

**FINAL REPORT**

**Crude MCHM**

**HAEL No. 97-0216**  
**CAS No. NA**

**EAN 972790**  
**PM No. 18717-00**

**DETERMINATION OF READY BIODEGRADABILITY (BIOTIC DEGRADATION)  
USING THE CO<sub>2</sub> EVOLUTION TEST (MODIFIED STURM)**

**GUIDELINES**

OECD 301B and EEC/Annex V C.4

**AUTHORS**

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**LABORATORY PROJECT ID**

Study No. EN-113-972790-A

**STUDY SPONSOR**

Eastman Chemical Company

**STUDY COMPLETION DATE**

December 3, 1997

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: MORRIS, J.D. PAGE 1  
ACCESSION NUMBER: 972790 11/18/97

STUDY TYPE: BIODEGRADATION (READY-MODIFIED STURM TEST)

M. L. James  
(AUDITOR, QUALITY ASSURANCE UNIT)

11/18/97  
DATE

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THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT. WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES.  
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INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
09/30/97	PROTOCOL APPENDIX/AMENDMENT SUBMISSION TEST SUBSTANCE WEIGH AND MIX WITH CARRIER TEST SUBSTANCE CARRIER MIXTURE ADDITION TO TEST SYSTEMS	11/18/97
11/18/97	FINAL REPORT REVIEW	11/18/97


## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT


This study was conducted according to:

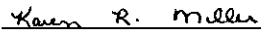
United States Food and Drug Administration, Good Laboratory Practice for  
Nonclinical Laboratory Studies, 21 CFR Part 58;

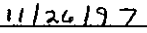
United States Environmental Protection Agency, Toxic Substances Control  
Act, Good Laboratory Practice Standards, 40 CFR Part 792;

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

  
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Study Director  
Jeffrey D. Morris, Ph.D.

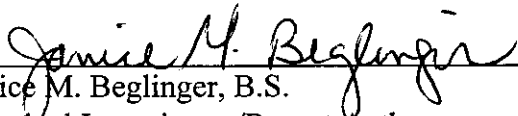
  
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Sponsor's Representative  
Karen R. Miller, Ph.D.

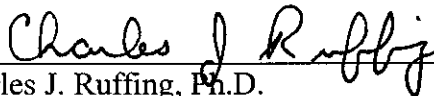
  
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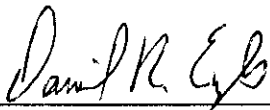
**DETERMINATION OF READY BIODEGRADABILITY (BIOTIC DEGRADATION)  
USING THE CO<sub>2</sub> EVOLUTION TEST (MODIFIED STURM)**

  
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Janice M. Beglinger, B.S.  
Principal Investigator/Report Author

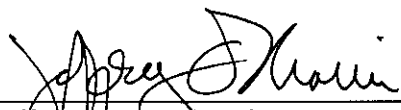
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**Crude MCHM**

**HAEL No. 97-0216**  
**CAS No. NA**

**EAN 972790**  
**PM No. 18717-00**

**DETERMINATION OF READY BIODEGRADABILITY (BIOTIC DEGRADATION)  
USING THE CO<sub>2</sub> EVOLUTION TEST (MODIFIED STURM)**

**ABSTRACT**

A 28-day biodegradation test was performed to determine the ready biodegradability of the test substance. A sample of activated sludge (mixed liquor suspended solids) from a domestic waste water treatment plant was used as the source of unacclimated microorganisms for the test. Biodegradability was determined by measuring the amount of carbon dioxide evolved from the test substance. The test substance at a theoretical concentration of 20 mg DOC/L showed 53% degradation (Test vessel #1), and 54% degradation (Test vessel #2) after 28 days, based on carbon dioxide evolution. In order for the test substance to be classified as readily biodegradable, it must first reach 10%. Then there is a 10-day time window in which 60% degradation must occur. A lag phase of 9 days occurred before biodegradation reached 10%. The test substance did not reach 60% within the 10-day time window requirement. These results indicate that the test substance was not readily biodegradable under the test conditions. DOC concentrations were determined for all test solutions at the test start and end. Loss of DOC for the test substance was 94% (Test vessel #1) and 99% (Test vessel #2) at test end.

It should be noted that the test substance, as received, is crude material containing significant impurities including water. If carbon dioxide evolution is normalized to the amount of active ingredient, the percent biodegradation calculated would suggest ready biodegradability. However, because some of the impurities contain significant amounts of carbon it is not possible to quantify theoretical CO<sub>2</sub> evolution in terms of active ingredient alone.

Although the test substance could not be classified as readily biodegradable, it is unlikely that the test substance would persist in an aquatic environment as evidenced by the significant carbon dioxide evolution.

## 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-113-972790-A

### Sponsor

Eastman Chemical Company  
Sponsor's Representative: Karen R. Miller, Ph.D.

### Study Dates

Study Initiation Date: September 29, 1997  
Sludge Preparation Date: September 29, 1997  
Experimental Start (Chemical Exposure) Date: September 30, 1997  
Experimental Termination Date: October 28, 1997

### Project Participants

#### Environmental Sciences Section

Study Director: J.D. Morris, Ph.D.  
Principal Investigator/Report Author: J.M. Beglinger, B.S.

### Test Substance Characterization

Test Substance Name:	Crude MCHM
HAEL No.:	97-0216
EAN:	972790
CAS No.:	NA
PM No.:	18717-00
SRID or Lot No.:	6-97
Physical State and Appearance:	Liquid, clear, colorless
Solubility:	Appreciable (in water)

### Positive Control

Identity: Sodium benzoate  
CAS Reg. No.: 000532-32-1  
Purity: 103%

## **Purity Analysis, Structure Confirmation, and Stability Determination**

Purity Analysis: 60.9% (area) by GC/FID, Appendix pp. 7 - 26.

Structure Confirmation: Confirmed by GC/MS, Appendix pp. 7 - 26.

Stability Determination: As part of this test, the test substance is exposed to activated sludge, which may degrade the test substance. This study is designed to measure biodegradation of the test substance and/or any decomposition products.

### **Acronyms**

DOC - dissolved organic carbon, measured as mg/L or ppm

ThCO<sub>2</sub> - theoretical amount of carbon dioxide a chemical could produce

BSM - basal salts medium

### **PURPOSE**

The purpose of this test is to determine the ready biodegradability of the test substance by unacclimated microorganisms by measuring the amount of carbon dioxide (CO<sub>2</sub>) evolved from the chemical over the 28-day test period.(1, 2) The degradation is defined as the CO<sub>2</sub> produced by the substance, as a percentage of the theoretical CO<sub>2</sub> it could have produced (ThCO<sub>2</sub>). The organic carbon content of the test substance is used to calculate ThCO<sub>2</sub>.

### **MATERIALS AND METHODS**

#### **Test System**

Activated sludge microorganisms were obtained from the Van Lare Treatment Plant, Rochester, New York. Viability of the microorganisms was checked using an Easicult® TTC dip slide to estimate microbe numbers. Microorganism activity was checked by means of a positive control. In studies of this sort no attempt is made to identify the large variety of organisms present in the samples or the degree of absorption of the test and control substance.

#### **Experimental Design**

##### Preparation of the Inoculum

On arrival at the laboratory, the activated sludge was aerated for about four hours. A sample (about 2000 mL) of the mixed liquor was collected and homogenized for two minutes with a mechanical blender. It was allowed to settle for approximately one hour. The supernatant was pipetted to provide sufficient volume of inoculum for each carboy. Viability of the supernatant microorganisms was confirmed by using an Easicult® TTC dip slide to estimate microbe numbers after inoculation of the test solutions.



### Preparation of Test Solutions

1. Distilled water was used for the preparation of the Basal Salts Medium (BSM), and the other solutions.
2. Basal Salts Medium (BSM) was prepared according to Table 1. The pH was not adjusted because it was within the required range of  $7.4 \pm 0.2$ .
3. A Positive Control Stock Solution of sodium benzoate was prepared (102.9 mg/500 mL purged BSM). The pH of the stock solution was not adjusted because it was within the required range of 3 to 10.
4. A Test Substance Stock Solution of 20 mg DOC/L was prepared (27 mg/L purged BSM). The pH of the stock solution was not adjusted because it was within the required range of 3 to 10.
5. Barium Hydroxide Solution, (0.0125M): 4.0 g of  $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$  was dissolved per liter of distilled water. The solution was filtered and the clear filtrate was stored in airtight containers to prevent absorption of  $\text{CO}_2$  from the air. A total of 12.0 L was prepared in order to have sufficient solution for the complete test.

### Test Procedure

The  $\text{CO}_2$  scrubbing apparatus was set up to remove  $\text{CO}_2$  from the air supply (at a constant rate) to the carboy solutions. This was done by diverting the air through a drying column (containing Drierite<sup>®</sup>), a  $\text{CO}_2$  absorption column (containing Ascarite II<sup>®</sup>), and flow meters before being bubbled through the test carboys. The air passes through the system at a rate of approximately 50-100 mL/min. (1-2 bubbles/sec), although this was not measured. To protect the test carboys from light, they were covered with aluminum foil for the duration of the study.

Five inoculated carboys were required for the testing of the test substance: two for the Inoculum Blank, one for the Positive Control, and two for the test substance. BSM was prepared first (see Table 1). The carboys were filled with 2400 mL of BSM. Next, 100 mL of the prepared inoculum supernatant was added to each carboy. This mixture was aerated with  $\text{CO}_2$ -free air for approximately 24 hours to purge the system of carbon dioxide.

After the aeration period, the test substance was added to two of the carboys to begin the test period. The chemical was tested at theoretically 20 mg DOC/L. The test substance was prepared in purged BSM (168 mg/L BSM) and added as appropriate aliquots (500 mL) to the test carboys. To the carboys used as Inoculum Blank controls (containing no test substance) 500 mL of purged BSM was added. Due to a concurrent test substance using a carrier, Blank #1 had approximately 3 g of glass chromatography beads added to the test solution. Another carboy was used for the Positive Control (20 mg DOC/L): 500 mL of the Positive Control Stock Solution (102.9 mg/500 mL) was added to this carboy. The final volume in each carboy was 3000 mL. Each carboy was agitated with a magnetic stir bar. A volume of 100 mL of 0.0125M  $\text{Ba}(\text{OH})_2$  was introduced into each of three  $\text{CO}_2$  absorber bottles and connected in series to the exit air line of each carboy. A 12 mL aliquot was withdrawn from all test vessels for DOC (dissolved organic carbon) analysis.

## **Experimental Design, continued**

At test start, CO<sub>2</sub>-free air was bubbled through the solutions at a rate of about 50 to 100 mL/minute (one to two bubbles per second) at a temperature of 20°C. The course of degradation was followed by performing titrations over the 28-day test period. The CO<sub>2</sub> produced in each carboy reacts with the barium hydroxide in the absorber bottles where it is precipitated as barium carbonate; the amount of CO<sub>2</sub> produced was determined by titrating the barium hydroxide remaining in solution with 0.05N standardized HCl.

Periodically, the CO<sub>2</sub> absorber bottle nearest the carboy was removed for titration. The remaining two absorber bottles were moved one place closer to the carboy, and a new absorber bottle containing 100 mL of fresh 0.0125M Ba(OH)<sub>2</sub> was placed at the far end of the series.

On the 27th day, titrations were performed as usual. A 10 mL aliquot of test solution was removed to measure pH. Next, 1 mL of concentrated HCl was added to each carboy to drive off inorganic carbonate. The carboys were aerated overnight. Final titrations were performed on day 28. Samples (12 mL) from the test carboys were removed for final DOC analysis on day 28.

## **Optional Procedures**

### Additional Controls

An additional set of 3 absorber bottles containing 0.0125M Ba(OH)<sub>2</sub> was connected directly to the scrubbed air line and titrated in the same manner as the test. These served as the air line control. The barium hydroxide used throughout the study was also monitored by titrating the stored solution at weekly intervals, and when first prepared. The two sets of titration data were compared in order to demonstrate that the air supply was free of carbon dioxide.

### Microbiology

An Easicult® TTC dip slide was used to estimate microbial numbers in the supernatant used to inoculate the test. The microbe count of the supernatant was 10<sup>5</sup> orgs/mL.

## **Analytical Measurements**

### Carbon Dioxide Determination

Titrations on the 100 mL Ba(OH)<sub>2</sub> solutions were made after removing the bottles closest to the carboys. The Ba(OH)<sub>2</sub> was titrated with 0.05N HCl, using phenolphthalein as an indicator. Titrations were made as deemed appropriate for the first 10 days, and then twice a week until the 27th day. Titrations were performed before the addition of acid on the 27th day. Final titrations were made on day 28.

## **Analytical Measurements, continued**

### Temperature and pH

Air temperature was recorded during the study on titration days. The pH of the BSM and of the Positive Control Stock Solution was measured at test start. The pH was measured in all carboys on day 27, before the addition of concentrated HCl.

### DOC Measurements

DOC concentrations were determined for all test solutions at the test start and end.

## **Data Storage**

All raw data and summaries of data will be retained for at least ten years in the testing facility archive managed under GLP-mandated conditions.

## **Data Evaluation and Interpretation**

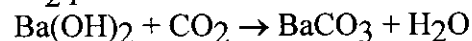
Lotus 1-2-3<sup>®</sup> was used for data calculations and tabulations, and also to generate the graph showing percent degradation over time.

Owing to the stringency of this test, a low result does not necessarily mean that the test compound is not biodegradable under environmental conditions. Test substances showing high biodegradation in this test should be regarded as readily biodegradable provided that this level (60%) is reached within the 10-day time window.<sup>(3)</sup>

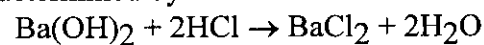
## **Calculations**

The total amount of CO<sub>2</sub> produced over the test period is calculated as a percentage of the total theoretical CO<sub>2</sub>. The first step in the calculation is to correct the test carboys for endogenous CO<sub>2</sub> production. The inoculum blank carboy serves as the control for this purpose. The amount of CO<sub>2</sub> produced by a test substance is determined by the difference (in mL of titrant) between the test and inoculum blank Ba(OH)<sub>2</sub> traps.

When using 0.05N HCl to titrate the absorber bottle, each 1.0 mL of HCl corresponds to 1.1 mg of CO<sub>2</sub> produced:



The BaCO<sub>3</sub> formed is insoluble and precipitates. The amount of Ba(OH)<sub>2</sub> remaining in solution is determined by titration with HCl according to the following equation:



### Calculations, continued

From the above two equations it can be seen that 1 mmol of CO<sub>2</sub> is produced for every 2 mmol of HCl. Therefore the number of mmol of CO<sub>2</sub> produced is:

$$\begin{aligned}\text{mmol CO}_2 &= \text{mmol HCl}/2 \\ &= (0.05 \times \text{mL of HCl titrated})/2 \\ \text{mg CO}_2 &= [(0.05 \times \text{mL of HCl titrated})/2] \times \text{mol. wt. of CO}_2 \\ &= 1.1 \times \text{mL of HCl titrated}\end{aligned}$$

$$\% \text{ Biodegradation} = \frac{(I_1 + I_2 + \dots + I_n) - (T_1 + T_2 + \dots + T_n)}{\text{ThCO}_2 \times \text{mg of test substance}} \times 1.1 \times 100$$

Where:

T<sub>1</sub> ... T<sub>n</sub> = mL of HCl titrated at time 1 ... time n for the Test

I<sub>1</sub> ... I<sub>n</sub> = mL of HCl titrated at time 1 ... time n for the Inoculum Blank

ThCO<sub>2</sub> = Theoretical CO<sub>2</sub> (mg CO<sub>2</sub>/mg test substance)

$$= \frac{\text{No. of carbon atoms in test substance} \times \text{mol. wt. of CO}_2}{\text{mol. wt. of test substance}}$$

The CO<sub>2</sub> concentrations were calculated to the nearest 0.1 mg/L. The biodegradation values were rounded up to the nearest whole percent.

### Test Validity

The positive control yielded ≥ 60% biodegradation (≈ 66%) by day 14. No significant amount of CO<sub>2</sub> (40 mg CO<sub>2</sub>/L of medium) was evolved from the Inoculum Blank during the test. These conditions fulfill the requirements for a valid test, as stated in the protocol (EN-113). In addition, the data from the air line controls (see Additional Controls, p. 10) show, by comparison with those of the barium hydroxide stock solution, that CO<sub>2</sub> was scrubbed properly from the incoming air line.

### Protocol and Standard Operating Procedure Deviations

The protocol states: "The test is run at a temperature of 22 ± 2°C." The test temperature on Day 20 fell to 19°C. This deviation does not affect the outcome nor integrity of the study.

## RESULTS

Cumulative biodegradation remained at zero until about Day 1. After Day 1, biodegradation began in both test vessels. Biodegradation in both test carboys reached 10% on Day 9. Ten days later, Test vessel #1 reached ≈42% degradation, while Test vessel #2 reached ≈45% degradation (refer to Figure 1). Test vessels #1 and #2 reached 53% and 54% degradation, respectively, at test end. The Positive Control, sodium benzoate, yielded 74% of the theoretical carbon dioxide possible over the course of the test. The graph showing percent biodegradation for both Test

vessels and the Positive Control is provided in Figure 1.

No unusual variation in pH was noted in any of the test carboys during the study. The DOC of the Positive Control fell from 19.5749 ppm to -0.2723 ppm over the test period, resulting in a 101% loss of DOC over the test period. The DOC of the test substance fell from 14.8252 ppm to 0.8789 ppm for Test vessel #1. Test vessel #2 DOC fell from 16.0958 ppm to 0.1624 ppm. The loss of DOC for the test substance was 94% (Test vessel #1) and 99% (Test vessel #2).

The Inoculum Blanks released an average of 32.5 mg CO<sub>2</sub> over the test period. The barium hydroxide stock solution needed  $49.5 \pm 0.2$  mL of titrant per titration compared to  $49.4 \pm 0.4$  mL for the air line control. This indicates that the air line did not contain carbon dioxide after scrubbing. The average temperature during the test period was  $20 \pm 0.6^\circ\text{C}$  (range 19 - 21°C).

## DISCUSSION

A 28-day test for ready biodegradability using unacclimated microorganisms as the inoculum showed 53% and 54% degradation of the test substance at 20 mg DOC/L (theoretical), based on carbon dioxide evolution. In order for the test substance to be classified as readily biodegradable, it must first reach 10%. Then there is a 10-day time window in which 60% degradation must occur. A lag phase of 9 days occurred before biodegradation reached 10%. The test substance did not reach 60% within the 10-day time window requirement. Due to the stringency of this test, low CO<sub>2</sub> evolution does not necessarily mean that the test substance is not degradable under environmental conditions, or after waste water treatment.

It should be noted that the test substance, as received, is crude material containing significant impurities including water. If carbon dioxide evolution is normalized to the amount of active ingredient, the percent biodegradation calculated would suggest ready biodegradability. However, because some of the impurities contain significant amounts of carbon it is not possible to quantify theoretical CO<sub>2</sub> evolution in terms of active ingredient alone.

## CONCLUSION

These results indicate that the test substance was not readily biodegradable under the conditions of this study. Although the test substance could not be classified as readily biodegradable, it is unlikely that the test substance would persist in an aquatic environment as evidenced by the significant carbon dioxide evolution.

## REFERENCES

1. Determination Of 'Ready' Biodegradability: Carbon dioxide Evolution (Modified Sturm Test), Method C.4-C, 1993. Official Journal of the European Communities, pp. 187-206.
2. OECD Guideline For Testing Of Chemicals, 1992. Ready Biodegradability Test Guideline 301B: CO<sub>2</sub> Evolution Test (Modified Sturm).
3. OECD, Paris, 1981, Test Guideline 301B, Decision of the Council C(81) 30, final.

**TABLE 1. Composition of Basal Salts Medium and Stock Solutions**

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Stock Solutions:

A. Phosphate Solution

$\text{KH}_2\text{PO}_4$  (potassium dihydrogen phosphate): 8.50 g

$\text{K}_2\text{HPO}_4$  (dipotassium hydrogen phosphate): 21.75 g

$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  (disodium monohydrogen phosphate dihydrate): 33.40 g

$\text{NH}_4\text{Cl}$  (ammonium chloride): 0.50 g

Dissolve in and make up to 1000 mL with distilled water.

The pH should be 7.4.

B. Calcium Chloride Solution

$\text{CaCl}_2$  (calcium chloride, anhydrous): 27.50 g

Dissolve in and make up to 1000 mL with distilled water.

C. Magnesium Sulfate Solution

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (magnesium sulfate heptahydrate): 22.50 g

Dissolve in and make up to 1000 mL with distilled water.

D. Ferric Chloride Solution

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (iron(III) chloride hexahydrate): 25 mg

Dissolve in and make up to 100 mL with distilled water.

Stock Solution Preparation: Stock solutions A-C may be made up in advance, filter sterilized through 0.45 $\mu\text{m}$  membrane, and refrigerated until needed (for a period up to 6 months). Stock solution D is made up immediately before use. If a precipitate forms in a stock solution, replace it with fresh.

Test Medium: BSM is prepared before use in distilled water and is prepared as follows: Mix 10 mL of solution A with 800 mL distilled water, then add 1 mL each of solutions B, C, and D. Bring volume to 1 L with distilled water. The pH value should be  $7.4 \pm 0.2$ . The BSM may be prepared in advance (up to 48 hours) as long as it is covered and refrigerated. If the solution is cloudy it should not be used.

**APPENDIX**

**DETERMINATION OF READY BIODEGRADABILITY (BIOTIC DEGRADATION)  
USING THE CO<sub>2</sub> EVOLUTION TEST (MODIFIED STURM)**

**TABLE OF CONTENTS FOR APPENDIX**

**DETERMINATION OF READY BIODEGRADABILITY (BIOTIC DEGRADATION)  
USING THE CO<sub>2</sub> EVOLUTION TEST (MODIFIED STURM)**

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Total Number of Appendix Pages: 26



STUDY SPECIFIC PROTOCOL AMENDMENTS

Protocol: Determination of Ready Biodegradability (Biotic Degradation) using the CO<sub>2</sub> Evolution Test (Modified Sturm) OECD 301B

*The following information pertains to corresponding sections of Protocol EN-113-XXXXXX-X.*

III. C. Dates of Experiment

Study Initiation Date: September 29, 1997

Estimated Experimental Start (Chemical Exposure) Date: September 30, 1997

Estimated Experimental Termination Date: October 28, 1997

Estimated Study Completion Date: December 28, 1997

IV. Test and Control Substances

A. Test Substance

Name: Crude MCHM

HAEL No.: 97-0216

EAN: 972790

PM No.: 18717-00

Sample Reference No.: 6-97

CAS Reg. No.: NA

Laboratory Project ID: Study No. EN-113-972790-A

Other Information (Composition, Stability, Purity, Solubility, etc.):

According to the MSDS solubility in water is appreciable. DOC will be performed for this test substance. A carrier will be added to one of the Blanks (but not the Positive Control) as a concurrent test substance will use a carrier.

Concentration Tested: 27.0 mg/L, 20 mg DOC/L

ThCO<sub>2</sub> 2.75 mg CO<sub>2</sub>/mg test article

V. Source of Inoculum

Fresh Activated Sludge: Van Lare Waste Water Treatment Plant

VI. Optional Procedures to be Performed

Additional System Controls Yes (Yes/No)

Microbial Counts for inoculum supernatant using dip slides Yes (Yes/No)

Concentration Determination No (Yes/No) If Yes, see Attachment 2.

VIII. Test Method

B. Test Procedure

Five carboys will be required: two for inoculated BSM (Inoculum Blank), one for inoculated Positive Control Solution, and the last two for inoculated replicate Test Solutions. First, the carboys are filled with 2400 mL of BSM. Then, 100 mL of the MLSS supernate inoculum are added to each carboy. This mixture is aerated with CO<sub>2</sub>-free air for approximately 24 hours, to purge the system of carbon dioxide.

XIV. Reporting and Records

B. Records

All records will be maintained by the Health and Environment Laboratories for at least 10 years.

Attachment 1

Test Medium: BSM is prepared before use in distilled water and is prepared as follows: Mix 10 mL of solution A with 800 mL distilled water, then add 1 mL each of solutions B, C, and D. Bring volume to 1 L with distilled water. The pH value should be  $7.4 \pm 0.2$ . The BSM may be prepared in advance (up to 48 hours) as long as it is covered and refrigerated. If the solution is cloudy it should not be used.

REMARKS:

Amount of test substance to be used in testing was calculated to be 27 mg/L. As the test substance is a liquid, it may not be possible to measure that exact amount. The exact amount used to make the stock solution will be recorded on the data sheets and used in the spreadsheet calculations. DOC samples will be taken at test start and end. Approximately 12 mL of test solution will be withdrawn from each test vessel and filtered using 0.22µm filters. Samples will be measured in duplicate using the OI Analytical Organic Carbon Analyzer.

Protocol No. EN-113-900220-A  
Revision No.

Attachment 2. Analytical Method for Test Article

GC \_\_\_\_\_ HPLC \_\_\_\_\_ UV-VIS \_\_\_\_\_ OTHER \_\_\_\_\_

Instrument: \_\_\_\_\_

Column: \_\_\_\_\_

Conditions: \_\_\_\_\_

Detection: \_\_\_\_\_

Sample Size: \_\_\_\_\_

Calibration: \_\_\_\_\_

Sample Prep.: \_\_\_\_\_

Remarks: \_\_\_\_\_

Jeffrey D. Morris  
Study Director

9/29/97  
Date

Kevin R. Miller  
Sponsor's Representative

10/6/97  
Date

EAN 972790

HAEI No. 97-0216

CO2 EVOLUTION (MODIFIED STURM) READY BIODEGRADABILITY TEST  
TITRATION DATA WORKSHEET

DATE	DAY	AIRLINE	BLANK	BLANK CUMUL CO2	CONTROL	CONTROL CUMUL % BIODEG.	TEST #1	TEST #1 CUMUL % BIODEG.	TEST #2	TEST #2 CUMULATIVE % BIODEG.
9/30/97	0			0.0		0		0		0
10/1/97	1	50.3	46.6	4.1	35.4	6	45.9	0	44.8	1
10/3/97	3	49.6	43.6	10.7	15.2	20	43.2	1	42.2	2
10/6/97	6	49.1	42.9	17.5	9.2	37	42.8	1	39.7	3
10/8/97	8	49.6	45.1	22.4	16.2	51	40.5	3	40.7	5
10/10/97	10	49.2	46.4	25.5	28.6	60	25.0	13	31.2	12
10/13/97	13	49.8	48.1	27.4	38.8	65	19.6	27	19.7	26
10/16/97	16	49.1	47.6	29.0	42.4	67	26.6	37	22.1	38
10/20/97	20	48.8	47.9	30.0	42.9	70	34.4	43	30.2	46
10/23/97	23	49.3	48.3	31.1	45.1	71	39.0	47	40.7	50
10/27/97	27	48.9	48.4	31.7	45.1	73	41.3	51	42.0	53
10/28/97	28	49.4	48.7	32.5	46.7	74	44.2	53	45.9	54

Airline, Blank, Control, Test #1, and Test #2 values are for mL of titrant (0.05 N HCl)

Blank Cumulative CO2 (mg) = (Airline - Blank) x 1.1

Cumulative % Biodeg. = [(Sum of mL Blank - Sum of mL Test or Control) x 1.1 x 100] / (ThCO2 x mg chemical)

Where mg Control = 102.90 and mg ThCO2/mg Control = 2.14  
 Where mg Test #1 = 84.00 and mg ThCO2/mg Test = 2.75  
 Where mg Test #2 = 84.00

Remarks:

Prepared by Jamie H. Beglinger

Date 10/29/97

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

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

Walter H. 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*

Nancy K. Porter, B.S.  
Study Director

*November 7, 1997*

Month/Day/Year

*Karen R. Miller*

Karen Miller, Ph.D.  
Sponsor Representative

*November 14, 1997*

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**

**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 (GC/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.

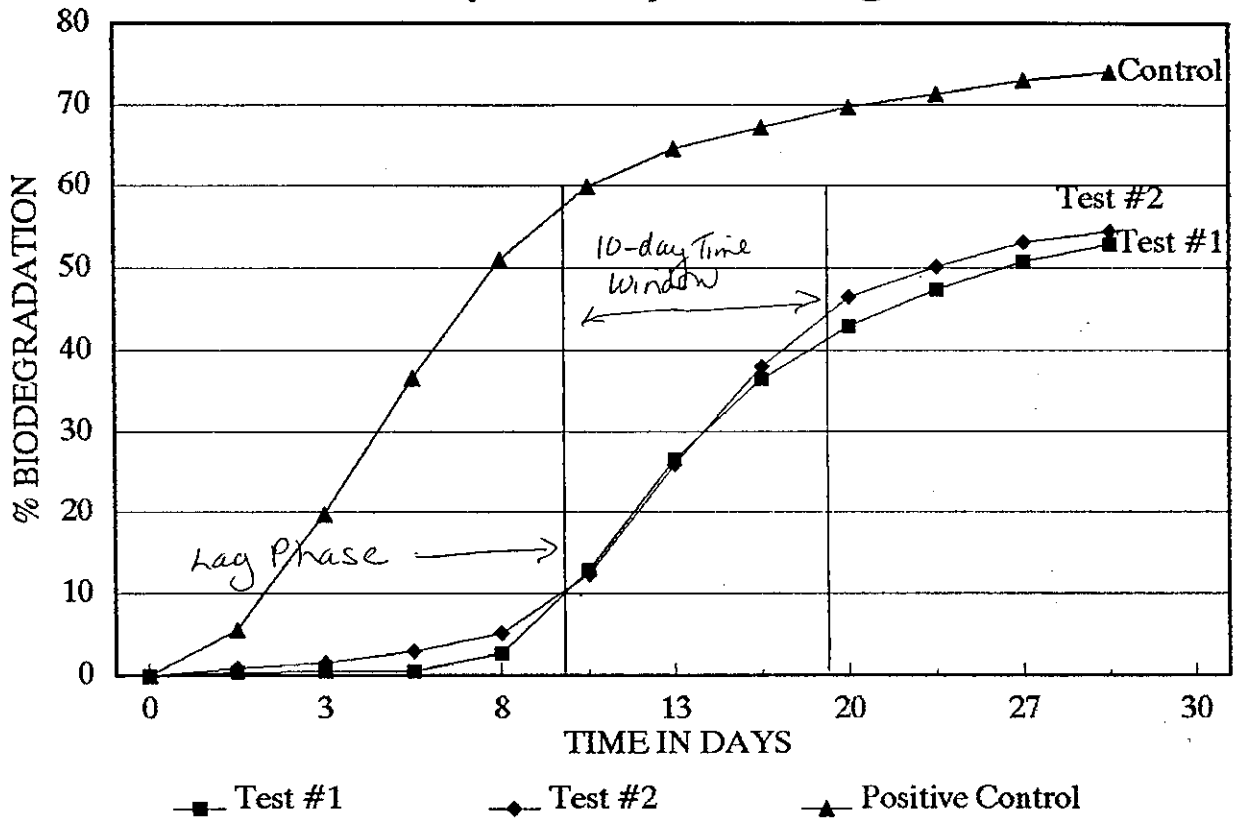
**Area Percent Purity Determination**

**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.

FIGURE 1. Graph Showing Percent Biodegradation

### CO2 Evolution (Sturm) Biodegradation Test



**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  $\mu$ 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  $\mu$ 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  $\mu$ m film thickness  
 Carrier Gas: Helium  
 Injection Size: 0.5  $\mu$ 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 H<sub>2</sub>O yields the ion at 110 m/z (see Figure V on page 19). The loss of CH<sub>2</sub>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<sub>4</sub>H<sub>7</sub>) (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

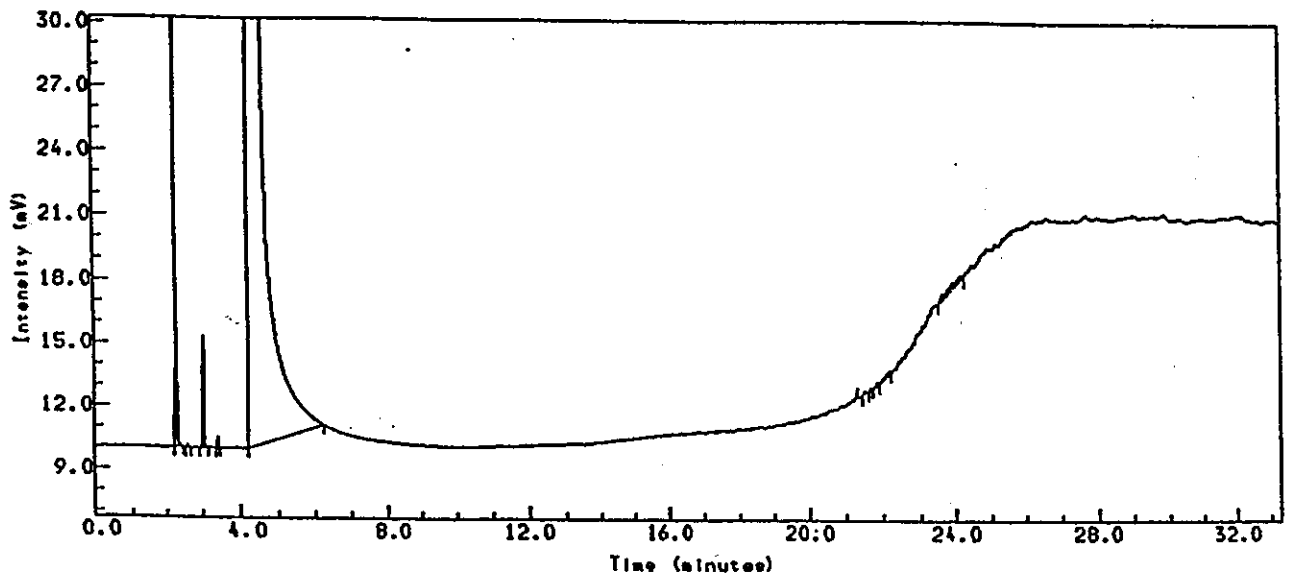


FIGURE II

Expanded Chromatogram of the Test Substance

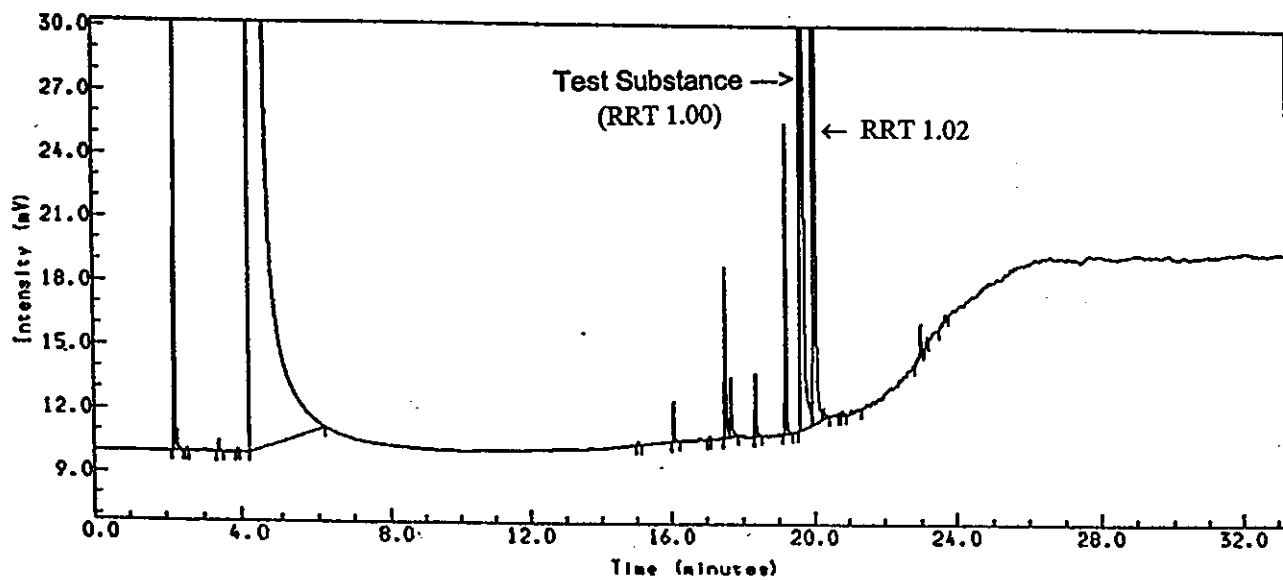


FIGURE III

Full Scale Chromatogram of the Test Substance

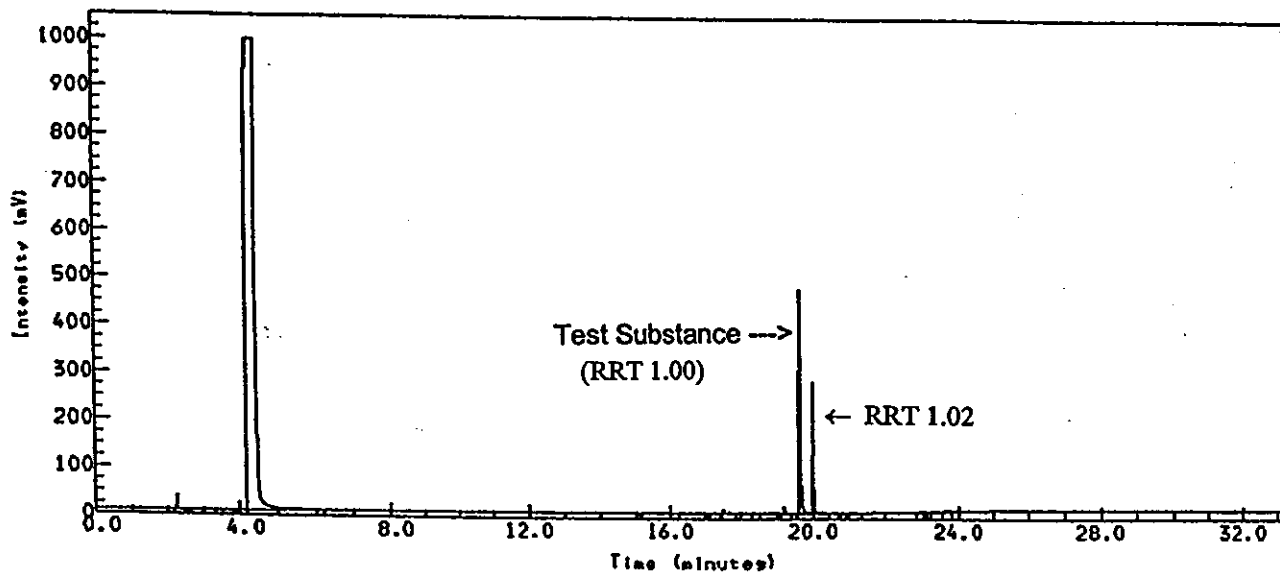


FIGURE IV

GC/MS Total Ion Chromatogram Of The Test Substance

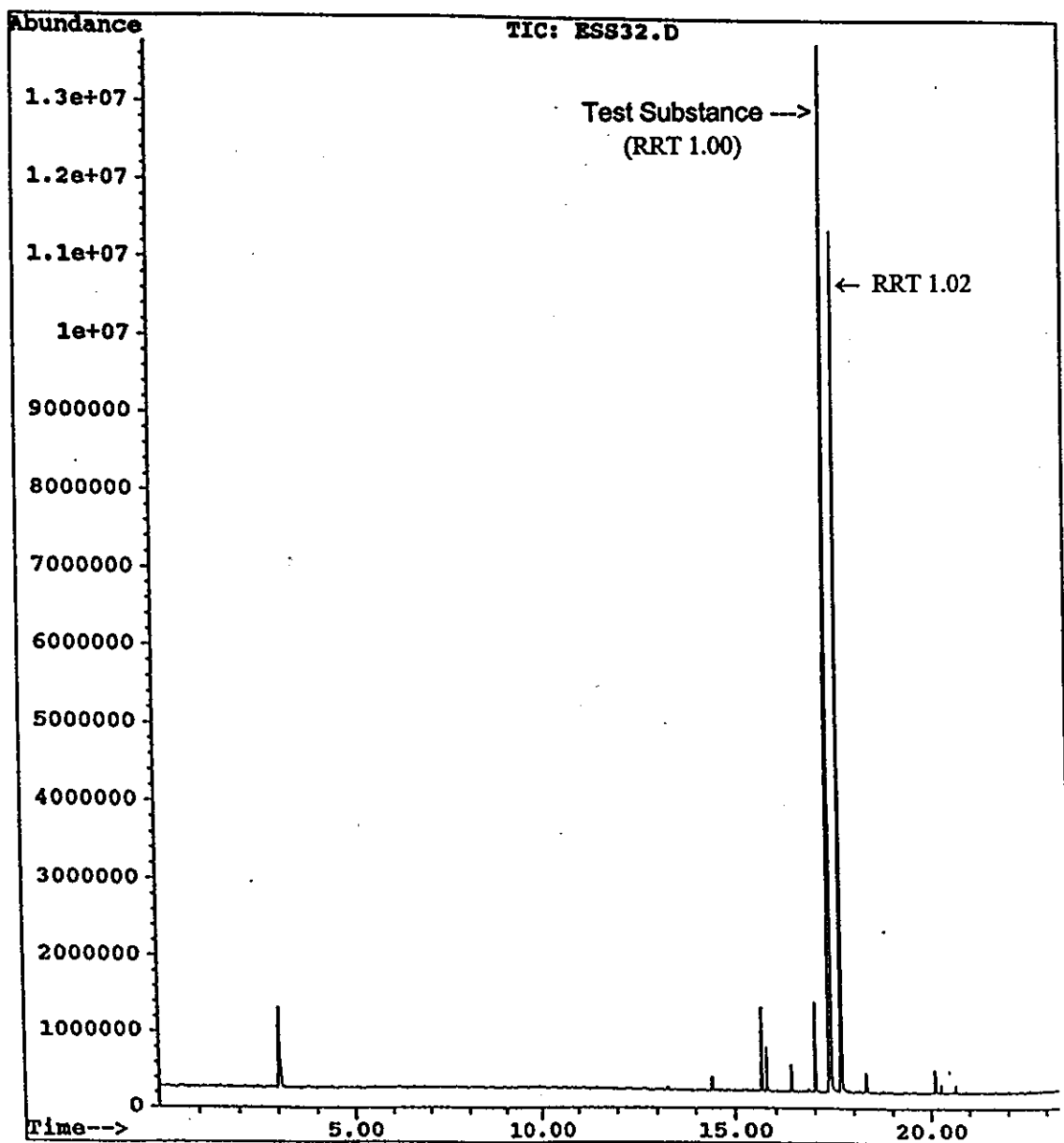


FIGURE V  
GC/MS Spectrum of the Test Substance

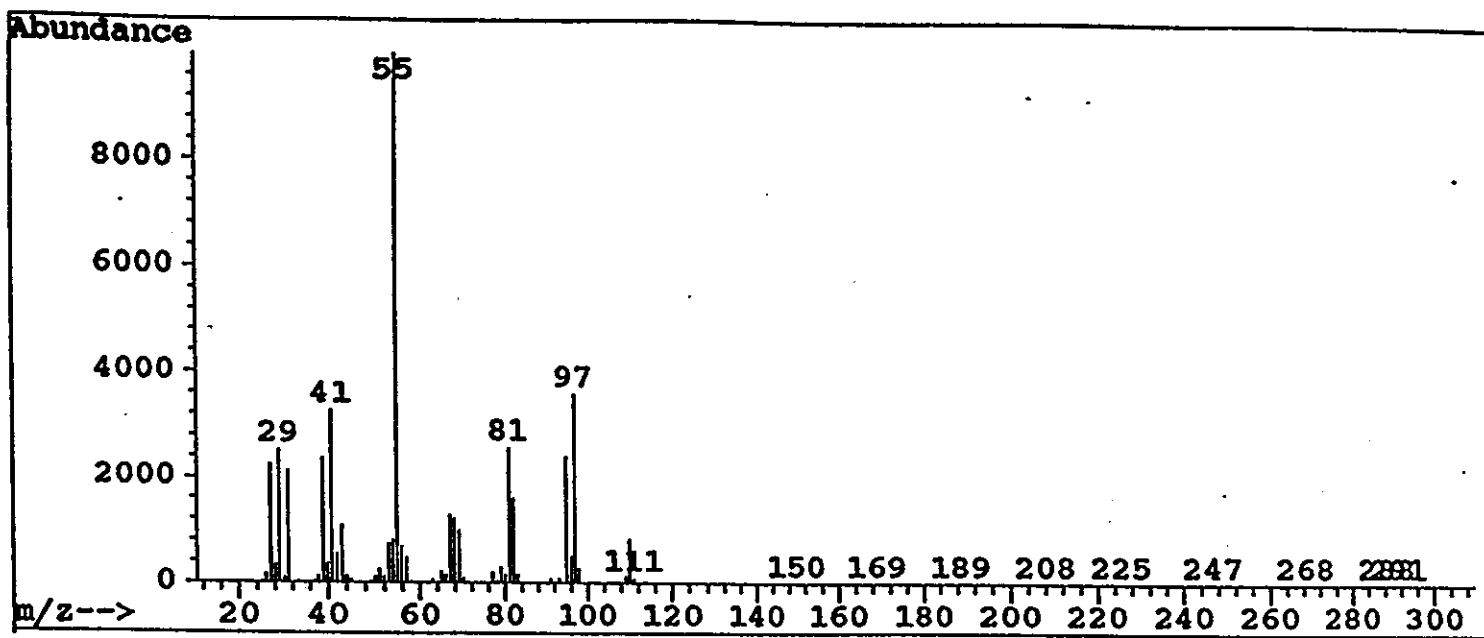


FIGURE VI  
GC/MS Spectrum of the RRT 1.02 Peak

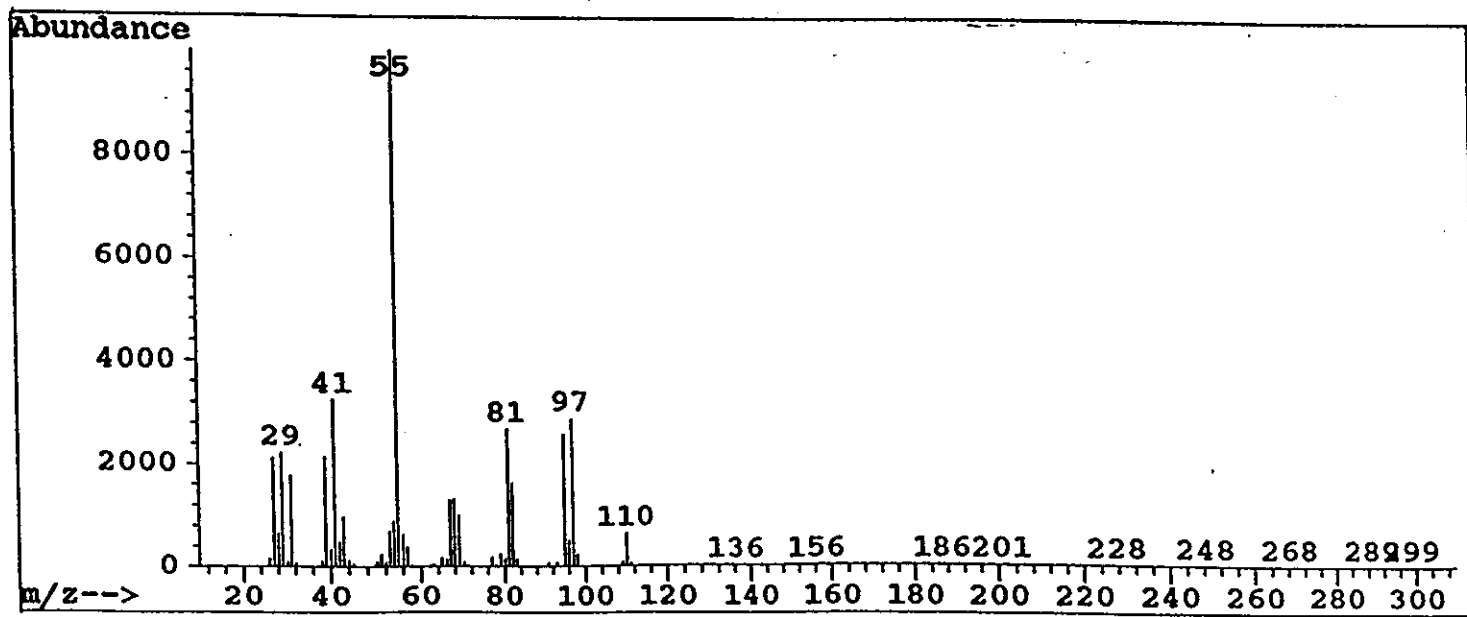




FIGURE VII

Library Reference Spectrum

