

FINAL REPORT

Crude MCHM

HAEL 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

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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))

STUDY: 97-0216-1 STUDY DIRECTOR: HIRSCH, M.P.
ACCESSION NUMBER: 972790

PAGE 1
01/21/98

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

M. James
(AUDITOR, QUALITY ASSURANCE UNIT)

1/21/98 98 *1/21/98 written error*
DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT. WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES.

INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
12/11/97	PROTOCOL APPENDIX/AMENDMENT SUBMISSION	
12/16/97	CLINICAL SIGNS AT 24 HRS.	01/21/98
01/21/98	FINAL REPORT REVIEW	01/21/98

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to:

United States Food and Drug Administration, 21 CFR Part 58 as revised September 4, 1987.

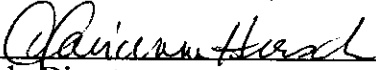
United States Environmental Protection Agency, 40 CFR Part 792 (TSCA) as revised August 17, 1989.

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.

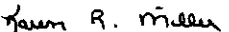
The uniformity and concentration of the test substance in the exposure solutions were not confirmed periodically throughout the test by analytical methods at the request of the sponsor.



Study Director
Marianne P. Hirsch, Ph.D.

2/9/98

Month/Day/Year



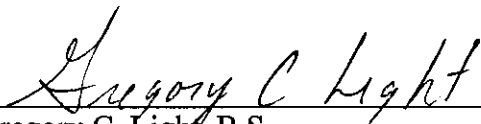
Sponsor's Representative
Karen R. Miller, Ph.D.

2/5/98

Month/Day/Year

SIGNATURE PAGE

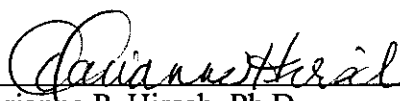
**AN ACUTE AQUATIC EFFECTS TEST WITH THE DAPHNID,
*Daphnia magna***



Gregory C. Light, B.S.
Principal Investigator/Report Co-author

1/14/98

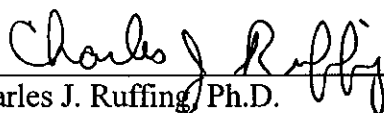
Month/Day/Year



Marianne P. Hirsch, Ph.D.
Study Director, Eco-Chem Testing Group

2/9/98

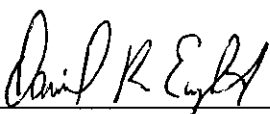
Month/Day/Year



Charles J. Ruffing, Ph.D.
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Month/Day/Year



Daniel R. English, Ph.D.
Unit Director, Environmental Sciences Section

January 14, 1998

Month/Day/Year

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Crude MCHM

HAEL No. 97-0216
CAS No. Not Available

EAN 972790
PM No. 18717-00

**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 chlorine. 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 EC₅₀ value to be 98.1 mg/L. The 48-hour NOEC value for this study was 50.0 mg/L.

CONCLUSION

The 48-hour EC₅₀ 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 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].

REFERENCES

1. Classification on the Basis of Environmental Effects. 1993. Official Journal of the European Communities, No. L110A, pp. 68-70.
2. Smrcek, J., Clements, R., Morcock, R., and Rabert, W., "Assessing Ecological Hazard Under TSCA: Methods and Evaluation of Data," Environmental Toxicology and Risk Assessment, ASTM STP 1179, Wayne Landis, Jane S. Hughes, and Michael A. Lewis, Eds., American Society for Testing and Materials, Philadelphia, 1993, pp. 22-39.
3. American Society for Testing and Materials (ASTM). 1995. Annual Book of ASTM Standards. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians, ASTM Standard E 729-88a; American Society for Testing and Materials, Philadelphia, PA. Volume 11.05, pp. 247-267.
4. American Public Health Association/American Water Works Association/Water Pollution Control Federation. 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. 40 CFR Part 797.1300 (Revised July 1, 1992), "*Daphnia magna* Acute Toxicity Test" (Amended 1987).
6. "*Daphnia* sp., Acute Immobilization Test and Reproduction Test," 1984. OECD Guideline for Testing of Chemicals. No. 202. pp. 1-16.
7. "Acute Toxicity for *Daphnia*. Methods for Determination of Ecotoxicity," 1993. Official Journal of the European Communities, Vol. L383A, pp. 172-178.
8. Department of the Environment. 1996. Guidance on the Aquatic Toxicity Testing of Difficult Substances. London, United Kingdom.

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

Nominal Conc. (mg/L)	Replicate	Temperature (° C)		Dissolved Oxygen (mg/L)		pH	
		0 hrs	48 hrs	0 hrs	48 hrs	0 hrs	48 hrs
DWC	A	19	20	9.2	8.8	8.4	8.5
DWC	B	19	20	9.4	8.7	8.3	8.5
6.25	A	19	20	9.4	8.8	8.2	8.5
6.25	B	19	20	9.4	8.9	8.2	8.5
12.5	A	19	20	9.4	8.8	8.3	8.5
12.5	B	19	20	9.4	8.9	8.1	8.5
25.0	A	19	20	9.4	8.8	8.1	8.5
25.0	B	19	20	9.4	8.8	8.0	8.5
50.0	A	19	20	9.6	8.8	8.1	8.5
50.0	B	19	20	9.4	8.8	8.1	8.5
100.0	A	19	20	9.4	8.8	8.1	8.5
100.0	B	19	20	9.5	8.9	8.2	8.5

DWC = Dilution Water Control

Table 2. *Daphnia magna* Mobility

Nominal Conc. (mg/L)	Replicate	Survival at time		
		4 hrs	24 hrs	48 hrs
DWC	A	10	10	10
DWC	B	10	10	10
6.25	A	10	10	10
6.25	B	10	10	10
12.5	A	10	10	10
12.5	B	10	10	10
25.0	A	10	10	10
25.0	B	10	10	10
50.0	A	10	10	10
50.0	B	10	10	10
100.0	A	10	10	6
100.0	B	10	10	3

DWC = Dilution Water Control

Table 3. Estimated EC₅₀ (95% Confidence Intervals) and Acute No-Observed-Effect Concentration Values for *Daphnia magna*

Time (hours)	EC ₅₀ Value (mg/L) (95% C.I.)	NOEC Value (mg/L)
24	> 100.0 (Not Estimable)	100.0
48	98.1 (86.4 & 111.3)	50.0

NOEC = No-Observed-Effect Concentration

C.I. = Confidence Interval

EC₅₀ 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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Daphnia magna

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Total Number of Pages: 25

APPENDIX 1. Purity and Structure Confirmation

FINAL REPORT

CRUDE MCHM

HAEL No.: 97-0216

EAN: 972790

CAS Registry No.: Not Available

PM No.: 18717-00

**PURITY AND
STRUCTURE CONFIRMATION**

AUTHOR

Beth Isaacs, B.S.

PERFORMING LABORATORY

Environmental Analytical Services
Chemicals Quality Services Division
Manufacturing Quality Assurance Organization
Eastman Kodak Company
Rochester, New York 14652-6276
USA

STUDY SPONSOR

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USA

Report No.: ESP-00798

STUDY COMPLETION DATE

November 7, 1997

ANALYTICAL QUALITY ASSURANCE INSPECTION STATEMENT
(CFR 58.35(B) (7) 792.35(B) (7) 160.35(B) (7))

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

STUDY TYPE: PURITY AND STRUCTURE CONFIRMATION

Glenn M. Patterson
(AUDITOR, QUALITY ASSURANCE UNIT)

November 7, 1997
DATE

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.

INSPECT DATES	REQUEST NUMBER	PHASE(S) INSPECTED	STATUS REPORT DATES
10/01/97		PROTOCOL SUBMISSION ECHEM/PURITY AND STRUCTURE/RFAC-HIRSCH	10/01/97
10/01/97		PHASE INSPECTION ECHEM/PURITY	10/01/97
10/03/97		PHASE INSPECTION ECHEM/STRUCTURE	10/03/97
11/07/97		TEST REPORT INSPECTION ECHEM/PURITY AND STRUCTURE	11/07/97

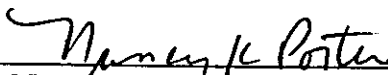
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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



Month/Day/Year



Karen Miller, Ph.D.
Sponsor Representative



Month/Day/Year

SIGNATURE PAGE

Beth Isaacs

November 3, 1997

Beth Isaacs, B.S.

Month/Day/Year

Report Author/Analyst

Nancy K. Porter

November 7, 1997

Nancy K. Porter, B.S.

Month/Day/Year

Study Director/Analyst

Karen R. Miller

November 14, 1997

Karen Miller, Ph.D.

Month/Day/Year

Sponsor Representative

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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
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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:

Instrument: Hewlett Packard 5890
Detector: Flame Ionization
Column: J + W DB Wax, 30 m x 0.32 mm i.d., 0.25 μ m film thickness
Carrier Gas: Helium
Detector Temperature: 250 $^{\circ}$ C
Injection Mode: Split
Injector Temperature: 100 $^{\circ}$ C
Initial Head Pressure: 8 psig
Split Flow: 60 cc/min.
Diluting Solvent: methylene chloride
Injection Volume: 2 μ L

Gradient:

Initial Temp. ($^{\circ}$ C)	Hold Time (min.)	Rate ($^{\circ}$ C/min.)	Final Temp ($^{\circ}$ C)	Final Time (min.)
40	10	15	240	10

Structure Confirmation

Sample Preparation

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

Instrument Conditions

Instrument: Hewlett Packard Model 5970 Quadrupole Mass Spectrometer with Hewlett Packard Model 5890 Series II GC and GC interface
Scan Range: 20 - 300 Daltons
Column: J+W DB Wax, 30 m x 0.32 mm i.d., 0.25 μ m film thickness
Carrier Gas: Helium
Injection Size: 0.5 μ L
Initial Head Pressure: 8 psig
Injection Mode: Split, with the split flow at 120 mL / minute
Injector Temperature: 100 $^{\circ}$ C
Detector Temperature: 250 $^{\circ}$ C
Interface Temperature: 250 $^{\circ}$ C

Oven conditions:

Initial Temp (°C)	Hold Time (min.)	Rate (°C/min.)	Final Temp (°C)	Final Time (min.)
40	10	15	240	0

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

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

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

$$\text{Area Percent Purity} = \frac{1169305}{1920601} \times 100 = 60.88$$

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 H₂O yields the ion at 110 m/z (see Figure V on page 19). The loss of CH₂OH 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 (C₄H₇) (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:

H. Budzikiewicz, Mass Spectrometry of Organic Compounds, Holden-Day, Inc., San Francisco, 1967

FIGURE I

Expanded Chromatogram of a Blank

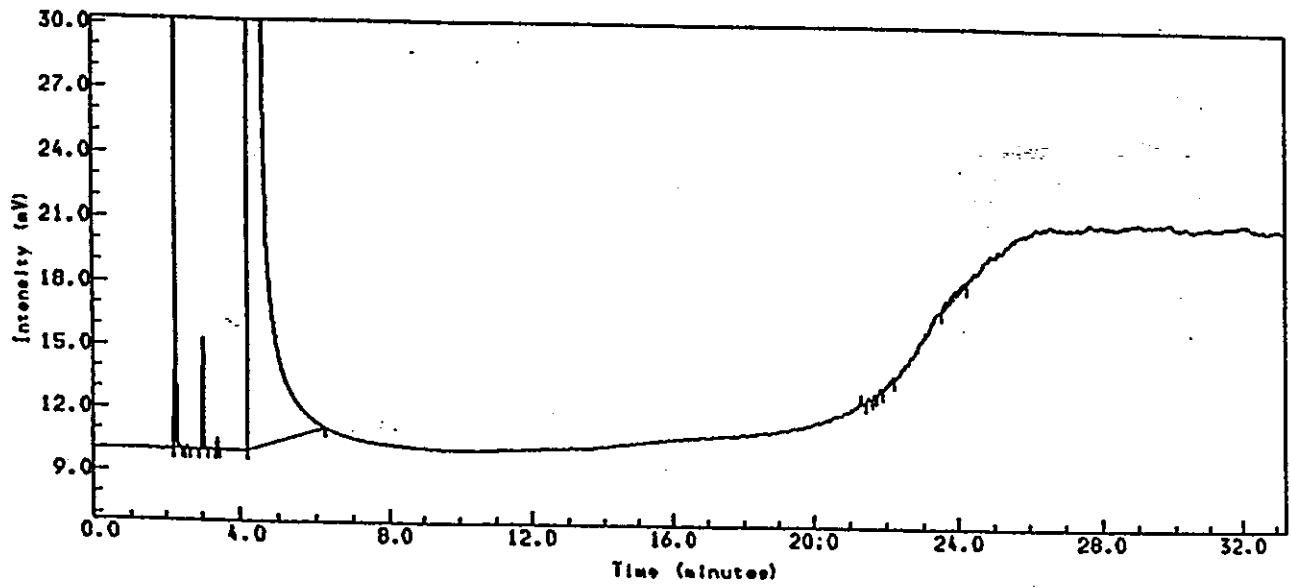


FIGURE II

Expanded Chromatogram of the Test Substance

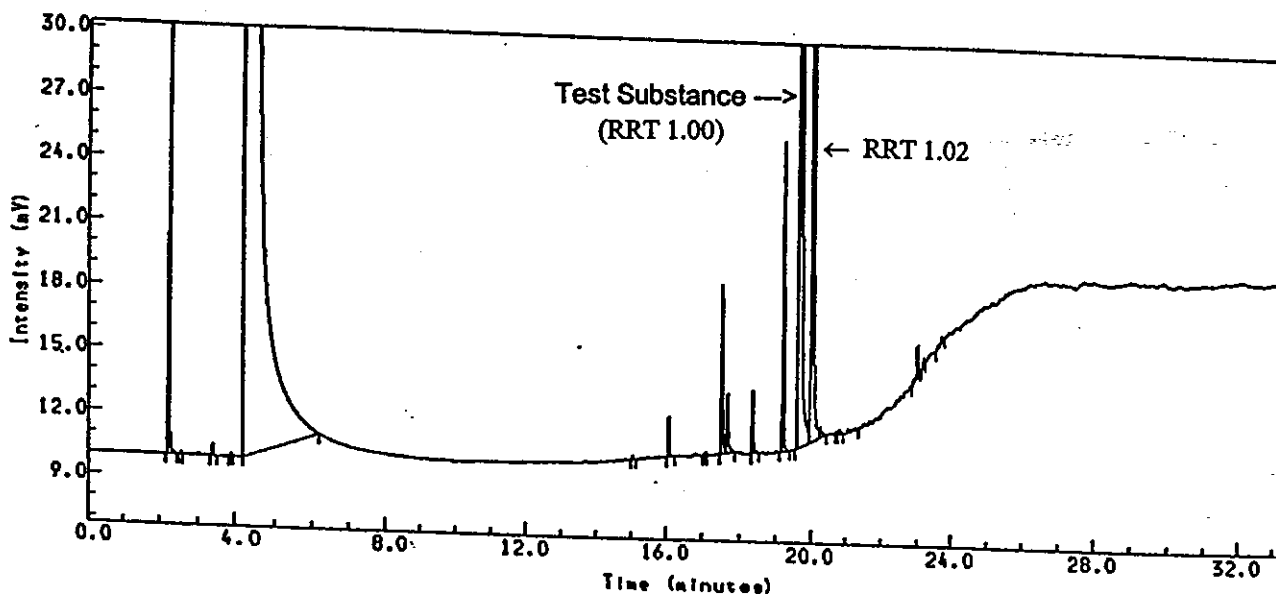


FIGURE III

Full Scale Chromatogram of the Test Substance

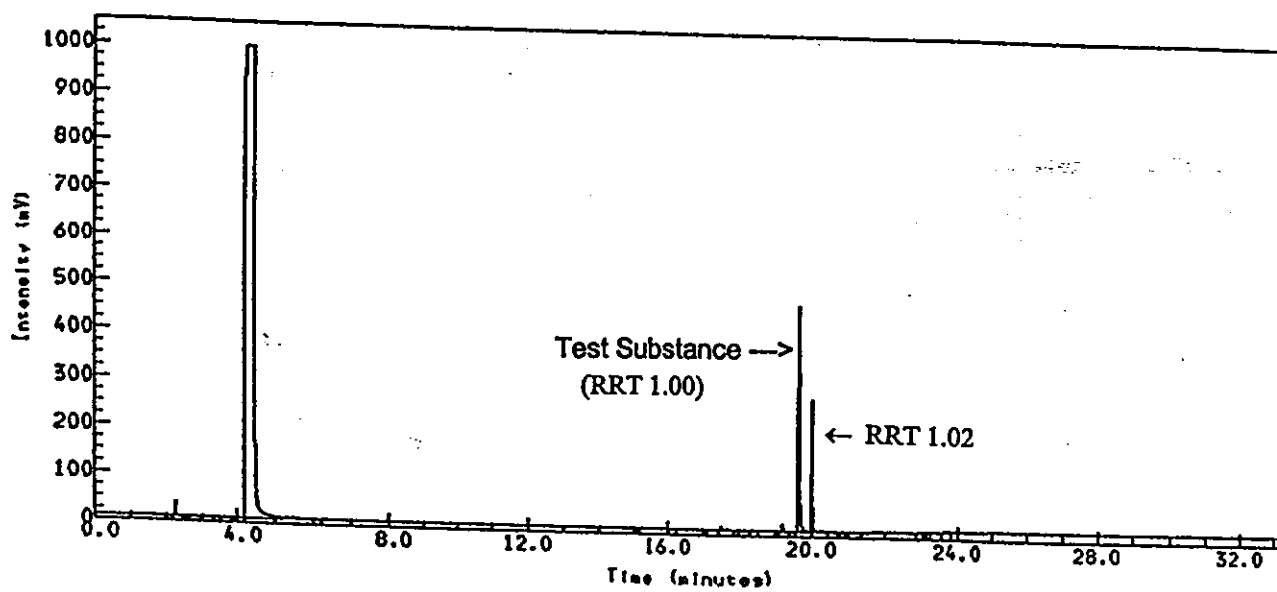


FIGURE IV

GC/MS Total Ion Chromatogram Of The Test Substance

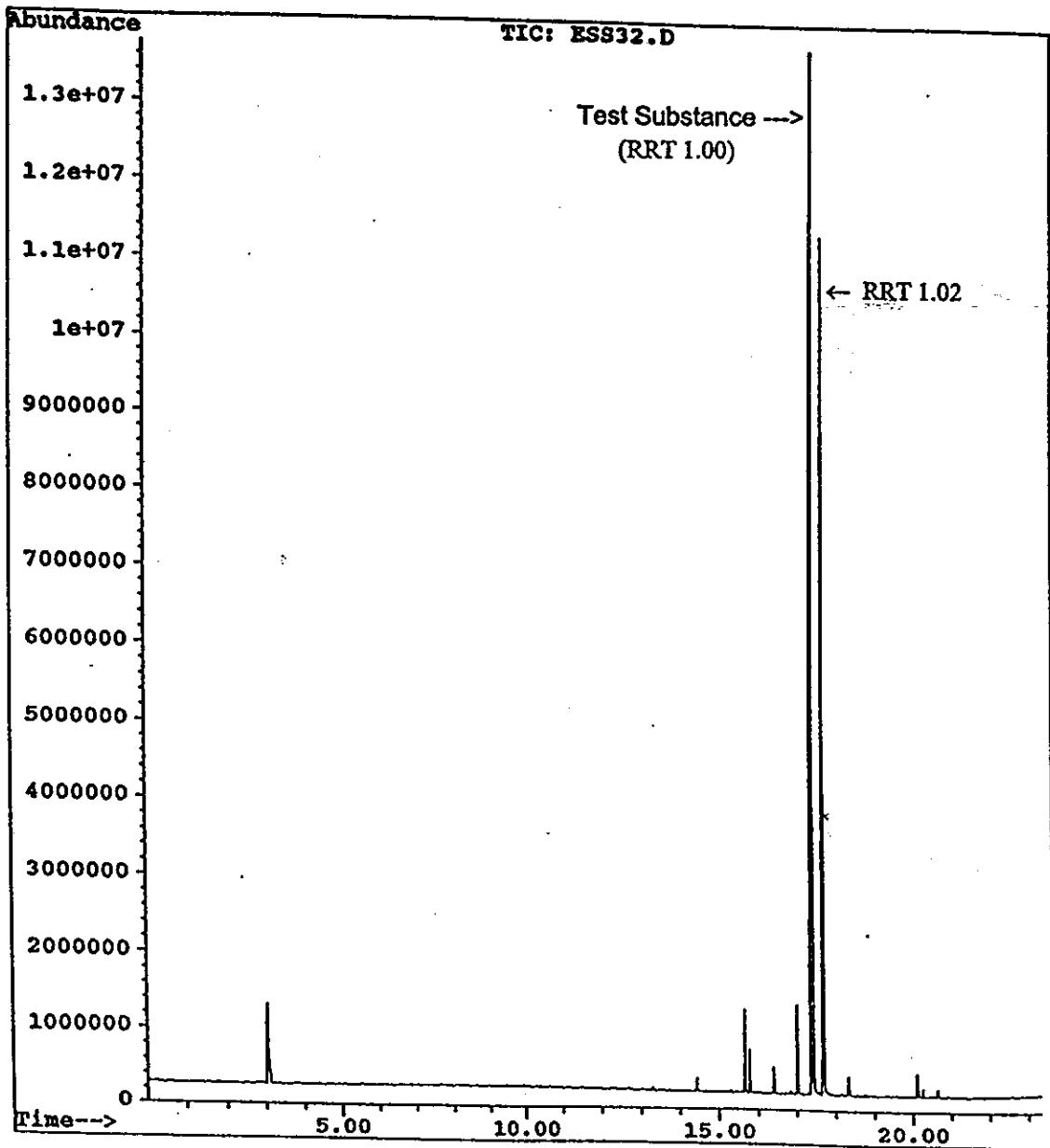


FIGURE V
GC/MS Spectrum of the Test Substance

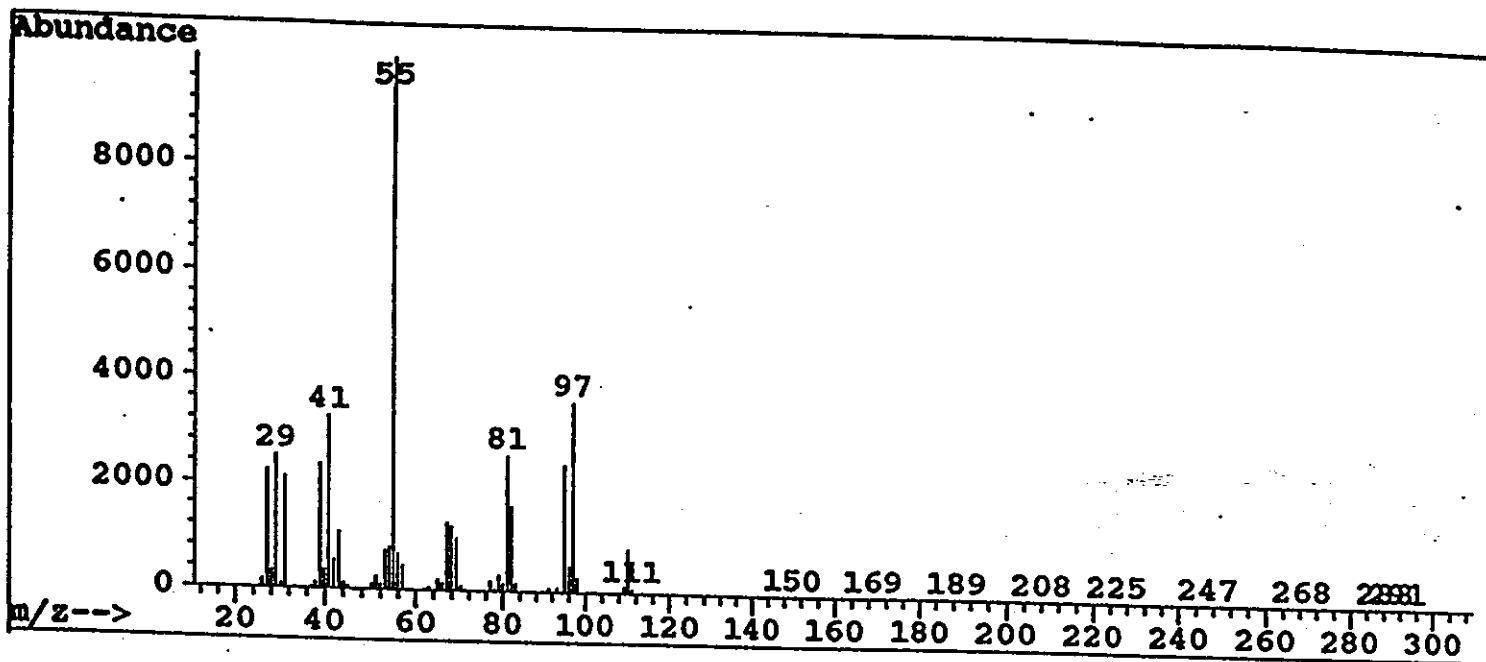


FIGURE VI
GC/MS Spectrum of the RRT 1.02 Peak

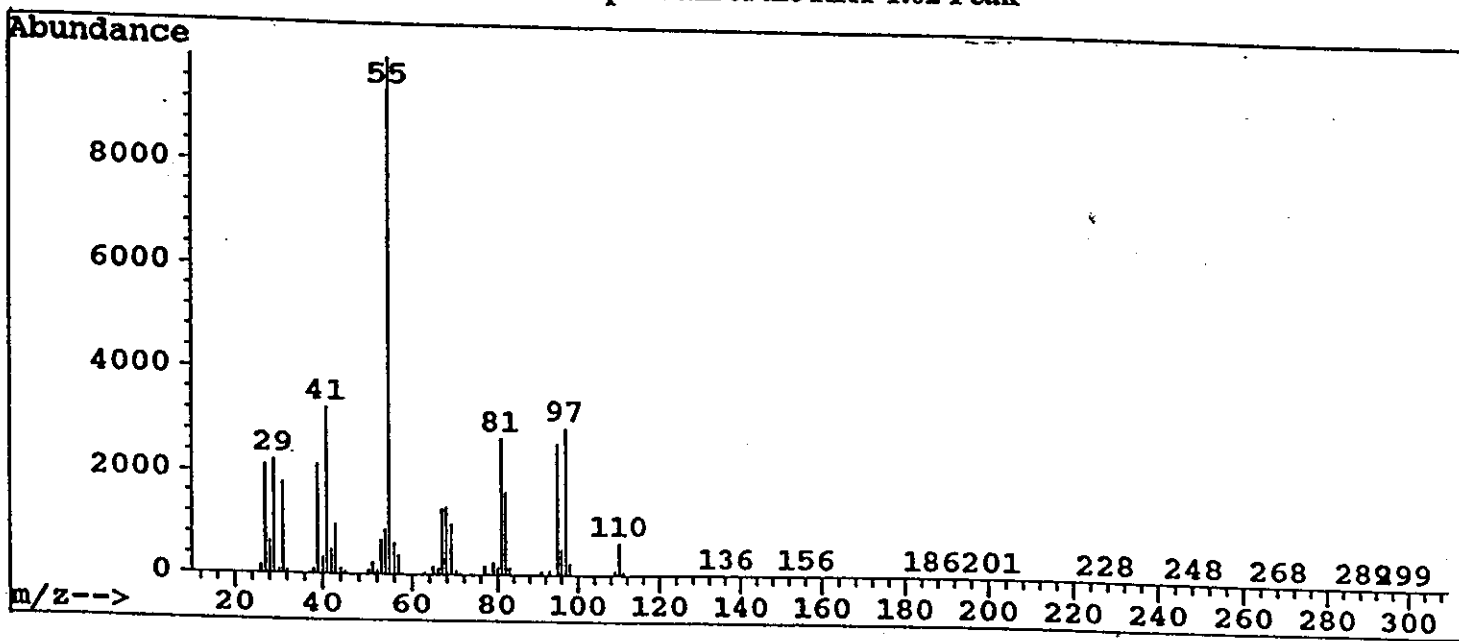
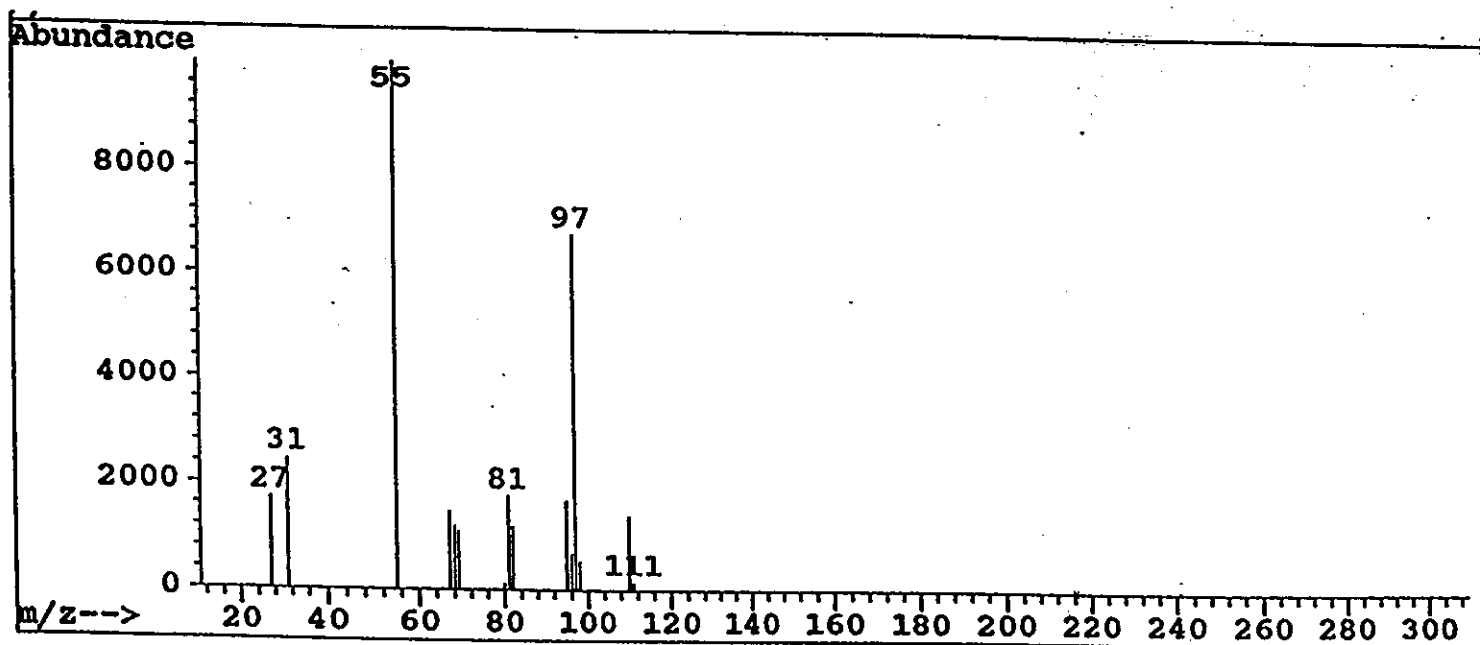


FIGURE VII

Library Reference Spectrum



APPENDIX 2. Summary of Chemical Characterization of Dilution Water

PARAMETER	MOST RECENT VALUE*	MEAN	S.D.	LOW	HIGH	N
pH (at 20 C)	7.90	7.714	0.347	6.580	8.400	11
Conductivity (umhos/cm)	300.00	304.545	17.851	270.000	350.000	11
Total Hardness (as CaCO3) (mg/L)	123.00	122.727	3.157	119.000	130.000	11
Total Organic Carbon (ppm)	2.28	2.430	0.476	1.700	3.200	11
Total Residual Cl2 (ug/L)	3.00	1.773	0.446	1.500	3.000	11
Acidity (as CaCO3) (ppm)	0.25	-55.045	40.215	-93.000	0.250	11
Alkalinity (as CaCO3) (ppm)	89.80	91.991	1.281	89.800	95.000	11
Non-carbonate Hardness (as CaCO3) (ppm)	33.20	30.982	2.296	25.000	35.000	11
Total Dissolved Solids (ppm)	180.00	171.818	13.719	110.000	190.000	11
Turbidity, (as NTU)	0.13	0.224	0.163	0.030	0.920	10
Total Suspended Solids (ppm)	7.40	2.467	2.600	0.190	7.400	11
Total NH3 (as N) (ppm)	0.49	0.143	0.114	0.033	0.485	11
Organic Nitrogen) (ppm)	0.40	0.843	0.919	0.090	5.400	11
NO3- (as N) (ppm)	0.34	0.413	0.219	0.005	1.000	11
NO2- (as N) (ppm)	0.06	0.017	0.016	0.005	0.060	11
Chloride by IC (Cl-) (ppm)	22.50	23.009	1.633	20.000	29.000	11
Fluoride by ISE (F-) (ppm)	0.12	0.134	0.040	0.046	0.250	11
Sulfate by IC (SO4-) (ppm)	27.90	26.645	1.559	24.000	29.000	11
Total Cyanide (CN-) (ppm)	0.01	0.031	0.047	0.002	0.290	11
Total Phosphorus (P) (ppm)	0.05	0.017	0.011	0.001	0.046	11
Dissolved Si (as SiO2) (ppm)	0.98	0.520	0.278	0.100	0.980	11
TOTAL METALS by ICP/AES						
Aluminum (mg/L)	0.11	0.122	0.063	0.005	0.302	11
Barium (mg/L)	0.01	0.056	0.040	0.014	0.100	11
Boron (mg/L)	0.01	0.026	0.015	0.005	0.061	11
Cadmium (mg/L)	0.00	0.005	0.005	0.003	0.030	11
Cobalt (mg/L)	0.01	0.005	0.001	0.005	0.008	11
Copper (mg/L)	0.00	0.002	0.002	0.001	0.013	11
Chromium (mg/L)	0.00	0.006	0.004	0.004	0.026	11
Iron (mg/L)	0.01	0.015	0.008	0.010	0.050	11
Lead (mg/L)	0.00	0.007	0.007	0.001	0.022	11
Manganese (mg/L)	0.00	0.001	0.001	0.001	0.008	11
Nickel (mg/L)	0.01	0.005	0.001	0.005	0.008	11
Silver (mg/L)	0.00	0.002	0.000	0.002	0.002	11
Zinc (mg/L)	0.00	0.014	0.013	0.002	0.060	11
Arsenic by ICP/AES (mg/L)	0.00	0.004	0.004	0.000	0.023	11
Mercury by CVAA (ug/L)	0.01	0.034	0.019	0.005	0.055	11

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)

PARAMETER	MOST RECENT			LOW	HIGH	N
	VALUE*	MEAN	S.D.			
Mercury (mg/L)	0.00500	0.0119	0.0157	0.0001	0.0550	11
PESTICIDES (ug/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
α -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 (ug/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 (ug/L)						
Bromoform	0.5000	0.5000	0.0000	0.5000	0.5000	11
Bromodichloromethane	6.1000	7.3273	1.9934	3.0000	11.0000	11
Chloroform	8.5000	10.8818	2.8562	5.4000	17.0000	11
Dibromochloromethane	3.7000	3.6727	1.2298	0.5000	6.1000	11
Methylene chloride	0.5000	0.5000	0.0000	0.5000	0.5000	11
Benzene	0.5000	0.5000	0.0000	0.5000	0.5000	11
Vinyl chloride	1.0000	1.0000	0.0000	1.0000	1.0000	11
Carbon tetrachloride	0.5000	0.5000	0.0000	0.5000	0.5000	11
1,2-Dichloroethane	0.5000	0.5000	0.0000	0.5000	0.5000	11
1,1-Dichloroethylene	0.5000	0.5000	0.0000	0.5000	0.5000	11
1,4-Dichlorobenzene	1.0000	1.0000	0.0000	1.0000	1.0000	11
1,1,1-Trichloroethane	0.5000	0.5000	0.0000	0.5000	0.5000	11
Carbon disulfide	0.5000	0.5000	0.0000	0.5000	0.5000	11
Trichloroethylene	0.5000	0.5000	0.0000	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)

Copyright (c) 1989-1994, by Luke Tierney.

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)

Copyright (c) 1989-1994, by Luke Tierney.

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

Test Substance HAEL #: 97-0216

Date/Time of Data Analysis: "Jan 13, 1998, 13:21:46"

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
