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 FATHEAD MINNOW, *Pimephales promelas*

GUIDELINES

OECD 203 and EEC/Annex V C.1.

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-430-972790-A

STUDY SPONSOR

Eastman Chemical Company
200 S. Wilcox Drive
Kingsport, TN 37660
U.S.A.

STUDY COMPLETION DATE

February 10, 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. PAGE 1
ACCESSION NUMBER: 972790 01/19/98

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

(AUDITOR, QUALITY ASSURANCE UNIT) 1/19/98 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	INSTRUMENT CALIBRATION INSPECTION CLINICAL SIGNS AT 24 HRS. PH, TEMPERATURE, AND DISSOLVED OXYGEN READINGS	01/19/98
01/19/98	FINAL REPORT REVIEW	01/19/98

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)].

The following exceptions were made to the above mentioned testing procedures:

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.

Marianne Hirsch — 2/9/98

Study Director
Marianne P. Hirsch, Ph.D.

Month/Day/Year

Karen R. Miller — 2/5/98

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

Month/Day/Year

SIGNATURE PAGE

AN ACUTE AQUATIC EFFECTS TEST WITH THE FATHEAD MINNOW,
Pimephales promelas

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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 FATHEAD MINNOW, *Pimephales promelas*

ABSTRACT

The acute toxicity of the test substance to the fathead minnow (*Pimephales promelas*) was determined by a 96-hour, static, aquatic effects test. The test substance exposures were prepared to contain nominally 6.25, 12.5, 25, 50 and 100 mg/L by adding the appropriate amount of test substance directly to test vessels containing 20 liters of laboratory dilution water. 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 96-hour LC_{50} value for the fathead minnow is 57.4 mg/L. The 96-hour No-Observed-Effect Concentration (NOEC) value was determined to be 25 mg/L. The minnows in the dilution water controls exhibited normal behavior and appearance throughout the test.

The 96-hour LC_{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].

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-430-972790-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 19, 1997

Project Participants

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

Juvenile fathead minnows (*Pimephales promelas*) were exposed to five concentration of the test substance in a 96-hour, static, aquatic effects test in order to assess the environmental impact of the test substance. The fathead minnow was chosen for this test based on its ecological importance as a representative freshwater species of fish which can be reared within the laboratory. The objectives of this test were: (1) to determine if acute exposure to a concentration of the test substance would affect fish adversely; and, (2) if appropriate, provide estimates of the 24-, 48-, 72-, and 96-hour LC_{50} values and the acute No-Observed-Effect Concentration (NOEC). In this test, the fathead minnows were observed for signs of stress, as well as mortality, when exposed to the test substance. For the purpose of calculating or estimating the 24, 48, 72, and 96-hour LC_{50} and NOEC values, mortality served as the requisite endpoint. The results of this test may be used to estimate the likelihood of an occurrence adverse effect if the test substance enters a freshwater environment.

MATERIALS AND METHODS

Test System

Phylum - Chordata
Class - Osteichthyes
Order - Cypriniformes
Family - Cyprinidae
Genus - *Pimephales*
Species - *promelas* (Rafinesque)

Experimental Design

Source of "*Pimephales promelas*"

Juvenile fathead minnows used in the test were obtained from Aquatic BioSystems , Inc., 1300 Blue Spruce Drive, Suite C, Fort Collins, Colorado 80524, U.S.A, and designated Lot # 082897ABS. The fathead minnows were hatched on August 28, 1997. The organisms were approximately 109 days old at the start of testing. Organism origination statements are kept on file in the Central File of Non-Test Data for the Acute Effects Testing Area in Room 2020, Building 320, Kodak Park.

Source of Dilution Water

The water used in this 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 ± 2°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 organisms for this test 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 of the aquatic organisms used in the test were maintained in this water for more than two weeks prior to testing.

Size and Number of Fathead Minnows Used at Each Concentration

Prior to test start, fourteen sets of fathead minnows were collected from the rearing tanks and held in small glass bowls. Each set consisted of seven minnows that were as uniform in size as possible. Sequential randomization was accomplished by allocating to each glass bowl no more than 50% of any one set of test organisms at a time. The biological loading of the test vessels was kept below 1.0 g of wet weight per liter of test solution. Two of the fourteen sets of minnows were euthanized at test start and used to determine the average wet weights and mean standard lengths of the test fish. The average wet weight per minnow for set #1 was 0.53 g and 0.39 g for set #2. The mean standard length for set #1 was 3.2 cm and 2.9 cm for set #2.

Preparation of Test Solutions

The test substance exposures, nominally 6.25, 12.5, 25, 50 and 100 mg/L, were prepared by adding the appropriate amount of test substance to cuboidal glass test vessels containing 20 liters of dilution water. Test substance exposures and controls were prepared in replicates of two. Prior to the addition of the organisms, the exposure solutions were vigorously stirred with a hand-held mixer for 2-3 minutes to enhance dissolution of the test substance.

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 seamless Pyrex® glass 30.5-cm cuboidal chromatography jars, each of which contained 20 liters 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 [3-7]. The test substance exposures were prepared by directly adding the test substance to the test vessels. Test organisms were observed for signs of adverse effects at 4, 24, 48, 72, and 96 hours. Water quality parameters and appearances of exposure solutions were recorded at test start and at 24-hour intervals.

Analytical Procedures

This test was performed using nominal concentrations of the test substance. The nominal concentrations of the test substance exposures were 6.25, 12.5, 25, 50 and 100 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 the Summary Report for Statistical Analyses found in Appendix 3.

Water Quality During the Test

The temperature of all exposures remained at 19°C throughout the test. The pH values ranged from 8.0 to 8.4 and dissolved oxygen concentrations ranged from 7.0 to 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 of the exposures.

Test Validity

The following criteria for a valid test were met during the study:

A. The control mortality 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.

Protocol and Standard Operating Procedure Deviations

There were no deviations from either the protocol or standard operating procedures used throughout this test.

RESULTS

Table 1 shows the water quality parameters of the exposures at times 0, 24, 48, 72, and 96 hours. Table 2 summarizes the observations of survival for each of the replicates at times 4, 24, 48, 72, and 96 hours. Table 3 contains the estimated 24, 48, 72, and 96 hour LC_{50} values and No-Observed-Effect Concentrations. The results of the purity and structure confirmation analyses are found in Appendix 1. Appendix 2 contains a summary of the chemical characterization of the dilution water. Appendix 3 contains the results of the statistical analyses of the survival data.

DISCUSSION

The acute toxicity of the test substance to the fathead minnow was determined by a 96-hour, static, aquatic effects test. The replicate test substance exposures were prepared to contain nominally 6.25, 12.5, 25, 50 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 value of the test substance solutions at test start.

Complete mortality was observed in the highest concentration (100 mg/L) within the first 4 hours of the study. At 24 hours, additional mortality (2 out of 14 fish) was observed in the test vessels containing nominally 50 mg/L. The minnows in the dilution water controls exhibited normal behavior and appearance throughout the test.

Statistical analysis of the survival data estimated the 24, 48, 72, and 96 hour LC_{50} values to be 57.4 mg/L. The NOEC value for these exposure periods was 25 mg/L.

CONCLUSION

The 96-hour LC_{50} value was estimated to be 57.4 mg/L. The 96-hour No-Observed-Effect Concentration (NOEC) value was estimated to be 25 mg/L. The 96-hour LC_{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

1. Classification on the Basis of Environmental Effects. 1993. Official Journal of the European Communities, No. L110A, pp. 68-70.

2. Smrchek, J., Clements, R., Morcock, R., and Rabert, W. 1993. "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, pp. 22-39.

3. American Society for Testing and Materials (ASTM). 1992. Annual Book of ASTM Standards. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macro-invertebrates, and Amphibians, ASTM Standard E 729-88a; American Society for Testing and Materials, Philadelphia, PA. Volume 11.04, pp. 403-422.

4. American Public Health Association/American Water Works Association/Water Pollution Control Federation. 1992. Standard Methods for Examination of Water and Wastewater, 18th ed. American Public Health Association, Washington, D.C.

5. 40 CFR Part 797.1400 (Revised July 1, 1992), "Fish Acute Toxicity Test" (Amended 1989)

6. Acute Toxicity for Fish. Methods for Determination of Ecotoxicity. 1984. Official Journal of the European Communities, Vol. 27, pp. 146-154.

7. Fish, Acute Toxicity Test. 1984. OECD Guideline for Testing of Chemicals. No. 203. pp. 1-12.

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

Nominal Conc.		Temperature (° C) at time				
(mg/L)	Replicate	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
DWC	A	19	19	19	19	19
DWC	B	19	19	19	19	19
6.25	A	19	19	19	19	19
6.25	B	19	19	19	19	19
12.5	A	19	19	19	19	19
12.5	B	19	19	19	19	19
25.0	A	19	19	19	19	19
25.0	B	19	19	19	19	19
50.0	A	19	19	19	19	19
50.0	B	19	19	19	19	19
100.0	A	19	--	--	--	--
100.0	B	19	--	--	--	--

Nominal Conc.		Dissolved Oxygen (mg/L) at time				
(mg/L)	Replicate	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
DWC	A	9.2	7.7	7.4	7.0	7.4
DWC	B	9.4	7.6	7.4	7.1	7.4
6.25	A	9.4	8.1	7.8	7.4	7.3
6.25	B	9.4	8.1	7.8	7.5	7.4
12.5	A	9.4	8.1	8.0	7.5	7.5
12.5	B	9.4	8.2	8.0	7.5	7.5
25.0	A	9.4	8.2	7.8	7.5	7.8
25.0	B	9.4	8.2	7.8	7.4	7.5
50.0	A	9.6	8.2	8.1	7.5	7.6
50.0	B	9.4	7.9	8.3	7.9	7.5
100.0	A	9.4	--	--	--	--
100.0	B	9.5	--	--	--	--

DWC = Dilution Water Control
NA = Not Applicable

TABLE 1. Temperature, Dissolved Oxygen, and pH Measurements of Exposure Solutions *(continued)*

Nominal Conc. (mg/L)	Replicate	pH at time 0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
DWC	A	8.4	8.1	8.1	8.0	8.0
DWC	B	8.3	8.1	8.0	8.0	8.0
6.25	A	8.2	8.2	8.2	8.0	8.0
6.25	B	8.2	8.0	8.1	8.0	8.0
12.5	A	8.3	8.1	8.1	8.0	8.0
12.5	B	8.1	8.1	8.2	8.1	8.0
25.0	A	8.1	8.0	8.0	8.0	8.0
25.0	B	8.0	8.0	8.0	8.0	8.0
50.0	A	8.1	8.0	8.1	8.0	8.0
50.0	B	8.1	8.0	8.1	8.0	8.0
100.0	A	8.1	--	--	--	--
100.0	B	8.2	--	--	--	--

DWC = Dilution Water Control

TABLE 2. *Pimephales promelas* Survival

Nominal Conc.		Survival at time				
(mg/L)	Replicate	4 hrs	24 hrs	48 hrs	72 hrs	96 hrs
DWC	A	7	7	7	7	7
DWC	B	7	7	7	7	7
6.25	A	7	7	7	7	7
6.25	B	7	7	7	7	7
12.5	A	7	7	7	7	7
12.5	B	7	7	7	7	7
25.0	A	7	7	7	7	7
25.0	B	7	7	7	7	7
50.0	A	7	6	6	6	6
50.0	B	7	6	6	6	6
100.0	A	0	--	--	--	--
100.0	B	0	--	--	--	--

DWC = Dilution Water Control

Table 3. Estimated LC_{50} (95% Confidence Intervals) and Acute No-Observed-Effect Concentration Values for *Pimephales promelas*

Time (hours)	LC_{50} Value (mg/L) (95% C.I.)	NOEC Value (mg/L)
24	57.4 (29.71 and 110.8 mg/L)	25
48	57.4 (29.71 and 110.8 mg/L)	25
72	57.4 (29.71 and 110.8 mg/L)	25
96	57.4 (29.71 and 110.8 mg/L)	25

NOEC = No-Observed-Effect Concentration
C.I. = Confidence Interval
LC_{50} and NOEC values were estimated using replicates A and B, pooled.

APPENDIX

AN ACUTE AQUATIC EFFECTS TEST WITH THE FATHEAD MINNOW, *Pimephales promelas*

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AN ACUTE AQUATIC EFFECTS TEST WITH THE FATHEAD MINNOW, *Pimephales promelas*

Total Number of Pages: 24

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
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STUDY SPONSOR

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

Karen 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 — November 7, 1997

Nancy K. Porter, B.S. — Month/Day/Year
Study Director

Karen R. Miller — November 14, 1997

Karen Miller, Ph.D. — Month/Day/Year
Sponsor Representative

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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LIST OF FIGURES

CRUDE MCHM

HAEL No.: 97-0216 **EAN: 972790**
CAS Registry No.: Not Available **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
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Rochester, New York 14652-6276
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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 µm film thickness
Carrier Gas:	Helium
Detector Temperature	250 °C
Injection Mode:	Split
Injector Temperature:	100 °C
Initial Head Pressure:	8 psig
Split Flow:	60 cc/min.
Diluting Solvent:	methylene chloride
Injection Volume:	2 µL

Gradient:

Initial Temp. (°C)	Hold Time (min.)	Rate (°C/min.)	Final Temp (°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 µm film thickness
Carrier Gas:	Helium
Injection Size:	0.5 µL
Initial Head Pressure:	8 psig
Injection Mode:	Split, with the split flow at 120 mL / minute
Injector Temperature:	100 °C
Detector Temperature:	250 °C
Interface Temperature:	250 °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 H_2O yields the ion at 110 m/z (see Figure V on page 19). The loss of CH_2OH 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_4H_7) (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

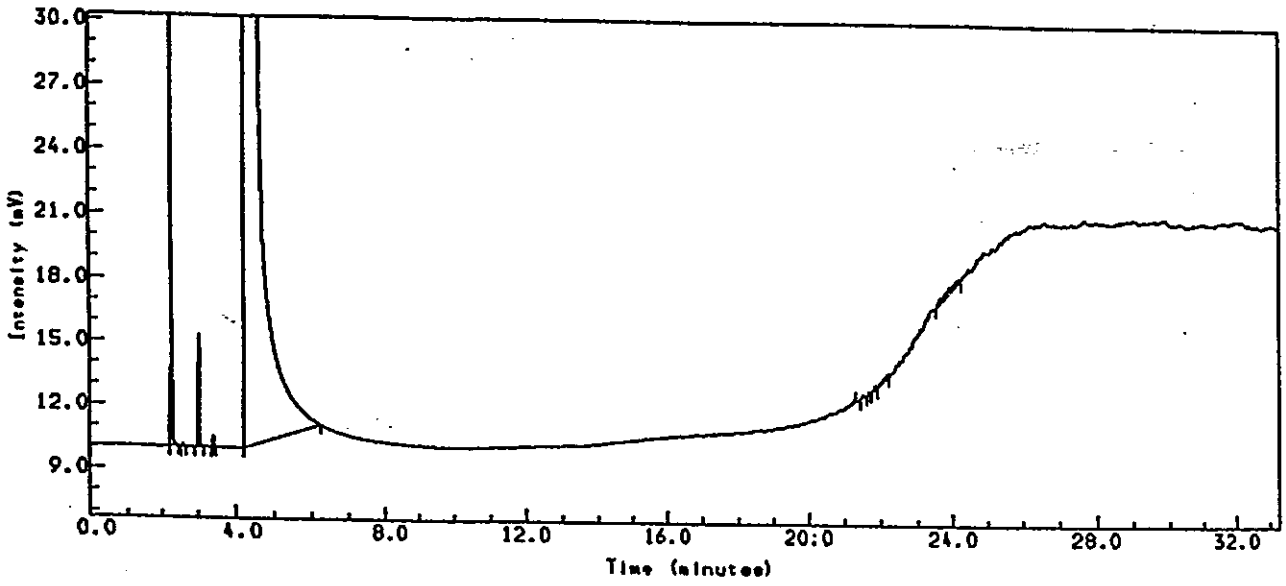


FIGURE II

Expanded Chromatogram of the Test Substance

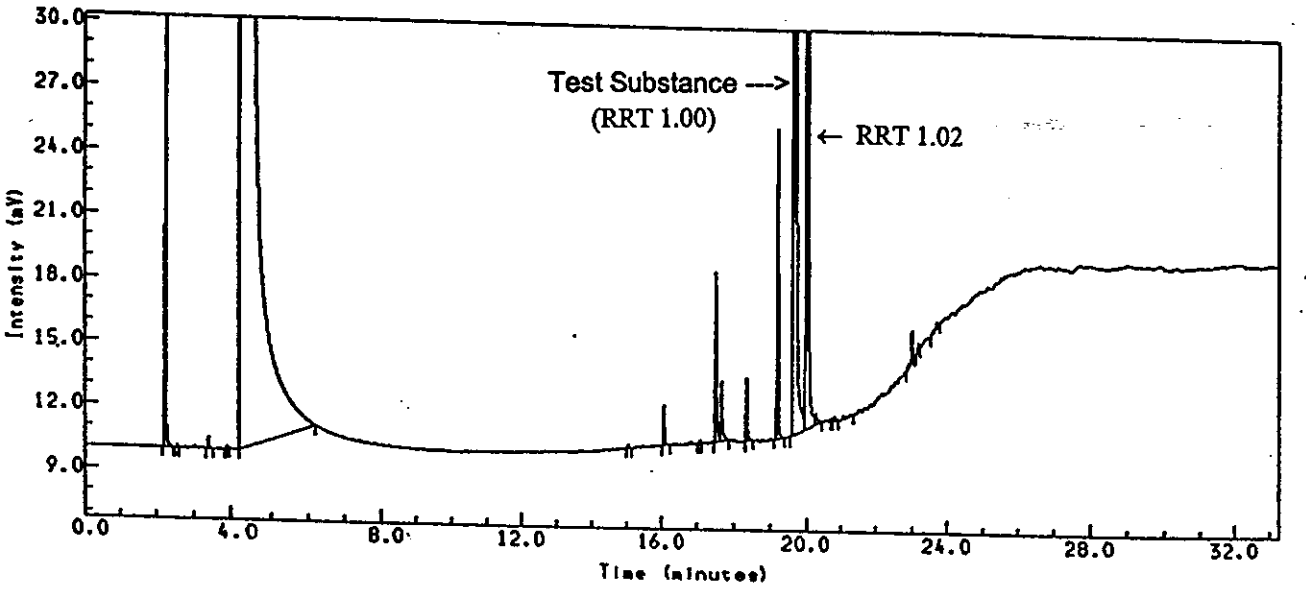


FIGURE III

Full Scale Chromatogram of the Test Substance

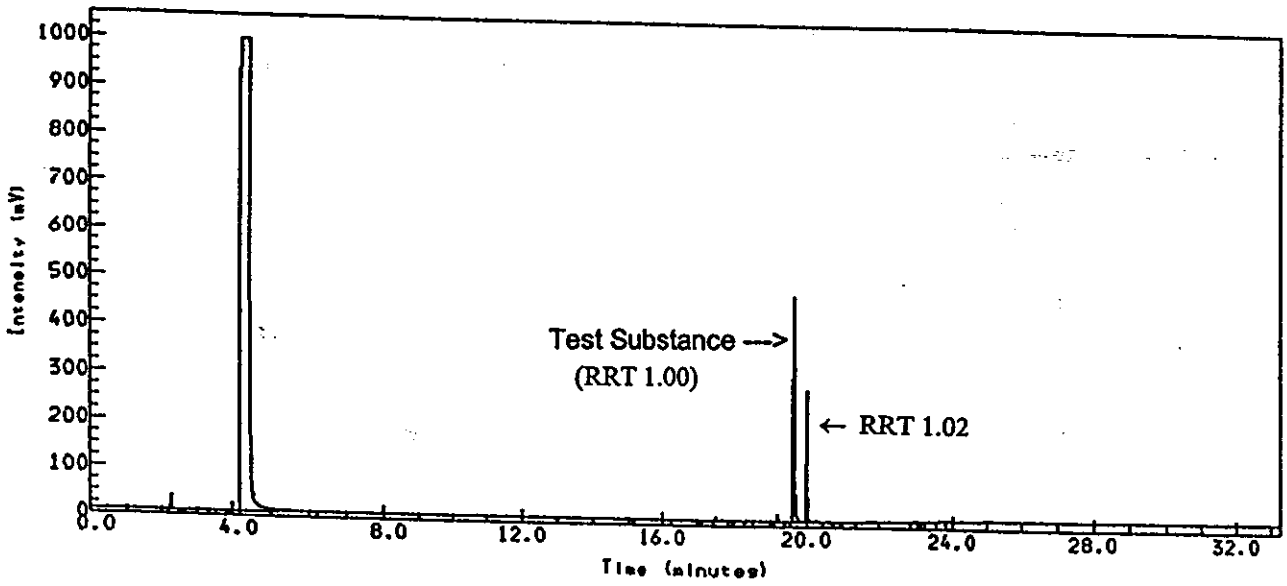


FIGURE IV

GC/MS Total Ion Chromatogram Of The Test Substance

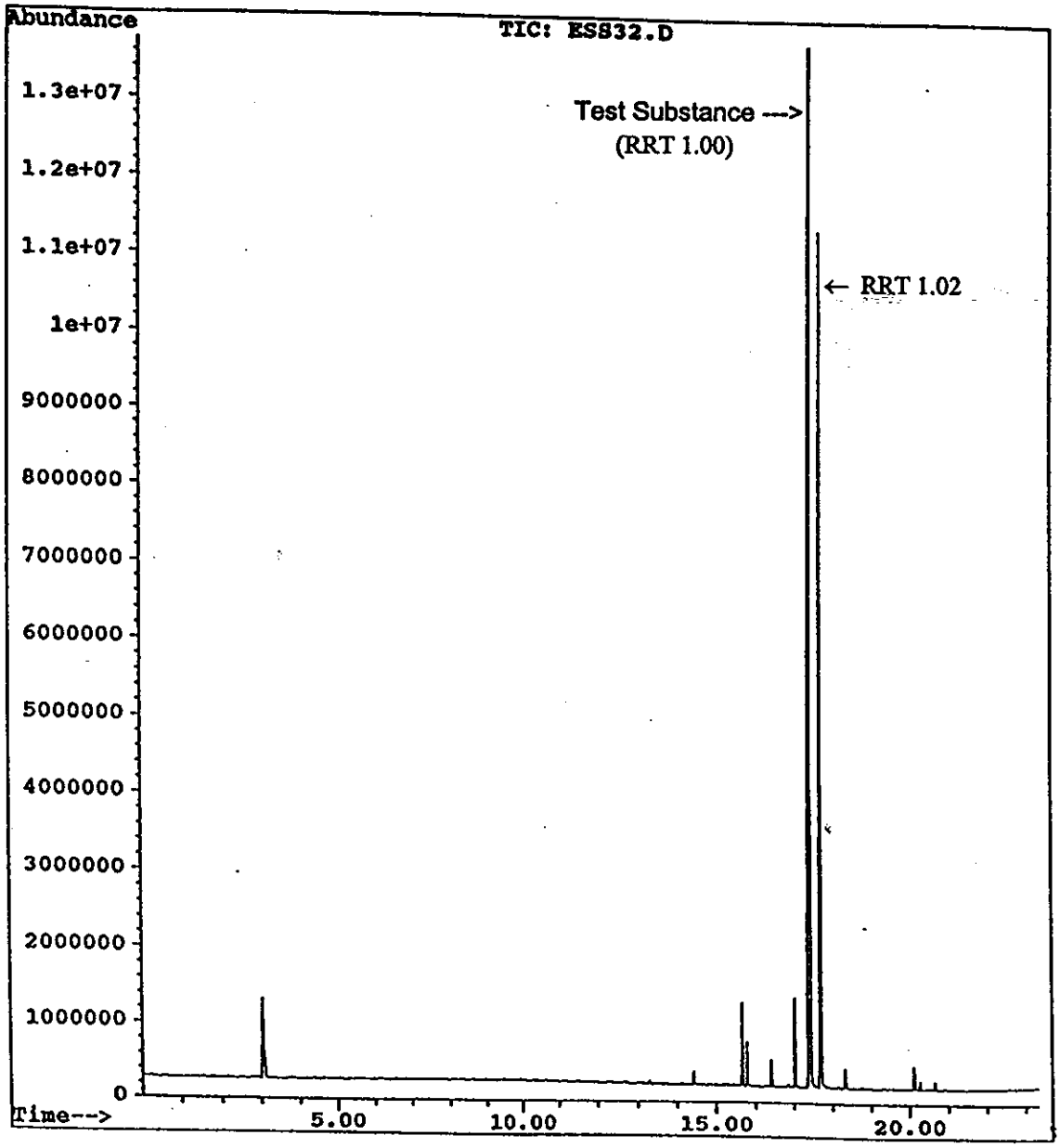


FIGURE V

GC/MS Spectrum of the Test Substance

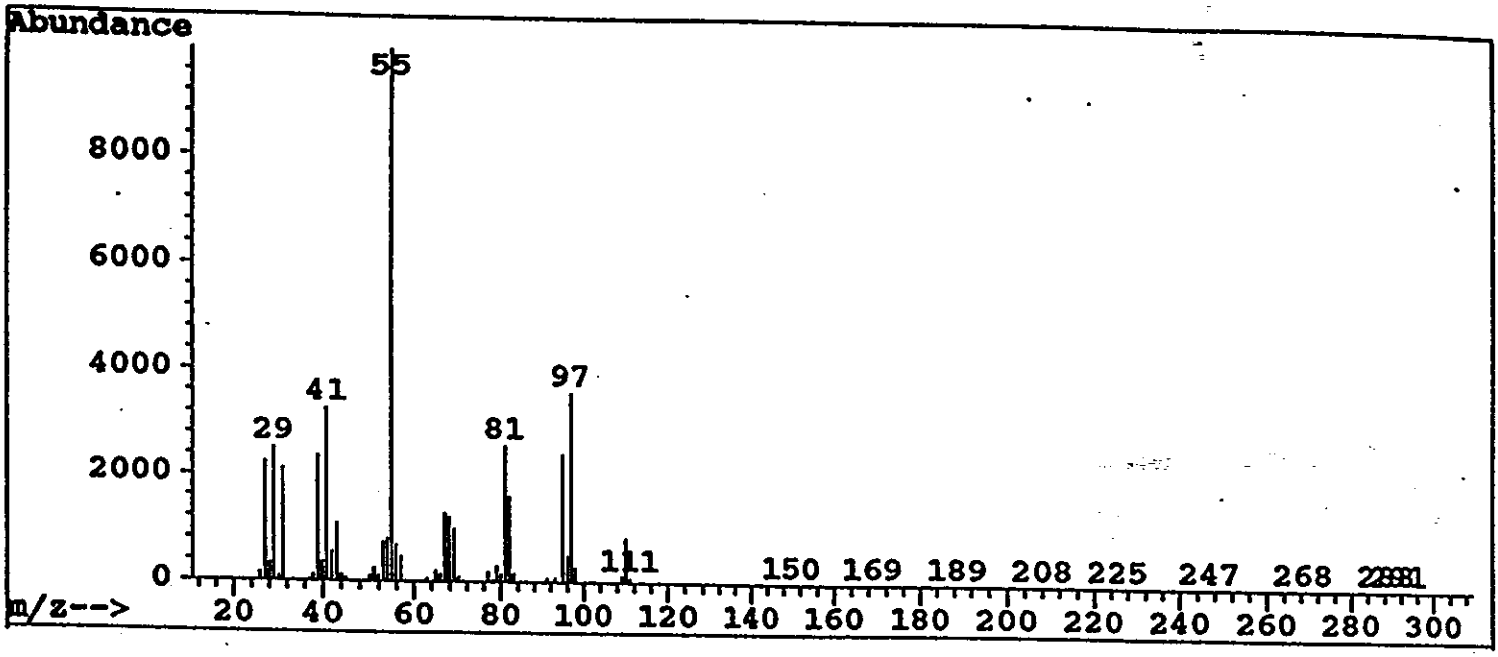


FIGURE VI

GC/MS Spectrum of the RRT 1.02 Peak

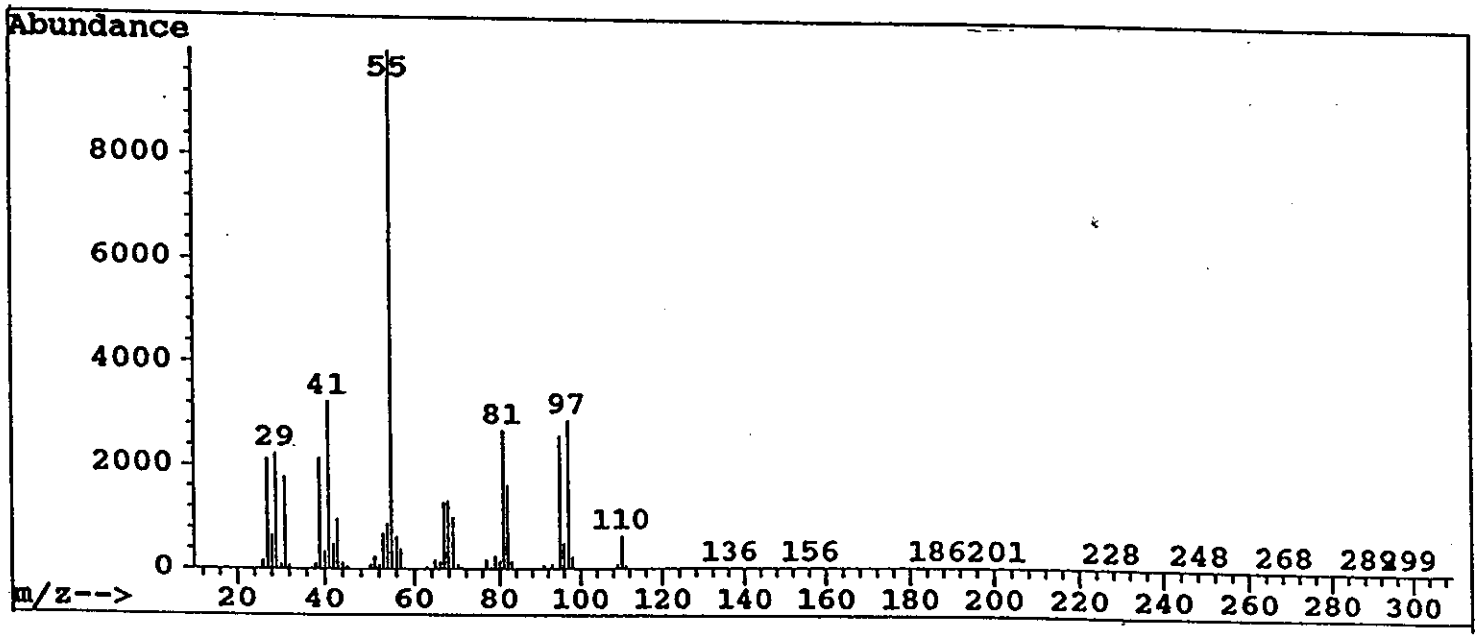
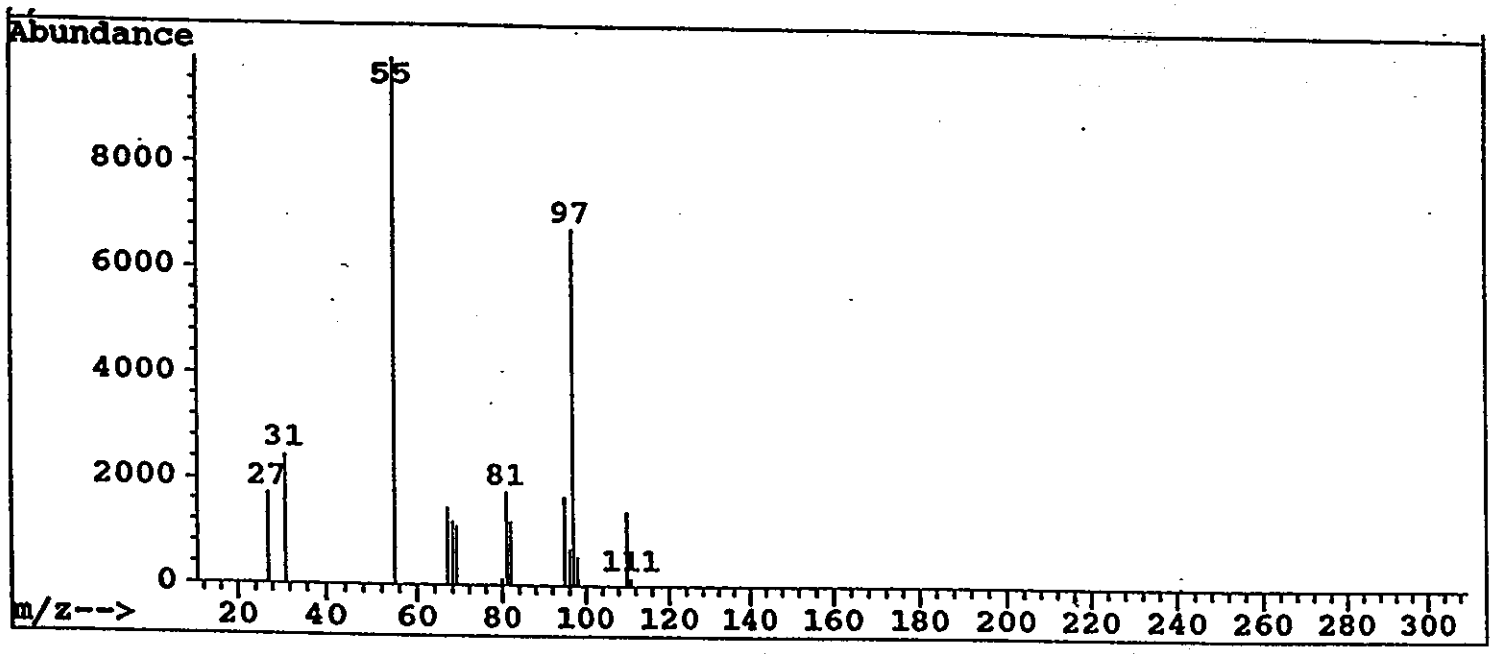


FIGURE VII

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 VALUE*	MEAN	S.D.	LOW	HIGH	N
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
a-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

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

Test Type: 96-hour Acute Minnow Test
24, 48, 72, & 96-hour Results
Test Substance HAEL #: 97-0216
Date/Time of Data Analysis: "Dec 24, 1997, 9:43:23"

LC_{50} Estimation Using Probit Model

Chi-Square Test for Lack of Model fit: p= 1.000
The lack of fit test indicates that the probit model is appropriate.

Concentrations Used:	6.25	12.5	25.0	50.0	100.0
# Dead per Conc.:	0	0	0	2	14
Total per Conc.:	14	14	14	14	14
Proportion Dead:	0	0	0	0.143	1.0

Estimated LC_{50}: 57.38
LC_{50} Standard Error: 1.40
The 95% Confidence Interval For The LC_{50}: 29.71 & 110.81