance is a crude mixture containing several components.

The results of this test indicate that the 48-hour EC50 value for the Daphnia magna is 98.1 mg/L. The 48-hour No-Observed-Effect Concentration (NOEC) value was determined to be 50.0 mg/L. The Daphnia magna in the dilution water controls exhibited normal behavior and appearance throughout the test.

The 48-hour EC50 value indicates that the test substance would be classified according to the European Union's labeling directive as "harmful to aquatic organisms" [1] and would correspond to a "moderate concern level" according to the U.S. EPA's assessment criteria [2].
STUDY AND TEST SUBSTANCE INFORMATION

Testing Facility

Environmental Sciences Section
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6278
USA

Laboratory Project ID: Study No. EN-431-972790-A

Sponsor

Eastman Chemical Company
Karen Miller, Ph.D. (Sponsor's Representative)

Study Dates

Study Initiation Date: December 11, 1997
Experimental Start Date: December 15, 1997
Experimental Termination Date: December 17, 1997

Project Participants

Environmental Sciences Section
Study Director Marianne P. Hirsch, Ph.D.
Principal Investigator Gregory C. Light, B.S.

Chemical Quality Services Division (CQSD)
Analytical Director/Analyst Nancy K. Porter, B.S.
Analyst Beth Isaacs, B.S.

Test Substance Characterization

Test Substance Name: Crude MCHM
HAEL No.: 97-0216
EAN: 972790
CAS No.: Not Available
PM No.: 18717-00
SRID or Lot No.: 6-97
Physical State and Appearance: Clear, colorless liquid
Solubility: Appreciable (per MSDS)
Purity Analysis, Structure Confirmation, and Stability

Purity Analysis: Gas chromatography with flame ionization detection (GC/FID) was used to assay the test substance. The result of the purity assay was 60.9 area percent based on the principal test substance peak eluting at 19.6 minutes. Several additional peaks were seen in the chromatogram, notably a peak with an average area of 34.6 percent at relative retention time (RRT) 1.02. Refer to Appendix 1.

Structure Confirmation: Gas chromatography with mass spectrometric detection (GS/MS) was used to perform the structure confirmation analysis. The spectrum of the test substance was determined to be consistent with the proposed structure based on comparison with reference library spectrum. Refer to Appendix 1.

Stability: Stability and estimated half-life of the test substance in the test system was not determined prior to test start. The Material Safety Data Sheet (MSDS) notes that substances containing similar structural groups are known to be normally stable.

PURPOSE

_Daphnia magna_ neonates (between 6 and 24 hours old) were exposed to five concentrations of the test substance in a 48-hour, static, aquatic effects test. _Daphnia magna_ were chosen for the test because they are representative of sensitive, freshwater invertebrates and can be reared within the laboratory. The objectives of the test were: (1) to determine if acute exposure to concentrations of the test substance would affect daphnids adversely; and, (2) if appropriate, provide an estimate of the 48-hour EC50 value and the highest acute No-Observed-Effect Concentration (NOEC). The daphnids were observed for signs of stress, as well as immobility, while exposed to the test substance. For the purpose of calculating or estimating the 24- and 48-hour EC50 and NOEC values, immobility would serve as the requisite endpoint. The study results can be used to estimate the likelihood of an adverse effect if the test substance enters a freshwater environment.

MATERIALS AND METHODS

Test System

Phylum - Arthropoda
Class - Crustacea
Order - Cladocera
Family - Daphnidae
Genus - _Daphnia_
Species - _magna_ (Straus)
Experimental Design

Source and Age of *Daphnia magna*

Adult *Daphnia magna* are continuously reared in 100-L culturing tanks located within the testing facility. The day before test start, approximately 100 gravid daphnids were transferred by net into two 20-cm diameter bowls containing approximately 1 liter of dilution water and 5 mL of food. The following day (test start) all of the adult daphnids were removed from the bowls, using nets and pipettes, and the remaining neonates were used for testing. The neonates used for this test were less than 24 hours old.

Source of Dilution Water

The water used in the test was pumped from Lake Ontario by the Kodak Park Lake Station Water Treatment Facility into a large underground storage reservoir located near the Environmental Sciences Section testing facility (Building 320, Kodak Park). This water subsequently was pumped into Building 320 where it first passed through polypropylene filter tubes, then a series of powdered, activated carbon filter tubes, and finally through another set of polypropylene filter tubes. The filtered water was then treated with sodium thiosulfate via a chemical injection system to further reduced trace levels of residual chlorinc. The filtered-treated water was then tempered to $20 \pm 2^\circ\text{C}$ by passage through a heat-exchange unit and distributed throughout the testing facility through stainless steel and PVC piping. Upon reaching the laboratory, the filtered-treated-tempered water cascaded through a column degassing unit into an open aeration basin for seasoning prior to use. Representative values for the hardness and total alkalinity (both as CaCO$_3$) for the study period were 123.0 mg/L and 89.8 mg/L, respectively. Refer to Appendix 2, Summary of Chemical Characterization of Dilution Water.

Acclimation Procedure

All test organisms were acclimated to the test dilution water prior to test start. The same dilution water used for this test is continuously supplied to the culturing tanks of the aquatic organisms. All aquatic organism used in the test were maintained in this water for more than two weeks prior to testing.

Number of Daphnids Used at Each Concentration

Daphnid neonates were collected by pipette and transferred directly into the exposure solutions. Sequential randomization was accomplished by allocating to each vessel no more than 50% of any one set of test organisms at a time. A total of 10 daphnids were placed into each of the replicate test and control vessels.
Preparation of Test Solutions

The test substance exposures, nominally 6.25, 12.5, 25.0, 50.0, and 100.0 mg/L, were prepared by adding the appropriate amount of the test substance to cuboidal glass test vessels containing 20-L of dilution water, which were used as test vessels in a concurrently run minnow test. After addition of the test substance, the exposure solutions were vigorously stirred with a hand-held mixer for 2-3 minutes to enhance dissolution. Immediately after mixing, 250-mL aliquots of each exposure solution were transferred from the 20-L test vessels to the 300 mL daphnid test vessels. All test substance and control exposures were prepared in replicates of two.

Test Substance Exposure Solution Appearance

The test substance and control exposures appeared clear and colorless throughout the test. There were no apparent particulates, surface slicks or precipitates observed.

Apparatus and Test Conditions

The test vessels were exposures were 300-mL Pyrex® glass beakers, each of which contained 250 ml of solution. Illumination during the test consisted of 16 hours of light and 8 hours of darkness, with a 30-minute transition period.

Test Procedures

The procedures used for the test were based upon accepted methodologies [3-8]. The test substance solutions were collected from corresponding 20-L test vessels that were prepared for a concurrently run fathead minnow test. Test exposures were prepared in replicates of two. Physical parameters (dissolved oxygen, pH, temperature) were recorded and ten daphnids were then placed into each of the test vessels. Physical parameters were taken again at test end, 48 hours. The daphnids were observed for signs of immobility and stress at times 0, 4, 24, and 48 hours.

Analytical Procedures

This test was performed using nominal concentrations of the test substance. The nominal concentrations of the exposure solutions were 6.25, 12.5, 25.0, 50.0, and 100.0 mg/L. The concentration of the test substance in the exposure solutions was not analytically verified because the test substance is a crude mixture containing several components.

Data Storage

All raw data and summaries of data, all protocols and protocol amendments, and all final reports will be maintained by the Health and Environment Laboratories, Eastman Kodak Company, Rochester, New York 14652-6269, for at least ten years.
Calculations

Refer to Appendix 3, Summary Report for Statistical Analyses.

Water Quality During the Test

The temperature of the exposure solutions in all vessels remained within the range of 19-20°C throughout the test. The extremes of pH values were 8.0 and 8.5. The extremes of the dissolved oxygen for all the exposure solutions were 8.7 and 9.6 mg/L. The temperature, pH, and dissolved oxygen values are all within the acceptable test criteria range for the test species [3-7]. Refer to Table 1 for a summary of the water quality measurements for the exposure solutions.

Test Validity

The following criteria for a valid study were met:

A. The control immobility was not greater than 10% when adverse effects were noted.
B. The dissolved oxygen level did not fall below 60% of the initial oxygen level when adverse effects were observed in the highest test concentration.
C. No abnormal occurrences (i.e., laboratory accidents) that might have influenced the outcome of the test were noted.
D. Daphnids in the control groups were not trapped at the surface of the water.

Protocol and Standard Operating Procedure Deviations

The protocol also states that the daphnids would be observed at 0, 24, and 48 hours. The daphnids were observed at these times, however an additional observation was taken 4 hours into the exposure. This deviation did not affect the outcome of the study.

RESULTS

Table 1 shows the water quality measurements of the exposure solutions at times 0 and 48 hours. Table 2 summarizes the observations of motility for each of the replicates at times 4, 24, and 48 hours. Table 3 contains the estimated 24 and 48 hour EC50 and No-Observed-Effect Concentration values. The results of the purity and structure confirmation are located in Appendix 1. Appendix 2 contains the results of the chemical characterization of the dilution water. Appendix 3 contains the results of the statistical analysis.
DISCUSSION

The acute toxicity of the test substance to the *Daphnia magna* was determined by a 48-hour, static, aquatic effects test. The replicate test substance exposures were prepared to contain nominally 6.25, 12.5, 25.0, 50.0 and 100 mg/L by adding the appropriate amount of test substance directly to test vessels containing laboratory dilution water and stirring them with a hand-held mixer. The concentration of the test substance in the exposure solutions was not analytically verified because the test substance is a crude mixture containing several components. Therefore the concentrations used throughout this report were based on the nominal values of the test substance solutions at test start.

At 48 hours, partial immobility (11 out of 20 daphnids) was observed in the test vessels containing nominally 100 mg/L. The daphnids in the dilution water controls exhibited normal behavior and appearance throughout the test.

Statistical analysis of the results estimates the 48-hour EC$_{50}$ value to be 98.1 mg/L. The 48-hour NOEC value for this study was 50.0 mg/L.

CONCLUSION

The 48-hour EC$_{50}$ value was determined to be 98.1 mg/L. The 48-hour No-Observed-Effect Concentration (NOEC) value was determined to be 50.0 mg/L. The 48-hour EC$_{50}$ value indicates that the test substance would be classified according to the European Union's labeling directive as "harmful to aquatic organisms" [1] and would correspond to a "moderate concern level" according to the U.S. EPA's assessment criteria [2].
REFERENCES


5. 40 CFR Part 797.1300 (Revised July 1, 1992), "Daphnia magna Acute Toxicity Test" (Amended 1987).


### TABLE 1. Temperature, Dissolved Oxygen, and pH Measurements of Exposure Solutions

<table>
<thead>
<tr>
<th>Nominal Conc. (mg/L)</th>
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<td>48 hrs</td>
<td>0 hrs</td>
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<td>A</td>
<td>19</td>
<td>20</td>
<td>9.2</td>
</tr>
<tr>
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<td>B</td>
<td>19</td>
<td>20</td>
<td>9.4</td>
</tr>
<tr>
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DWC = Dilution Water Control
Table 2. *Daphnia magna* Mobility

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DWC = Dilution Water Control
Table 3. Estimated EC$_{50}$ (95% Confidence Intervals) and Acute No-Observed-Effect Concentration Values for *Daphnia magna*

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<th>Time (hours)</th>
<th>EC$_{50}$ Value (mg/L) (95% C.I.)</th>
<th>NOEC Value (mg/L)</th>
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NOEC  = No-Observed-Effect Concentration  
C.I.   = Confidence Interval  
EC$_{50}$ and NOEC values were estimated using replicates A and B, pooled.
APPENDIX

AN ACUTE AQUATIC EFFECTS TEST WITH THE DAPHNID,
Daphnia magna
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AN ACUTE AQUATIC EFFECTS TEST WITH THE DAPHNID,
_Daphnia magna_

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APPENDIX 1. Purity and Structure Confirmation
FINAL REPORT

CRUDE MCHM

HAEL No.: 97-0216
CAS Registry No.: Not Available
EAN: 972790
PM No.: 18717-00

PURITY AND STRUCTURE CONFIRMATION

AUTHOR
Beth Isaacs, B.S.

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Kingsport, Tennessee 37662-5280
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Report No.: ESP-00798

STUDY COMPLETION DATE
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STUDY: 97-0216  STUDY DIRECTOR: PORTER, N.
ANALYTICAL DIRECTOR:
KAN : 972790
CQS JOB NUMBER:

STUDY TYPE:  PURITY AND STRUCTURE CONFIRMATION

CLARA M. PATTERSON
(AUDITOR, QUALITY ASSURANCE UNIT)

November 7, 1997

THE FOLLOWING PHASES OF THIS STUDY WERE INSPECTED BY ONE OR MORE PERSONS OF
THE QUALITY ASSURANCE UNIT ON THE DATES LISTED BELOW. WRITTEN STATUS REPORTS
WERE SUBMITTED TO THE STUDY DIRECTOR AND APPROPRIATE MANAGEMENT.

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to:

United States Food and Drug Administration, Good Laboratory Practice for Nonclinical Laboratory Studies, 21 CFR Part 58;

United States Environmental Protection Agency, Toxic Substances Control Act, Good Laboratory Practice Standards, 40 CFR Part 792;

Annex 2, Organization for Economic Cooperation and Development, Guidelines for the Testing of Chemicals [C(81)30(Final)].

Nancy K. Porter, B.S.
Study Director

Karen Miller, Ph.D.
Sponsor Representative

November 7, 1997

November 14, 1997
SIGNATURE PAGE

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Report Author/Analyst

Nancy K. Porter, B.S.
Study Director/Analyst

Karen Miller, Ph.D.
Sponsor Representative

November 3, 1997
Month/Day/Year

November 7, 1997
Month/Day/Year

November 14, 1997
Month/Day/Year
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CRUDE MCHM

HAEL No.: 97-0216
CAS Registry No.: Not Available
EAN: 972790
PM No.: 18717-00

ABSTRACT

The test substance was assayed for area percent purity on October 2, 1997 using gas chromatography with flame ionization detection (GC/FID). The result of the purity assay was 60.9 area percent based on the principal test substance peak eluting at 19.6 minutes. Several additional peaks were seen in the chromatogram, notably a peak with an average area of 34.6 percent at relative retention time (RRT) 1.02.

A structure confirmation analysis using gas chromatography with mass spectrometric detection (GS/MS) was performed on October 3, 1997. The test substance was injected neat (without prior dilution in a solvent) and spectra were obtained using a range of 20 to 300 Daltons. The spectrum of the test substance was determined to be consistent with the proposed structure based on comparison with reference library spectrum. The peak at RRT 1.02 had a similar spectrum and may be an isomer of the test substance.
STUDY AND TEST SUBSTANCE INFORMATION

Testing Facility
Environmental Analytical Services
Chemicals Quality Services Division
Eastman Kodak Company
Rochester, New York 14652-6276
USA

Sponsor
Eastman Chemical Company
PO Box 431
Kingsport, Tennessee 37662-5280
USA

Sponsor Representative
Karen Miller, Ph.D.
Eastman Chemical Company
PO Box 431
Kingsport, Tennessee 37662-5280
USA

Study Dates:
Study Start Date: September 30, 1997

Purity:
Experimental Start Date: October 2, 1997
Experimental Completion Date: October 2, 1997

Structure Confirmation:
Experimental Start Date: October 3, 1997
Experimental Completion Date: October 3, 1997

Project Participants
Study Director: Nancy K. Porter, B.S.
Analysts:
Beth Isaacs, B.S.
Nancy K. Porter, B.S.

Report Author:
Beth Isaacs, B.S.
Test Substance Characterization
Test Substance Name: Crude MCHM
EAN: 972790
HAEL No.: 97-0216
Sample Reference No.: 6-97
CAS Registry No.: Not Available
PM No.: 18717-00
Submitted to Laboratory: September 30, 1997

Test Substance Storage
The test substance was stored at room temperature in a locked cabinet before and after use.

PURPOSE
The purpose of this study was to determine the purity and structure of the test substance.

MATERIALS AND METHODS
The following procedures were used to obtain the data presented in this report.

Area Percent Purity Determination

Sample Preparation
Approximately 0.6 g of the test substance was weighed into a 100-mL volumetric flask and diluted to volume with methylene chloride.
**Instrument Conditions**
The purity determination was performed using GC/FID under the following test conditions:

<table>
<thead>
<tr>
<th>Instrument:</th>
<th>Hewlett Packard 5890</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detector:</td>
<td>Flame Ionization</td>
</tr>
<tr>
<td>Column:</td>
<td>J+W DB Wax, 30 m x 0.32 mm i.d., 0.25 μm film thickness</td>
</tr>
<tr>
<td>Carrier Gas:</td>
<td>Helium</td>
</tr>
<tr>
<td>Detector Temperature</td>
<td>250 °C</td>
</tr>
<tr>
<td>Injection Mode:</td>
<td>Split</td>
</tr>
<tr>
<td>Injector Temperature:</td>
<td>100 °C</td>
</tr>
<tr>
<td>Initial Head Pressure:</td>
<td>8 psig</td>
</tr>
<tr>
<td>Split Flow:</td>
<td>60 cc/min.</td>
</tr>
<tr>
<td>Diluting Solvent:</td>
<td>methylene chloride</td>
</tr>
<tr>
<td>Injection Volume:</td>
<td>2 μL</td>
</tr>
</tbody>
</table>

**Gradient:**

<table>
<thead>
<tr>
<th>Initial Temp. (°C)</th>
<th>Hold Time (min.)</th>
<th>Rate (°C/min.)</th>
<th>Final Temp (°C)</th>
<th>Final Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>10</td>
<td>15</td>
<td>240</td>
<td>10</td>
</tr>
</tbody>
</table>

**Structure Confirmation**

**Sample Preparation**
There was no sample preparation, the test substance was analyzed neat using the following conditions:

**Instrument Conditions**

<table>
<thead>
<tr>
<th>Instrument:</th>
<th>Hewlett Packard Model 5970 Quadrupole Mass Spectrometer with Hewlett Packard Model 5890 Series II GC and GC interface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scan Range:</td>
<td>20 - 300 Daltons</td>
</tr>
<tr>
<td>Column:</td>
<td>J+W DB Wax, 30 m x 0.32 mm i.d., 0.25 μm film thickness</td>
</tr>
<tr>
<td>Carrier Gas:</td>
<td>Helium</td>
</tr>
<tr>
<td>Injection Size:</td>
<td>0.5 μL</td>
</tr>
<tr>
<td>Initial Head Pressure:</td>
<td>8 psig</td>
</tr>
<tr>
<td>Injection Mode:</td>
<td>Split, with the split flow at 120 mL / minute</td>
</tr>
<tr>
<td>Injector Temperature:</td>
<td>100 °C</td>
</tr>
<tr>
<td>Detector Temperature:</td>
<td>250 °C</td>
</tr>
<tr>
<td>Interface Temperature:</td>
<td>250 °C</td>
</tr>
</tbody>
</table>
Oven conditions:

<table>
<thead>
<tr>
<th>Initial Temp (°C)</th>
<th>Hold Time (min.)</th>
<th>Rate (°C/min.)</th>
<th>Final Temp (°C)</th>
<th>Final Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>10</td>
<td>15</td>
<td>240</td>
<td>0</td>
</tr>
</tbody>
</table>

Calculations

Area Percent Purity:
The area percents of all peaks specific to the test substance were calculated by comparing a chromatogram of a blank (methylene chloride) with a chromatogram of the test substance. Peak areas common to the test substance chromatograms and blank chromatograms were subtracted from the test substance integration data, leaving the total peak area attributable to the test substance. The area percents of the remaining peaks were calculated by dividing the area of the peak of interest by the total peak area attributable to the test substance and multiplying the quotient by 100. The area percent purity of the test substance was calculated using the principal test substance peak at a retention time of 19.6 minutes.

Protocol and Standard Operating Procedure Deviations
There were no deviations noted.

Data Storage and Record Retention
All original protocols, raw data, and reports will be stored for at least ten years by the Chemicals Quality Services Division, building 320 of the Eastman Kodak Company, Kodak Park, Rochester, New York 14652-6276.
RESULTS

Area Percent Purity Determination
The results of the purity analysis were as follows:

Injection 1 = 60.88 area %
Injection 2 = 60.95 area %
Injection 3 = 60.91 area %

mean = 60.9 area %
std. dev. = 0.04
n = 3

Fifteen additional peaks were detected in the sample at approximate relative retention times (RRT) of 0.20, 0.77, 0.82, 0.87, 0.89, 0.90, 0.94, 0.98, 1.02, 1.04, 1.05, 1.06, 1.17, 1.18, and 1.21 minutes. Twelve of the fifteen additional peaks had individual mean area percents of less than 0.5%. The remaining three additional peaks had mean area percents of 1.0 (RRT 0.89), 1.8 (RRT 0.98), and 34.6% (RRT 1.02), respectively.

Structure Confirmation
The mass spectrum of the submitted sample was consistent with the known structure of the test substance based on comparison with reference library spectra. The mass spectrum of the 1.02 RRT peak was similar to the spectrum of the test substance. It is probable that the peak at 1.02 RRT is an isomer of the test substance.

CALCULATIONS

Area Percent Purity = \frac{\text{area of principal test substance peak}}{\text{sum of areas of all peaks relating to the sample}} \times 100

e.g. Area Percent of Test Substance in methylene chloride, Injection 1

\text{Area Percent Purity} = \frac{1169305}{1920601} \times 100 = 60.88 \text{ area %}
Relative Retention Time = \frac{\text{retention time of peak of interest (minutes)}}{\text{retention time of test substance peak (minutes)}}

e.g. Relative retention time of additional component at 19.2 minutes, injection 1

\[
\text{Relative Retention Time} = \frac{19.2}{19.6} = 0.98
\]

**DISCUSSION**

The purity of the test substance was determined using GC/FID with a mean result of 60.9 area percent. Fifteen additional peaks were detected in the sample at approximate relative retention times (RRT) of 0.20, 0.77, 0.82, 0.87, 0.89, 0.90, 0.94, 0.98, 1.02, 1.04, 1.05, 1.06, 1.17, 1.18, and 1.21 minutes. Twelve of the fifteen additional peaks had individual mean area percents of less than 0.5%. The remaining three additional peaks had mean area percents of 1.0 (RRT 0.89), 1.8 (RRT 0.98), and 34.6% (RRT 1.02), respectively. Expanded chromatograms of the test substance and a blank solution are shown in Figure I and Figure II (pages 15-16). The full scale chromatogram of the test substance is shown in Figure III (page 17).

The spectrum for the test substance was consistent with the known structure of the test substance based on comparison with reference library spectra. The molecular ion at 128 m/z is not seen in the test substance spectrum. The loss of H$_2$O yields the ion at 110 m/z (see Figure V on page 19). The loss of CH$_2$OH yields the ion at 97 m/z (see Figure V on page 19). The most abundant ion occurs at 55 m/z and appears to consist of mostly the ring carbons and hydrogens (C$_4$H$_4$) (see Figure V on page 19). The GC/MS total ion chromatogram of the test substance is shown in Figure IV on page 18. There were two major peaks in the total ion chromatogram with RRT of 1.00 and 1.02. Both peaks have spectra consistent with isomers of the test substance, based on comparison with reference library spectra (see Figures V, VI, and VII).

**CONCLUSION**

The purity of the test substance was found to be 60.9 area percent using GC/FID. The GC/MS of the test substance was consistent with the known structure of the test substance, based on comparison with reference library spectra.
REFERENCES

The following references were used as spectral interpretation aids:

FIGURE I

Expanded Chromatogram of a Blank
FIGURE II

Expanded Chromatogram of the Test Substance

Test Substance → (RRT 1.00)    ← RRT 1.02

Intensity (aU)

Time (minutes)
FIGURE III

Full Scale Chromatogram of the Test Substance

Test Substance → (RRT 1.00)

← RRT 1.02
FIGURE IV

GC/MS Total Ion Chromatogram Of The Test Substance

Test Substance ->
(RRT 1.00)

↔ RRT 1.02
FIGURE VII

Library Reference Spectrum
APPENDIX 2. Summary of Chemical Characterization of Dilution Water

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>MOST RECENT VALUE*</th>
<th>MEAN</th>
<th>S.D.</th>
<th>LOW</th>
<th>HIGH</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (at 20 C)</td>
<td>7.90</td>
<td>7.714</td>
<td>0.347</td>
<td>6.580</td>
<td>8.400</td>
<td>11</td>
</tr>
<tr>
<td>Conductivity (umhos/cm)</td>
<td>300.00</td>
<td>304.645</td>
<td>17.651</td>
<td>270.000</td>
<td>350.000</td>
<td>11</td>
</tr>
<tr>
<td>Total Hardness (as CaCO3) (mg/L)</td>
<td>123.00</td>
<td>122.727</td>
<td>3.157</td>
<td>110.000</td>
<td>130.000</td>
<td>11</td>
</tr>
<tr>
<td>Total Organic Carbon (ppm)</td>
<td>2.28</td>
<td>2.430</td>
<td>0.476</td>
<td>1.700</td>
<td>3.200</td>
<td>11</td>
</tr>
<tr>
<td>Total Residual CI2 (ug/L)</td>
<td>3.00</td>
<td>1.773</td>
<td>0.446</td>
<td>1.500</td>
<td>3.000</td>
<td>11</td>
</tr>
<tr>
<td>Acidity (as CaCO3) (ppm)</td>
<td>0.25</td>
<td>-55.045</td>
<td>40.215</td>
<td>-83.000</td>
<td>0.250</td>
<td>11</td>
</tr>
<tr>
<td>Alkalinity (as CaCO3) (ppm)</td>
<td>19.80</td>
<td>81.981</td>
<td>1.261</td>
<td>89.800</td>
<td>95.000</td>
<td>11</td>
</tr>
<tr>
<td>Non-carbonate Hardness (as CaCO3) (ppm)</td>
<td>33.20</td>
<td>30.682</td>
<td>2.290</td>
<td>25.000</td>
<td>35.000</td>
<td>11</td>
</tr>
<tr>
<td>Total Dissolved Solids (ppm)</td>
<td>180.00</td>
<td>171.818</td>
<td>13.719</td>
<td>110.000</td>
<td>190.000</td>
<td>11</td>
</tr>
<tr>
<td>Turbidity, (as NTU)</td>
<td>0.13</td>
<td>0.224</td>
<td>0.163</td>
<td>0.030</td>
<td>0.920</td>
<td>10</td>
</tr>
<tr>
<td>Total Suspended Solids (ppm)</td>
<td>7.40</td>
<td>2.467</td>
<td>2.600</td>
<td>0.190</td>
<td>7.400</td>
<td>11</td>
</tr>
<tr>
<td>Total NH3 (as N) (ppm)</td>
<td>0.49</td>
<td>0.143</td>
<td>0.114</td>
<td>0.033</td>
<td>0.485</td>
<td>11</td>
</tr>
<tr>
<td>Organic Nitrogen (ppm)</td>
<td>0.40</td>
<td>0.843</td>
<td>0.919</td>
<td>0.050</td>
<td>5.400</td>
<td>11</td>
</tr>
<tr>
<td>NO3- (as N) (ppm)</td>
<td>0.34</td>
<td>0.413</td>
<td>0.219</td>
<td>0.005</td>
<td>1.000</td>
<td>11</td>
</tr>
<tr>
<td>NO2- (as N) (ppm)</td>
<td>0.08</td>
<td>0.017</td>
<td>0.016</td>
<td>0.005</td>
<td>0.060</td>
<td>11</td>
</tr>
<tr>
<td>Chloride by IC (Cl-) (ppm)</td>
<td>22.50</td>
<td>23.000</td>
<td>1.033</td>
<td>23.000</td>
<td>29.000</td>
<td>11</td>
</tr>
<tr>
<td>Fluoride by ISE (F-) (ppm)</td>
<td>0.12</td>
<td>0.134</td>
<td>0.040</td>
<td>0.048</td>
<td>0.250</td>
<td>11</td>
</tr>
<tr>
<td>Sulfate by IC (SO4-) (ppm)</td>
<td>27.90</td>
<td>26.845</td>
<td>1.559</td>
<td>24.000</td>
<td>29.000</td>
<td>11</td>
</tr>
<tr>
<td>Total Cyanide (CN-) (ppm)</td>
<td>0.01</td>
<td>0.031</td>
<td>0.047</td>
<td>0.002</td>
<td>0.290</td>
<td>11</td>
</tr>
<tr>
<td>Total Phosphorus (P) (ppm)</td>
<td>0.05</td>
<td>0.017</td>
<td>0.011</td>
<td>0.001</td>
<td>0.046</td>
<td>11</td>
</tr>
<tr>
<td>Dissolved Si (as SiO2) (ppm)</td>
<td>0.98</td>
<td>0.520</td>
<td>0.278</td>
<td>0.100</td>
<td>0.980</td>
<td>11</td>
</tr>
<tr>
<td>TOTAL METALS by ICP/AES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminum (mg/L)</td>
<td>0.11</td>
<td>0.122</td>
<td>0.063</td>
<td>0.005</td>
<td>0.302</td>
<td>11</td>
</tr>
<tr>
<td>Barium (mg/L)</td>
<td>0.01</td>
<td>0.056</td>
<td>0.040</td>
<td>0.014</td>
<td>0.100</td>
<td>11</td>
</tr>
<tr>
<td>Boron (mg/L)</td>
<td>0.01</td>
<td>0.026</td>
<td>0.015</td>
<td>0.005</td>
<td>0.061</td>
<td>11</td>
</tr>
<tr>
<td>Cadmium (mg/L)</td>
<td>0.00</td>
<td>0.005</td>
<td>0.005</td>
<td>0.003</td>
<td>0.030</td>
<td>11</td>
</tr>
<tr>
<td>Cobalt (mg/L)</td>
<td>0.01</td>
<td>0.005</td>
<td>0.001</td>
<td>0.001</td>
<td>0.013</td>
<td>11</td>
</tr>
<tr>
<td>Copper (mg/L)</td>
<td>0.00</td>
<td>0.002</td>
<td>0.002</td>
<td>0.001</td>
<td>0.013</td>
<td>11</td>
</tr>
<tr>
<td>Chromium (mg/L)</td>
<td>0.00</td>
<td>0.006</td>
<td>0.004</td>
<td>0.004</td>
<td>0.026</td>
<td>11</td>
</tr>
<tr>
<td>Iron (mg/L)</td>
<td>0.01</td>
<td>0.015</td>
<td>0.008</td>
<td>0.010</td>
<td>0.050</td>
<td>11</td>
</tr>
<tr>
<td>Lead (mg/L)</td>
<td>0.00</td>
<td>0.007</td>
<td>0.007</td>
<td>0.001</td>
<td>0.022</td>
<td>11</td>
</tr>
<tr>
<td>Manganese (mg/L)</td>
<td>0.00</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.008</td>
<td>11</td>
</tr>
<tr>
<td>Nickel (mg/L)</td>
<td>0.01</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.008</td>
<td>11</td>
</tr>
<tr>
<td>Silver (mg/L)</td>
<td>0.00</td>
<td>0.002</td>
<td>0.000</td>
<td>0.002</td>
<td>0.002</td>
<td>11</td>
</tr>
<tr>
<td>Zinc (mg/L)</td>
<td>0.00</td>
<td>0.014</td>
<td>0.013</td>
<td>0.002</td>
<td>0.060</td>
<td>11</td>
</tr>
<tr>
<td>Arsenic by ICP/AES (mg/L)</td>
<td>0.00</td>
<td>0.004</td>
<td>0.004</td>
<td>0.000</td>
<td>0.023</td>
<td>11</td>
</tr>
<tr>
<td>Mercury by CVAA (ug/L)</td>
<td>0.01</td>
<td>0.034</td>
<td>0.019</td>
<td>0.005</td>
<td>0.055</td>
<td>11</td>
</tr>
</tbody>
</table>

For calculation purposes, values reported to be less than the detection limit for the analytical method used were assigned a value equal to one-half the detection limit. (In accordance with the Central Limit Theory for Averages.)

* Most recent sample date: July 23, 1997
APPENDIX 2. Summary of Chemical Characterization of Dilution Water (continued)

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>MOST RECENT VALUE*</th>
<th>MEAN</th>
<th>S.D.</th>
<th>LOW</th>
<th>HIGH</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury (mg/L)</td>
<td>0.0050</td>
<td>0.0119</td>
<td>0.0157</td>
<td>0.0001</td>
<td>0.0550</td>
<td>11</td>
</tr>
</tbody>
</table>

**PESTICIDES (µg/L)**

- Diazinon          | 0.1750             | 0.1111 | 0.0722 | 0.0100 | 0.1750 | 11  |
- Malathion          | 0.0750             | 0.0473 | 0.0307 | 0.0050 | 0.0750 | 11  |
- Parathion          | 0.0750             | 0.0473 | 0.0307 | 0.0050 | 0.0750 | 11  |
- Aldrin             | 0.0025             | 0.0045 | 0.0037 | 0.0025 | 0.0250 | 11  |
- o-BHC              | 0.0025             | 0.0045 | 0.0037 | 0.0025 | 0.0250 | 11  |
- Chlordane          | 0.0050             | 0.0143 | 0.0149 | 0.0025 | 0.0500 | 11  |
- 4,4'-DDT           | 0.0050             | 0.0091 | 0.0074 | 0.0050 | 0.0500 | 11  |
- Dieldrin           | 0.0050             | 0.0314 | 0.0431 | 0.0050 | 0.2500 | 11  |
- Endrin             | 0.0050             | 0.0091 | 0.0074 | 0.0050 | 0.0500 | 11  |
- Gamma-BHC (Lindane)| 0.0025             | 0.0111 | 0.0141 | 0.0025 | 0.0750 | 11  |
- Mirex              | 0.0050             | 0.0043 | 0.0010 | 0.0025 | 0.0050 | 11  |

**PCBs (µg/L)**

- PCB-1016           | 0.0250             | 0.0545 | 0.0483 | 0.0250 | 0.2500 | 11  |
- PCB-1221           | 0.0500             | 0.1023 | 0.0992 | 0.0250 | 0.5000 | 11  |
- PCB-1232           | 0.0250             | 0.0545 | 0.0483 | 0.0250 | 0.2500 | 11  |
- PCB-1242           | 0.0250             | 0.0545 | 0.0483 | 0.0250 | 0.2500 | 11  |
- PCB-1248           | 0.0250             | 0.0545 | 0.0483 | 0.0250 | 0.2500 | 11  |
- PCB-1254           | 0.0500             | 0.1091 | 0.0967 | 0.0500 | 0.5000 | 11  |
- PCB-1260           | 0.0500             | 0.1091 | 0.0967 | 0.0500 | 0.5000 | 11  |

**VOLATILE ORGANICS (µg/L)**

- Bromoform          | 0.5000             | 0.5000 | 0.5000 | 0.5000 | 0.5000 | 11  |
- Bromodichloromethane | 6.1000             | 7.3273 | 3.9334 | 11.0000 | 11.0000 | 11  |
- Chloroform         | 8.5000             | 10.8818 | 2.8562 | 5.4000 | 17.0000 | 11  |
- Dibromochloromethane | 3.7000             | 3.6727 | 1.2283 | 6.0000 | 11.0000 | 11  |
- Methylene chloride | 0.5000             | 0.5000 | 0.5000 | 0.5000 | 0.5000 | 11  |
- Benzene            | 0.5000             | 0.5000 | 0.5000 | 0.5000 | 0.5000 | 11  |
- Vinyl chloride     | 1.0000             | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 11  |
- Carbon tetrachloride | 0.5000             | 0.5000 | 0.5000 | 0.5000 | 0.5000 | 11  |
- 1,2-Dichloroethane | 0.5000             | 0.5000 | 0.5000 | 0.5000 | 0.5000 | 11  |
- 1,1-Dichloroethylene | 0.5000             | 0.5000 | 0.5000 | 0.5000 | 0.5000 | 11  |
- 1,4-Dichlorobenzene | 1.0000             | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 11  |
- 1,1,1-Trichloroethane | 0.5000             | 0.5000 | 0.5000 | 0.5000 | 0.5000 | 11  |
- Carbon disulfide   | 0.5000             | 0.5000 | 0.5000 | 0.5000 | 0.5000 | 11  |
- Trichloroethylene  | 0.5000             | 0.5000 | 0.5000 | 0.5000 | 0.5000 | 11  |

*Most Recent Sample Date: July 23, 1997*
APPENDIX 3. Statistical Analysis of Test Results

******************************************************************************
ECOTOXSTAT 0.1
June 1997
Written using: XLISP-STAT Release 3.50 (Beta)
******************************************************************************
Test Type: 48-hour Acute Daphnid Test
48-hour Results
Test Substance HAEL #: 97-0216
Date/Time of Data Analysis: "Dec 17, 1997, 15:39:46"

EC50 Estimation Using Probit Model

Chi-Square Test for Lack of Model fit: p = 0.985
The lack of fit test indicates that the probit model is appropriate.

Concentrations Used:  
6.25  12.5  25.0  50.0  100.0
# Immobile per Conc.:  
0    0    0    0    11
Total per Conc.:  
20   20   20   20   20
Proportion Immobile:  
0    0    0    0    0.55

Estimated EC50:  98.06
EC50 Standard Error:  1.07
The 95% Confidence Interval For The EC50: (86.39, 111.30)
APPENDIX 3. Statistical Analysis of Test Results (continued)

******************************************************************************

ECOTOXSTAT 0.1
June 1997
Written using: XLISP-STAT Release 3.50 (Beta)
******************************************************************************

Test Type: "48-hour Acute Daphnid Test
48-hour Results"

Test Substance HAEL #: 97-0216

Dunnett’s Test Using Arc Sin Transformation for Comparing
the Control to 5 Treatments

Test Results Summary
Concentrations used: DWC 6.25 12.5 25.0 50.0 100.0
Number immobile: 0 0 0 0 0 11
Number on test/conc: 20 20 20 20 20 20
Proportion immobile: 0 0 0 0 0 0.55

Statistical Tests Summary
Contrast          Difference

-----------------------------
DWC vs. 6.25          0.00
DWC vs. 12.50         0.00
DWC vs. 25.00         0.00
DWC vs. 50.00         0.00
DWC vs. 100.00        0.68

If the difference exceeds 0.25, then the contrast is significant at alpha = 0.05

The NOEC and LOEC have been set accordingly to:
NOEC:  50.00
LOEC:  100.00