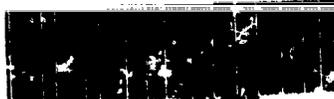


CODING FORMS FOR SRC INDEXING

Microfiche No.		OTS0001055	
New Doc ID	FYI-OTS-0794-1055	Old Doc ID	84950000004
Date Produced	02/13/89	Date Received	07/14/94
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Submitting Organization		GREAT LAKES CHEM CORP	
Contractor			
Document Title		INITIAL SUBMISSION: LETTER FROM GREAT LAKES CHEM CORP TO CORP/USEPA SUBMITTING INFO RE HEXABROMOCYCLODODECANE AND BIS (TRIBROMOPHENOXY) ETHANE W/ATTCHMTS, DATED 2/13/89	
Chemical Category		HEXABROMOCYCLODODECANE; BIS (TRIBROMOPHENOXY)	



Great Lakes
Chemical Corporation



74I-0794-001055
INIT 07/14/94

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February 13, 1989



84958000004

Ms. Roberta Wedge
Dynamac
11140 Rockville Pyke
Rockville, MD 20852

Dear Ms. Wedge:

Copies of the following study reports which you requested are enclosed:

Hexabromocyclododecane (CD-75P)

Acute toxicity of HBCD to the bluegill sunfish.
HBCD - biodegradation study.
Partition coefficients of dicamba, endrin, VEL 3510 and several industrial chemicals and flame retardants.
Water solubility of several industrial chemicals, flame retardants and a herbicide VEL-3510.
Hydrolysis of Firemaster 100.

Bis(tribromophenoxy)ethane (FF-680)

Acute toxicity (TL50) study in the rainbow trout.
Fish bioaccumulation with 1,2-Bis(tribromophenoxy)ethane.
Biodegradability study with ¹⁴C-tagged MC-680 (November 1975).
Biodegradability study with ¹⁴C-tagged MC-680 (June 1976).
Partition coefficient of MC-680, chlorendic anhydride and chlorendic acid.
Water solubility of several flame retardants and industrial chemicals.
Solubility of MC-680 in various class solvents.
Photolysis of Firemaster 680.
Rate of hydrolysis studies.

From the nature of the studies requested, you are obviously evaluating the possible impact of environmental releases of these compounds. We are aware of only a very few reports in which these compounds have been identified in the environment. This is not surprising, considering the ways in which these chemicals are produced, distributed, and used.

Both of these chemicals are non-volatile substances which are manufactured in closed systems fully equipped with devices to control emissions. Wastes from the processes are either recycled or disposed of in approved hazardous waste landfills. Equipment now under construction and expected to start up in March of this year will eliminate the use of landfill disposal

Both CD-75 and FF-680 are used as flame retardant additives for plastics. Incorporation into the polymer matrix immobilizes these compounds and further diminishes the likelihood of environmental release.

I hope you find these reports useful. Please let me know if I can be of further assistance.

Sincerely,



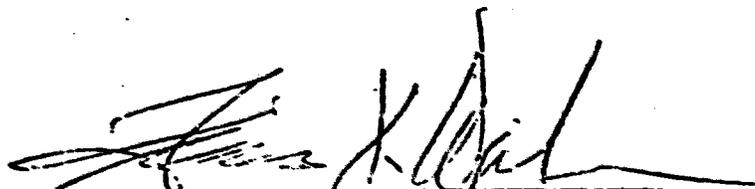
Dennis L. McFadden
Product Safety
Coordinator

DLM:sb:78
Enclosures

International Research and Development Corporation

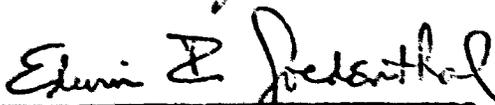
SPONSOR: Michigan Chemical Corporation
COMPOUND: Firemaster® 680, Lot No. 761-7
SUBJECT: Acute Toxicity (TL₅₀) Study
in the Rainbow Trout.

1,2-Bis-(tribromophenoxy)ethane
CAS# 37853-59-1



Francis X. Wazeter, Ph.D.
President

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Edwin I. Goldenthal, Ph.D.
Director of Research

Collaborator:

W. P. Dean

Date: December 13, 1974

NOTICE

These papers contain confidential matters which are the exclusive property of the Michigan Chemical Corporation.

No extract therefrom or any use or disclosure thereof may be made except upon proper authorization and for the sole benefit of the corporation.

134-014

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II. Compound.	2
III. Acute (TL ₅₀) Study in Rainbow Trout	3
A. Method.	3
B. Results	4

Table No.

1. Acute Toxicity (TL ₅₀) in Rainbow Trout.	6
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I. SYNOPSIS

An acute toxicity (TL₅₀) study in Rainbow Trout was conducted using Firemaster® 680 as the test material. Based upon the results obtained, the following (TL₅₀) values were derived.

24 hours: greater than 3160 mg/liter

48 hours: 2612 (2491-2738) mg/liter

96 hours: 1410 (1215-1637) mg/liter

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II. COMPOUND

The test compound was received from the Michigan Chemical Corporation, St. Louis, Michigan on October 17, 1974. It was identified as "Firemaster[®] 680, Lot No. 761-7" and was received as a white powder.

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III. ACUTE (TL₅₀) STUDY IN RAINBOW TROUT

A. Method:

Eighty Rainbow Trout obtained from a commercial trout producer in Tacoma, Washington, were used in this study. The fish had a mean weight of 1.90 grams and a mean length of approximately 45 mm.

The test fish were conditioned in the laboratory facilities for 21 days prior to testing, and were maintained on a standard fish food diet (PR-6, Glencoe Mills). No fish from the test group succumbed during the 8 day period immediately preceding the initiation of the test procedure. Fish selected for the bioassay were judged to be in an excellent state of health.

The bioassay was conducted in 5 gallon glass bioassay jars which were held in a constant temperature ($\pm 0.5^{\circ}\text{C}$) water bath. The trout were tested at 13°C .

The test water consisted of 16 liters of deionized water of at least 1 million ohms resistivity. The test water was reconstituted by adding the following per liter of water:

3 mg potassium chloride
30 mg calcium sulfate
30 mg magnesium sulfate
48 mg sodium bicarbonate

The pH of the test water was 6.2. The test water was aerated immediately prior to the filling of the jars and placing of the test fish into the bioassay containers.

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Page 4

A single introduction of the test material was made and the toxicity evaluated at 24 hour intervals for a total of 96 hours.

Fish used were of approximately the same weight and length ($\pm 20\%$). The fish were conditioned to the test (reconstituted) water over a 4 day period. The fish were not fed for a 3 day period prior to the initiation of the bioassay nor were they fed during the 96 hour study period.

The test material was suspended in reconstituted water, using sonification, in concentrations enabling the constant administration of 1 ml of the test material and diluent, per 16 liters of test water. Ten fish were used at each concentration. The mass/volume ratio did not exceed 1.19 grams of fish per liter of test water.

The test fish were placed in the bioassay jars 16 hours prior to the introduction of the test material.

Six concentrations of the test material were employed in a geometric progression, which allowed the calculation of a (TL₅₀). Two groups of 10 fish each were employed as negative (untreated) controls.

B. Results:

Based upon the results obtained, the acute toxicity (TL₅₀) for Firemaster 680 in Rainbow Trout was calculated to be as follows:

	(TL ₅₀) Value
	(Confidence Limits)
24 hours:	greater than 3160 mg/liter
48 hours:	2612 (2491-2738) mg/liter
96 hours:	1410 (1213-1637) mg/liter

There were no mortalities in the negative (untreated) control groups.

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Table 1 presents the (TL₅₀) values for 24, 48 and 96 hours and the observed percent mortalities for 24, 48, 72 and 96 hours.

Dissolved oxygen and pH were monitored during the test period. Dissolved oxygen levels were maintained above 5.0 mg/liter, aeration being conducted on the day of dosing (just prior to the introduction of the test material) and at 48 hours; pH ranged from 7.0 to 7.5.

The behavioral pattern exhibited by the fish at each concentration tested was as follows:

Concentration mg/liter	Observation Period			
	24 hours	48 hours	72 hours	96 hours
464	normal	normal	normal	normal
681	normal	normal	normal	9) normal 1) swimming in side-up position
1000	normal	normal	normal	9) normal 1) dead
1470	normal	5) normal 5) swimming in side-up position	7) normal 3) swimming in side-up position	1) lying on bottom and increased gill movement 4) swimming in side-up position 5) dead
2150	5) normal 5) swimming or lying upside down on bottom	10) lying on bottom and increased gill movement	8) lying on bottom and increased gill movement 2) swimming or lying upside down on bottom	10) dead
3160	10) lying on bottom and increased gill movement	10) dead	CONFIDENTIAL This document contains confidential information which is the property of Velsicol Chemical Corporation. It must be accounted for and returned, without reproduction, upon completion of the work involved or sooner upon request.	
Negative Control (1)	normal	normal	normal	normal
Negative Control (2)	normal	normal	normal	normal

Firemaster® 680:

Table 1. Acute Toxicity (TL₅₀) of Firemaster® 680 to Rainbow Trout at 24, 48 and 72 hours and Percent Mortality Observed at 24, 48, 72 and 96 hours.

Concentration mg/L.	Percent Mortality			
	24 hours	48 hours	72 hours	96 hours
464	0	0	0	0
681	0	0	0	0
1000	0	0	0	10
1470	0	0	0	50
2150	0	0	0	100
3160	0	100	100	100
Negative Control (1)	0	0	0	0
Negative Control (2)	0	0	0	0

*Statistical Reference:

Thompson, W. R., Bact. Rev., 11: 115-147, 1947 (Modified).

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APPENDIX I

Acute Toxicity (TL50) Study in the Rainbow Trout
with Firemaster[®] 680, Lot No. 761-7

Bromine Analysis

Bromine analysis was conducted for water samples collected from the bioassay test containers at 1 and 96 hours. The water samples were analyzed by Neutron Activation for bromine content. The values obtained are shown in the attached table.

Based upon the data obtained, bromine values markedly decreased during the 96 hour study period.

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Firemaster® 680: Acute Toxicity (TL₅₀) Study in the Rainbow Trout.

TABLE Bromine Content, ppm.

Test Compound Concentration mg/L.	Analysis of Bromine in Bicassay Containers	
	1 Hour	96 Hours
Control	1.0	0.2
464	2.0	3.3
681	2.5	1.1
1000	15.5	2.8
1470	38.4	5.4
2150	10.6	2.5
3160	276.0	8.9

134-014

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Fish Bioaccumulation with 1,2-Di-(tribromophenoxy)ethane

TEST REPORT

CAS # 37853-59-1

CHEMICAL BIOTESTING CENTER

CHEMICALS INSPECTION & TESTING

INSTITUTE, JAPAN

0039

attached paper

No. 1

METHOD FOR TESTING THE DEGREE OF ACCUMULATION
OF CHEMICAL SUBSTANCES IN FISH BODY

I. Scope of application

The standardized method for testing the degree of accumulation of chemical substances in fish body is as follows.

II. Terminology

The terminology used in this test method is in accordance with that used in the Japanese Industrial Standards (hereinafter referred to as the "JIS").

III. Test method

III-1. Test concentration of the test compound

Prior to initiation of the test on the degree of accumulation, an acute toxicity test is made as provided below the result of which is used as reference in determining the concentration of the compound to be subjected to the test.

1. Fish used in the acute toxicity test

Mature orangehead killifish (*Orizias Latipes*) (weighing 0.15 g to 0.5 g) is used. Diseased fish or those with abnormal external appearance and behavior should not be used.

2. Test water

The test water should be fresh underground water or dechlorinated city water sufficiently aerated so that the dissolved oxygen concentration is kept at about 7 ppm.

3. Test on acute toxicity (determination of TLm)

According to the method provided in JIS K 0102-§55, the test compound is dissolved in the test water (Note 1) and the 48-hours TLm (the concentration expressed in mg/l of the test compound at which 50 per cent of the test fish will die in 48 hours) is estimated and the TLm value is used as reference in determining the test concentration of the

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attached paper
No. 2

compound to be subjected to the test on the degree of accumulation.

(Note 1) If the test compound has little solubility in water, suitable solubilizers (such as ethyl alcohol, fatty acid esters of polyoxyethylene sorbitan, etc.) can be used.

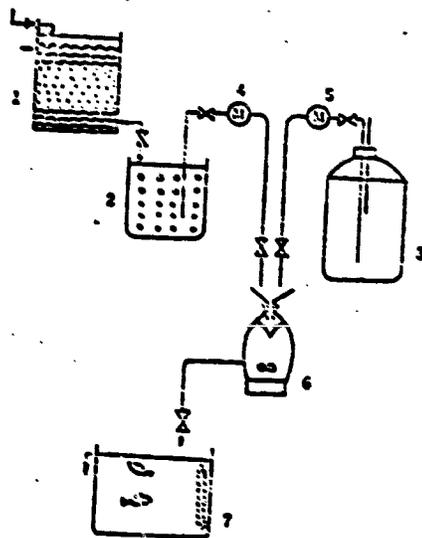
Fig-2. Method of test on the degree of accumulation

This test is carried out in flowing water and the degree to which the test compound is accumulated in fish bodies is examined.

1. Apparatus and equipment

(1) Apparatus

The apparatus is outlined as follows :



1. Active carbon tank for dechlorination of city water
2. Diluting-water tank in which temperature is controlled and water aerated
3. Tank for stock of solution of the test compound

attached paper
No. 3

4. Flow meter
5. Flow meter
6. Mixing tank
7. Aquarium of volume of 3 to 10 liters per fish, provided with temperature regulator

(2) Aquarium

The aquarium should be glass made clean and of sufficient volume for rearing the test fish.

(3) Other apparatus

The apparatus used for water supply or dilution of the test compound should be glass made and clean as possible.

Use of plastics should be limited to the parts where it is inevitable, such as joints.

2. Test fish

Carp weighing 20 to 40 g and measuring about 10 cm in body length (Note 2) are used.

(Note 2) the length between the tip of the mouth and the base of the caudal fin.

3. Rearing and acclimating

The carp are reared in a suitable fish pond. While rearing the diseased, the weakened and those showing other abnormal signs should be eliminated. Then external or internal parasitic pathogens are exterminated by sterilizer bathing or medication to keep the fish in good body condition and after that they are transferred to an acclimating tank.

If there is a difference between the test temperature and the water temperature of the fish pond, the fish are acclimated in the acclimating tank by the method provided in (1) or (2) below and in the meantime the fish with damages in the gills or the skin or diseased are eliminated. It is desirable

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that the water in the fish pond and acclimating tank is always flowing.

(1) If the test temperature is higher than the water temperature of the fish pond, fish are acclimated for not less than a day in a temperature higher by 5°C or less than the water temperature of the fish pond. Then the temperature is gradually raised at the rate not more than 2°C/day and finally the fish are reared in the test temperature for 5 to 7 days.

(2) If the test temperature is lower than the water temperature of the fish pond, fish are acclimated for not less than a day in a temperature lower by 2°C or less than the water temperature of the fish pond, then the temperature is gradually lowered at the rate not more than 2°C/day and finally fish are reared in the test temperature for 7 to 10 days.

4. Feeding

Pelleted feed is used. (Note 3) Fish are fed 2 to 3 times daily. In order to avoid any leftover, the feed should be given slowly so that the portion of the feed floating on the water surface is entirely eaten by the test fish before it reaches the bottom of the aquarium.

(Note 3) The compound feed tainted with residual agricultural chemicals or other chemicals that might cause serious effects on the test must not be used.

5. Assessment of adequacy of test fish

The carp in the acclimating tank in which not less than 5 per cent of the whole fish have weakened or died within one week before the start of the test should not be used.

6. Test procedure

(1) Test water and the concentration of the test compound

The test water is as provided in II-1-2. Two levels of concentration as low as possible within the analytical limit (one of the two levels of concentration is ten times higher than the other) should be prepared. (Note 4)

The test is carried out in each of two concentration levels mentioned above. (Note 5) and at the same time blank test is made in the water in which the test compound is not dissolved. During the test period, test water is controlled so that the concentration of dissolved oxygen is kept at about 7 ppm.

(Note 4) Two concentration levels as low as possible within the analytical limit, among the levels of 1/100, 1/1000 and 1/10000 of 48-hour TLm determined in II-1-4, will serve as a guide.

(Note 5) If the test compound has little solubility in water, suitable solubilizers (such as ethyl alcohol, fatty acid esters of polyoxyethylene sorbitan, etc.) are used.

(2) Test temperature

The test water is kept at $25 \pm 2^\circ\text{C}$.

(3) Test period

The test should be continued for 8 weeks, in principle.

However, in the case that the concentration factor calculated by the equation provided in II-2-8(1) exceeds a given value or in the case it gives a maximum value in either one of the two levels of test concentration, the test may be discontinued.

(4) Procedure

The test fish that have met the assessment provided in II-2-5, are reared under the conditions provided in (1)

through (3) using the apparatus and equipment provided in II-2-1. Excretions of the carp and smudges on the wall of the aquarium should be removed about once a day throughout the test period.

7. Analysis of test fish

(1) Every one or two weeks two to three test fish are taken out not less than ten hours after feeding and subjected to analysis. The fish from the water concentration level in which the test fish have showed distinct abnormal signs during the test should not be served for analysis.

(2) The surface of fish body is wiped lightly with gauze and each fish is weighed accurately to the unit of 0.1 g.

In the next place, the whole body of each fish, in principle, is homogenated and a suitable pretreatment such as extraction and concentration, depending upon the chemical properties of the test compound, is made to prepare a sample for quantitative analysis.

The analysis is made as directed in the General Rules of Analysis provided in the JIS (gas chromatography, absorptionometry, mass spectrometry, atomic absorption spectrophotometry, etc.).

8. Calculating method of the test result

(1) The degree of accumulation, expressed by concentration factor, is calculated by the following equation.

$$CF_n = \frac{F_n - FB}{W}$$

CF_n: Concentration factor after n weeks

F_n: Concentration of the test compound in the fish body after n weeks in the test period

FB: Arithmetical mean concentration of the test compound

attached paper
No.7

in the fish body at the start of, and at the termination
of the blank test (Note 6)

W : Mean concentration of the test compound in the
aquarium (Note 7)

(Note 6) In the case CF_n is calculated during the test
period, the concentration of the test compound in fish
bodies before the start of test may be quoted as
tentative FB .

(Note 7) The concentration of the test compound in the
water of the aquarium is measured periodically and
the arithmetical mean value of measured concentra-
tions is used. However, the calculated concentration
may be used instead if inevitable.

(2) Draw a correlation curve of the values calculated in (1),
against time.

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OSAKA, KOBE, TOYO-OKA, OKAYAMA,
TAKAMATSU, HIROSHIMA, KURUME.

5.1.2. Analytical instrument

Gas chromatograph, JGC-20K.

(Japan Electron Optics Laboratory Co., Ltd.)

5.2. Test Condition

5.2.1. Measurement of 48-hours TL_m value

(a) Test fish

Mature orange-red killfish (*Orizias latipes*), Passed mercuric chloride test.

Mean Weight 0.2g.

(b) Solubilizers and dissolving method

Dimethyl sulfoxide.

Hydrogenated Castor Oil (HCO-40).

The amount of solubilizer to test sample were 22 times the quantity of dimethyl sulfoxide and 20 times of the quantity of Hydrogenated Castor Oil in weight ratio. The mixture of sample and solubilizer is irradiated with supersonic wave for 10 minutes, and dissolved in water. The concentration of the stock solution is 500ppm.

Refer to Kenji Tebata : "The proposal for standard TL_m test using Orange-Red Killfish as test fish,"
Journal of Water and Waste, 14, 1297 - 1303 (1972)

(c) Test temperature

25 ± 2 °C.

(d) Result

48-hours TL_m value : 230ppm (w/v)

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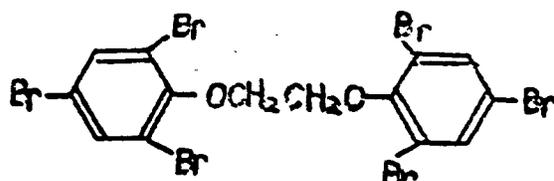
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OSAKA, KOBE, TOYO-OKA, OKAYAMA,
TAKAMATSU, HIROSHIMA, KURUME.

Test Report

1. Applicant : Ube - Cycon Co., Ltd.
2. Test Sample : Bis - 1,2 - (2,4,6 - Tribromophenoxy) - ethane.
structural formula



3. Scope : Measurement of the degree of accumulation of the test sample in fish body.
4. Test period : Jul. 5, 1976 - Nov. 5, 1976
5. Test method : Refer to the Items of Article 2. of the Order Prescribing the Items of the Test Relating to New Chemical Substances (Order of the Prime Minister, the Minister of Health & Welfare and the Minister of International Trade & Industry No. 1.)
" Method for testing the degree of accumulation of chemical substance in fish body."
See the attached paper.

5.1. Equipment and Analytical Instrument

5.1.1. Aquatron

Sample to be tested were dispersed in water with solubilizer and supplied continuously to the mixing tank, where diluted with 100 times the volume water and introduced into test tank.

Both room and test tank temperature were controlled within 25 ± 2 C.

The figures above each peak show the peak height (mm)

Upper concentration region after 34 days

Lower concentration region after 34 days

Standard solution 0.2ppm

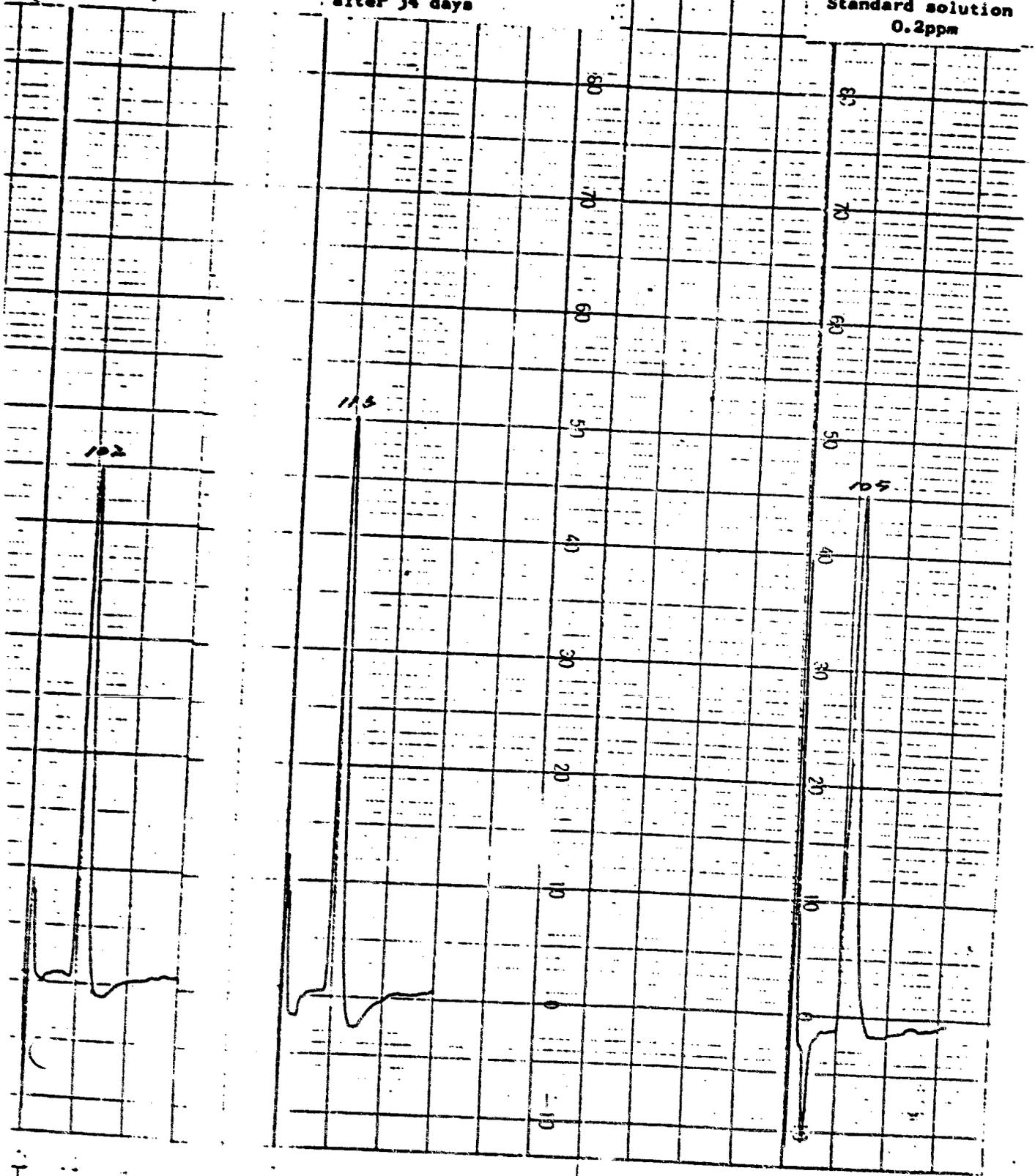


Fig - 11

No. Water 2

(20, 27. SEP)
Date 19. 12. 1976

1976

Room Temp. 25 °C

Model JGC - 20K

Sample S - 82

GLS 4 ml (2) mg.

Solvent Benzene

Column L. 0.3 m. I.D. 3 mm.

Temp. 215 °C → °C

Temp. Rate °C/min.

Liq. Phase DC - 200

wt. % 2

Support Gas - chrom Q

Mesh 80 - 100

Treatment

Ref. Column

Carrier Gas N₂

Flow Rate A : 2.0 kg/cm² (ml/min)

B : kg/cm² (ml/min)

H₂ Flow Rate A : kg/cm² (ml/min)

B : kg/cm² (ml/min)

Air Flow Rate kg/cm² (l/min)

Detector ECD

Rad. Source 63 Ni

Range 1, 1 mV

Sensitivity Ni x 8

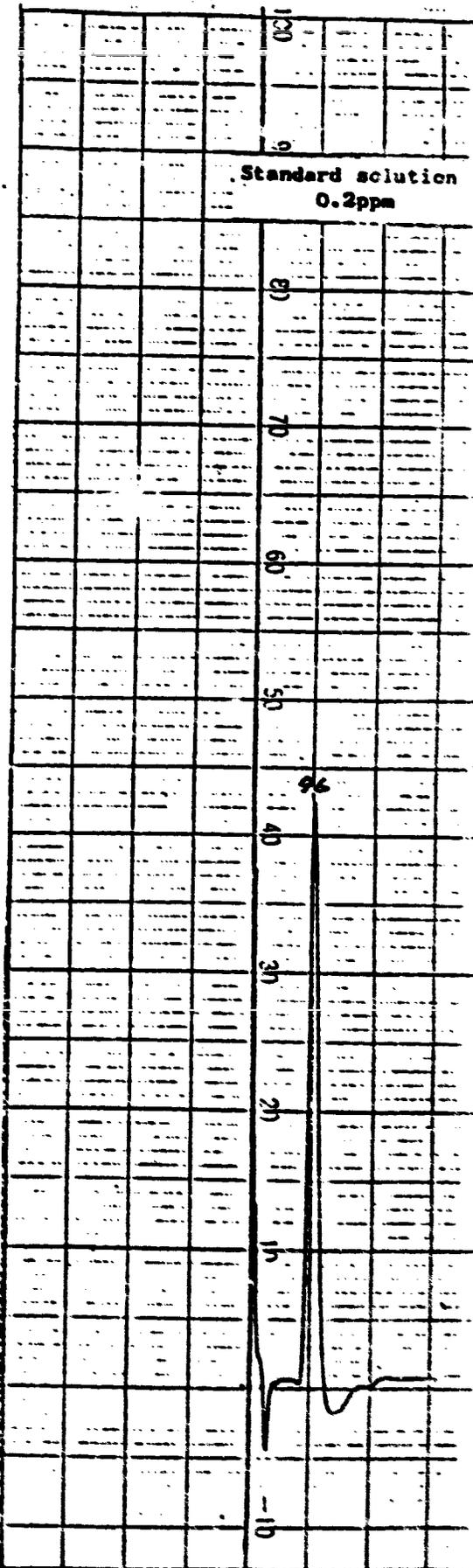
Detector Temp. 320 °C

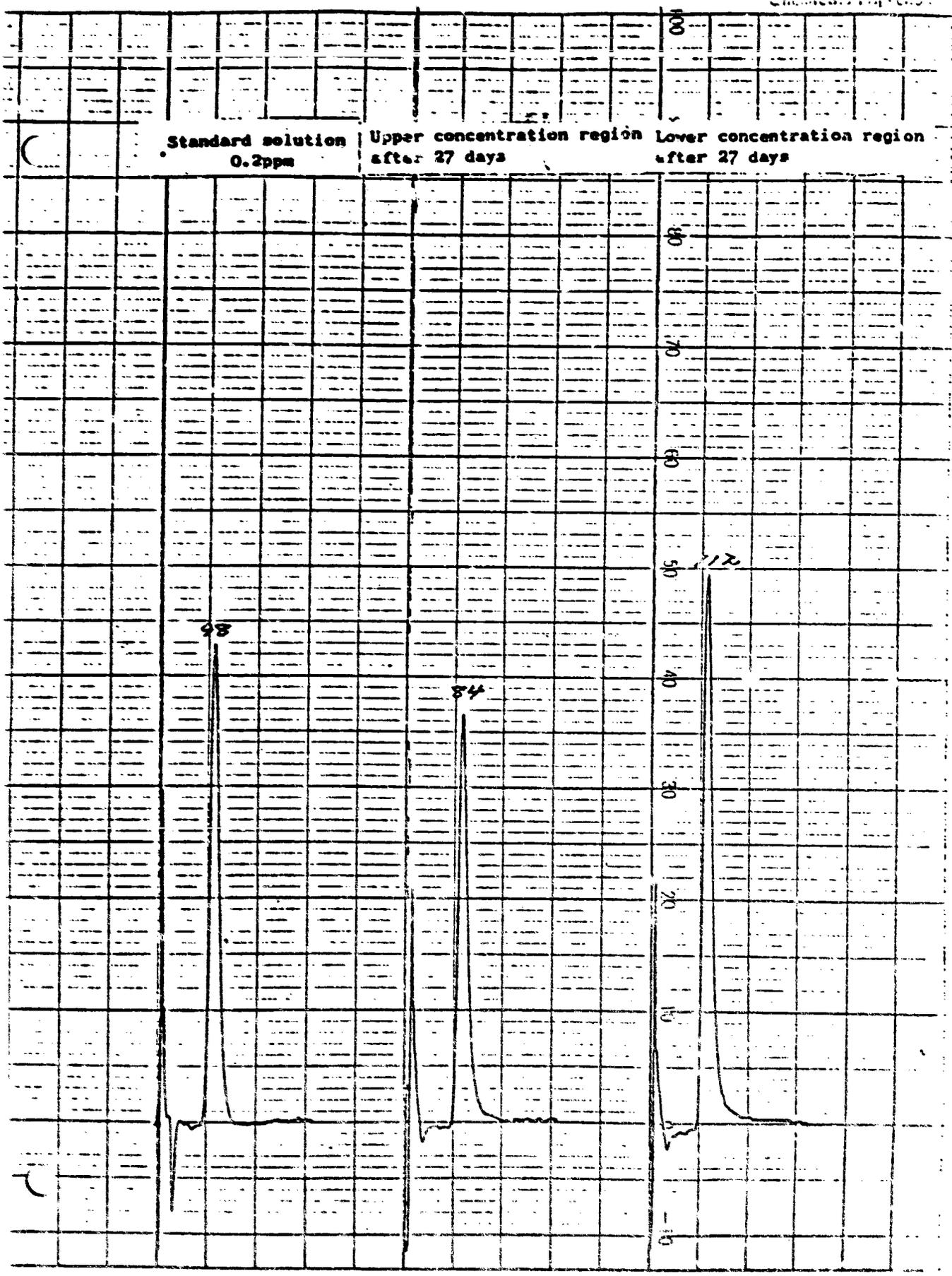
Injection Temp. 320 °C

Chart Speed 5 mm/min.

Note :

Operator A Group





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OSAKA, KOBE, TOYO-OKA, OKAYAMA,
TAKAMATSU, HIROSHIMA, KURUME.

• Column chromatography conditions.

(1) Adsorbent

Silica gel, washed with conc. hydrochloric acid.

(2) Conditioning

Activated at 110°C for 24 hrs.

5% of water in weight ratio was added to activated silica gel, and kept at least for 12 hrs before use.

(3) Chromatographic column

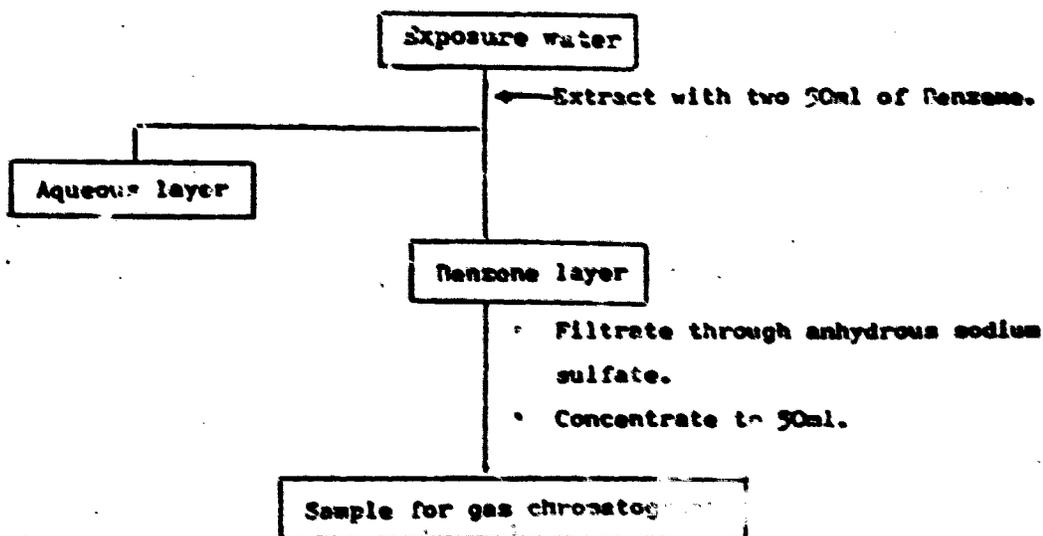
Glass column, 10mm in diameter, 15g of the conditioned silica gel packed in.

(4) Eluent

Benzene.

•• The first 50ml of fraction was provided for gaschromatography analysis.

b) Exposure water



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OSAKA, KOBE, TOYO-OKA, OKAYAMA,
TAKAMATSU, HIROSHIMA, KURUME.

5.2.4. Analytical conditions for gas chromatography

Column : Glass, 2mm^φ x 30cm
Stationary phase : Silicon DC200, 2%/Gas chrom Q 80/100 mesh
Column temp : 235°C
Carrier gas : N₂
Detector : ECD (⁶³Ni)

6. Test results

Table - 2. Concentration factor

Exposure levels \ Week	2W	4W	6W	8W
Higher	56.6	10.3	6.7	8.6
	26.6	27.9	5.2	27.1
Lower	19.0	11.9	26.1	43.6
	19.7	37.4	29.2	25.4

_____ Last Item _____

Chemical Biotesting Center

Chemicals Inspection & Testing Institute, Japan
4-1-1, Higashi-mukojiima, Suzidaku,
Tokyo, Japan

Director, Toshiro Arai

Toshiro Arai

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ASHIKAGA, TOKYO, SHIZUOKA, NAGOYA,
OSAKA, KOBE, TOYO-OKA, OKAYAMA,
TAKAMATSU, HIROSHIMA, KURUME.

Table - 1.

Mean of test sample concentration in exposure water for the calculation
of the concentration factor

Exposure levels \ Week	2W	4W	6W	8W
Higher	0.282	0.268	0.270	0.276
Lower	0.0250	0.0255	0.0259	0.0260

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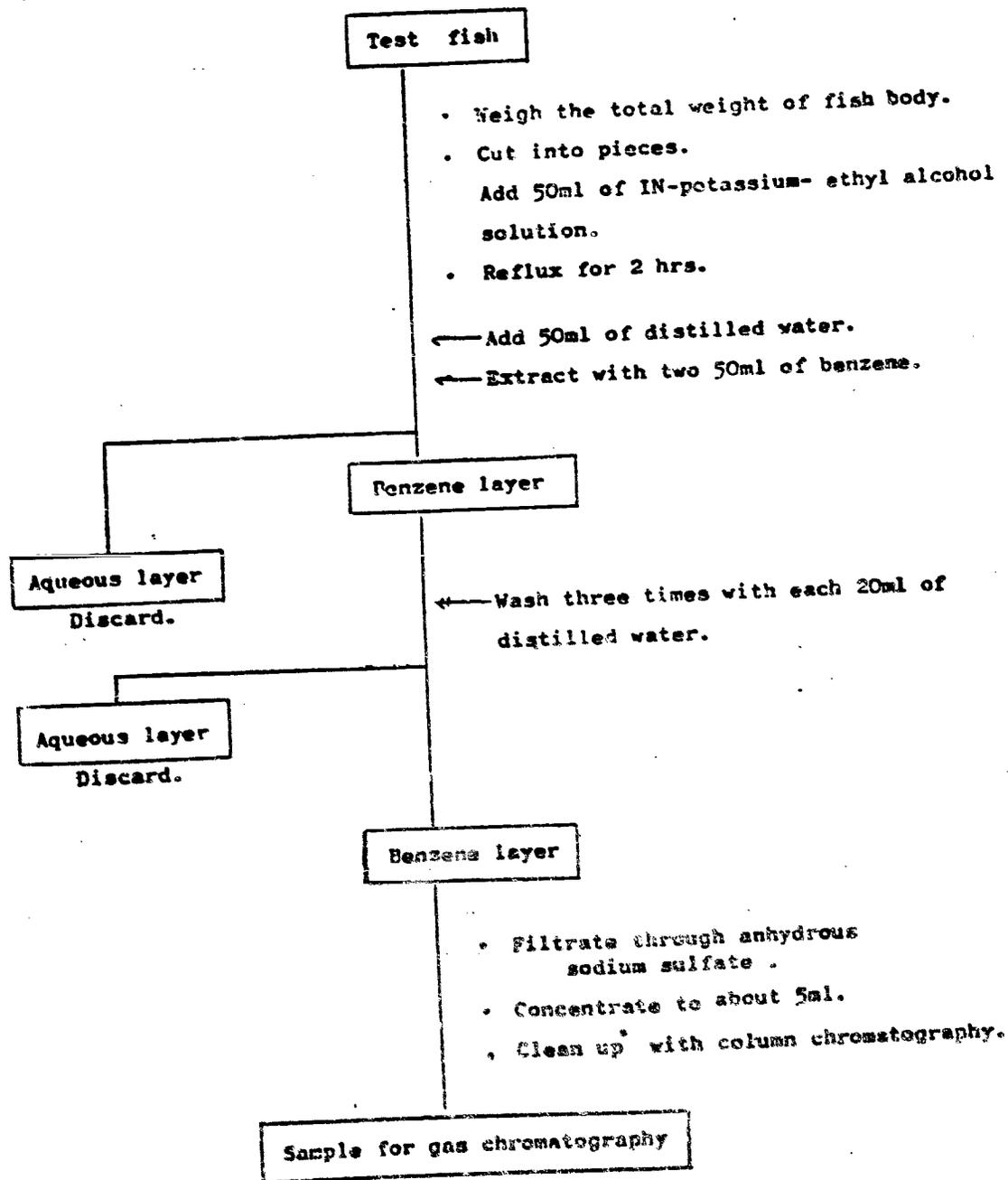
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LABORATORY
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5.2.3. Analytical procedure

a) Test fish



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TAKAMATSU, HIROSHIMA, KURUME.

5.2.2. Test of the degree of accumulation

(a) Disinfection

- (1) The test fishes were bathed in the aqueous solution containing 30g of CuSO_4 and 36ml. of acetic acid in 60 l. of water for 30 seconds, and then 10ppm solution of chlorotetracycline hydrochloride for 24 hours.

- (2) Acclimation, $25 \pm 2^\circ\text{C} \times 14$ days.

(b) Test tank

Glass, about 100 l. in volume.

Flow rate of test water, 576 l. per day.

(c) Test fish

Carp (*Cyprinus carpio*).

Mean weight, 30g.

Mean length, 10cm.

(d) Solubilizer

Same to 5.2.1. (b)

(e) Test Temperature

$25 \pm 2^\circ\text{C}$.

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TAKAMATSU, HIROSHIMA, KURUME.

(f) Exposure level of test sample

Two exposure levels that is 1/1,000 and 1/10,000 of 48 hrs
TLm value were chosen.

$$\text{Higher} \quad 230 \times \frac{1}{1,000} = 300 \times \frac{1}{1,000} = 0.3$$

$$\text{Lower} \quad 230 \times \frac{1}{10,000} = 300 \times \frac{1}{10,000} = 0.03 \text{ ppm}$$

Exposure levels	Test sample	Dimethyl sulfoxide	Hydrogenated Castor Oil (HCO 40)
Higher	0.3	0.6	6.0
Lower	0.03	0.06	0.6

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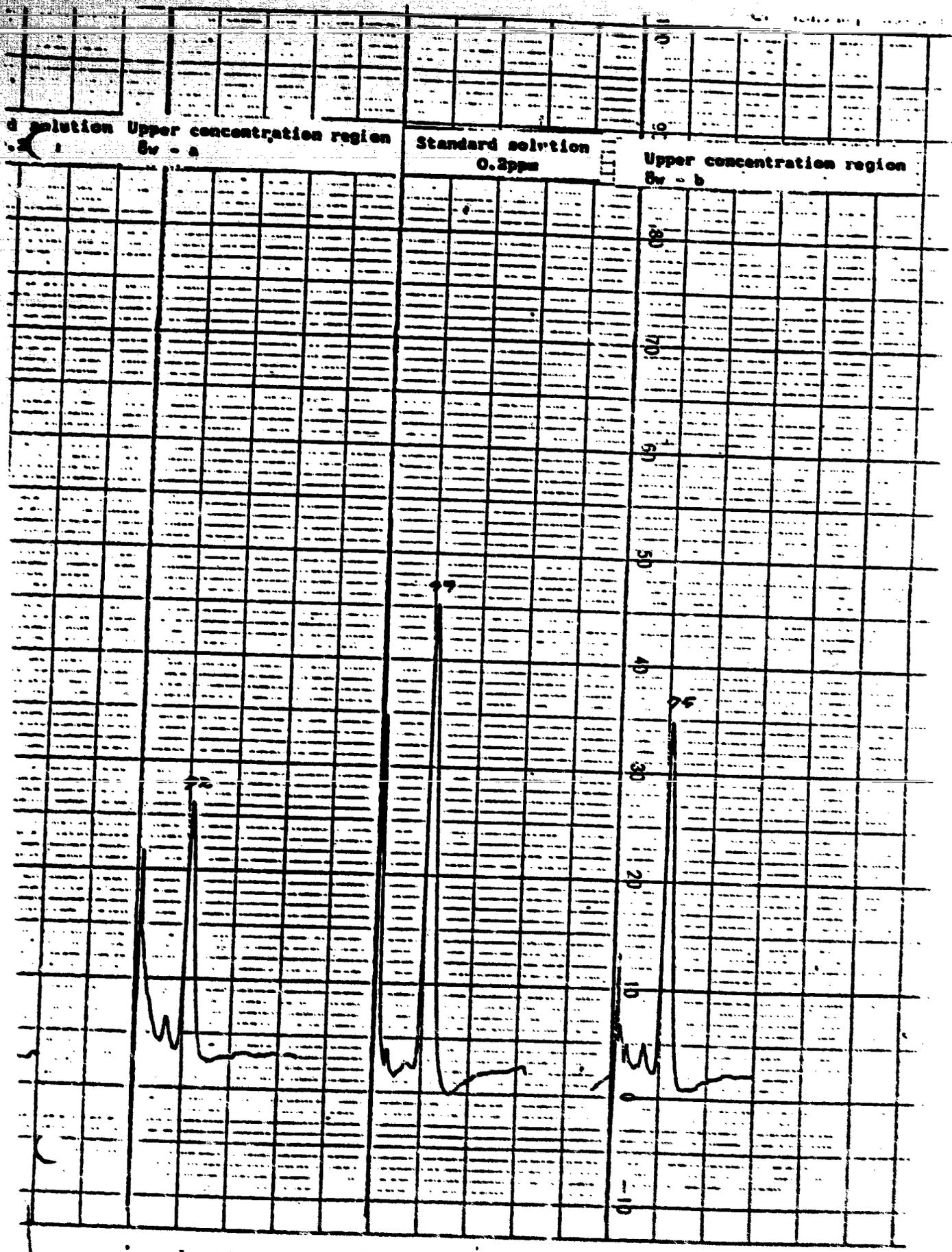
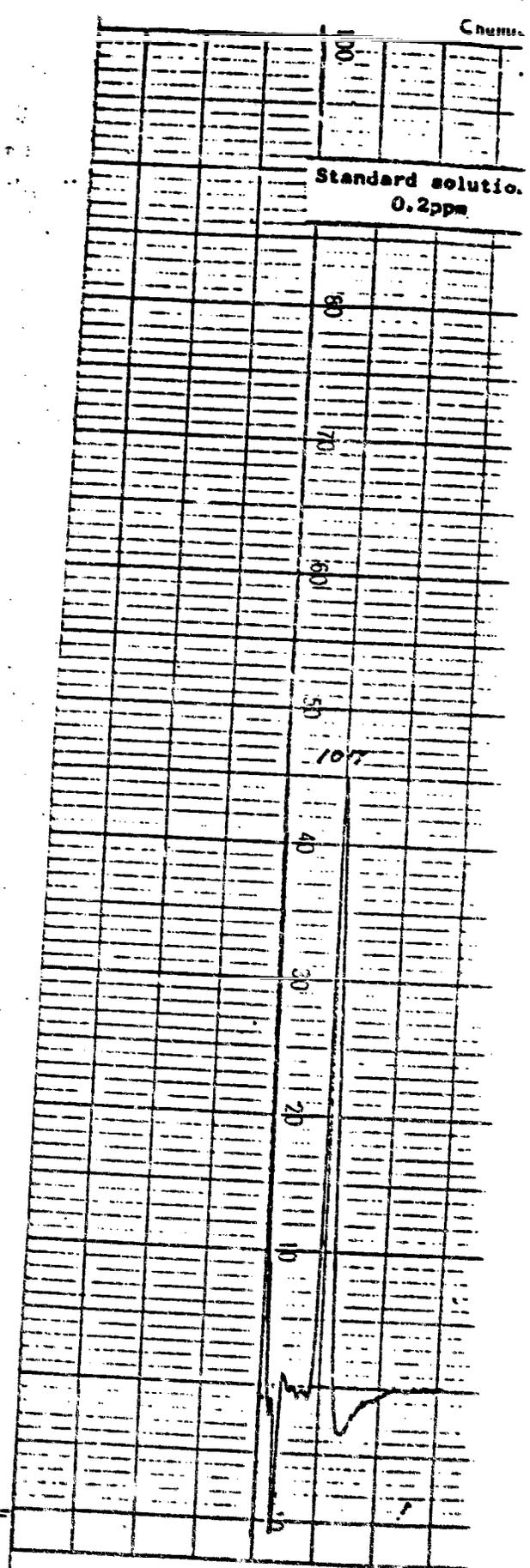


Fig - 6
 No. Fish 2
 Date (31. AUG) (12. OCT) 1976
 (14. 28. SEP)
 Room Temp. 25 °C
 Model JGC - 20K
 Sample S - 82
 GLS 4 ml (1) mg.
 Solvent Benzene
 Column L. O. 3 m. I. D. 2 mm.
 Temp. 215 °C → °C
 Temp. Rate °C/min.
 Liq. Phase DC - 200
 wt. % 2
 Support Gas - chrom Q
 Mesh 80 - 100
 Treatment
 Ref. Column
 Carrier Gas N₂
 Flow Rate A: 2.0 kg/cm² (ml/min)
 B: kg/cm² (ml/min)
 H₂ Flow Rate A: kg/cm² (ml/min)
 B: kg/cm² (ml/min)
 Air Flow Rate kg/cm² (l/min)
 Detector ECD
 Rad. Source 63 Ni
 Range 1.1 mV/V
 Sensitivity Hi x 8
 Detector Temp. 320 °C
 Injection Temp. 320 °C
 Chart Speed 5 mm/min.
 Note:
 Operator A Group



Industrial **BIO-TEST Laboratories, Inc.**

1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

STATUS REPORT TO

MICHIGAN CHEMICAL CORPORATION

BIODEGRADATION STUDY WITH ¹⁴C-TAGGED MC-680

NOVEMBER 20, 1975

IBT NO. 632-07189

CAS# 37853-59-1

*1,2-Bis-(tribromophenoxy)-
ethane*

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Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

November 20, 1975

Dr. F. A. Daniher, Manager
Research & Development
Michigan Chemical Corporation
1975 Green Road
Ann Arbor, Michigan 48105

Dear Dr. Daniher:

Re: IBT No. 632-07189 - Biodegradation Study with
¹⁴C-Tagged MC-680

We are submitting herewith our laboratory report prepared
in connection with the above study.

Very truly yours,

J. C. Calandra

J. C. Calandra
President

JCC:bp

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STATUS REPORT TO
MICHIGAN CHEMICAL CORPORATION
BIODEGRADATION STUDY WITH ^{14}C -TAGGED MC-680

NOVEMBER 20, 1975

IBT NO. 632-07189

I. Introduction

A sample of ^{14}C -labeled MC-680 with a specific activity of 34 micro-curies(μc) per mg was prepared by Amersham/Searle Corporation.

At the request of Michigan Chemical Corporation a biodegradation study was initiated to determine biodegradability of the flame-retardant material MC-680. The status report describes the test procedures and presents the results obtained during 7 weeks of testing. The test material is presently being tested at 3 different concentrations using a simple shake flask system to monitor CO_2 liberation.

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II. Summary

Tests of aerobic biodegradability are presently conducted by exposing ^{14}C -tagged MC-680 to acclimated sewage and garden soil microorganisms in a shake flask system. Each reaction flask (125 ml Erlenmeyer flask) is equipped with a small center cup containing the KOH-Solution for absorption of the respired $^{14}\text{CO}_2$ due to biodegradation of the labeled test material by the microorganisms present. The current experiment is now in progress for a period of 8 weeks for test groups I and II, and 4 weeks for test group III, not including previous microbial acclimation.

During an 18 day acclimation period microorganisms derived from sewage and garden soil were exposed to nonlabeled MC-680. The adapted bacterial seed organisms were then used to prepare the test media. Three different concentrations of ^{14}C -tagged test material in microbial media were prepared (1.0 and 0.01 percent, and 1.0 ppm). Control tests consisted of reaction flasks containing ^{14}C tagged D-Glucose to demonstrate microbial activity of the culture toward a known biodegradable compound. In another control sample ^{14}C MC-680 was suspended in distilled water (with addition of HgCl_2) eliminating the presence of microorganisms.

$^{14}\text{CO}_2$ liberation from universally radiocarbon labeled MC-680 was evident in all test samples throughout the study period reported. Degradation, however, is considered slow. A graphic illustration (Figure 2) demonstrates the daily rate of $^{14}\text{CO}_2$ respiration expressed as percent of theoretical substrate activity.

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During the past 4 weeks of incubation, $^{14}\text{CO}_2$ evolution appears to stabilize at a low rate ranging from 0.002 to 0.007 percent (T-I and T-II). The third group (lowest concentration - 1.0 ppm) is indicating similar tendencies, but percent recovery of ^{14}C -activity is slightly higher than in 1.0 and 0.01 percent MC-680.

Tables III through V present the radiometric results of tests T-I, T-II, and T-III for each sampling period. Table VI presents the results of all control samples.

Respectfully submitted,

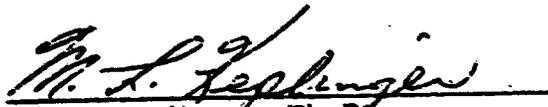
INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:



Irene A. Dressler, M. S.
Group Leader
Metabolic Studies

Report approved by:



M. L. Keplinger, Ph. D.
Manager, Toxicology

lam:bp

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III. Experimental Procedures

A. Preparation of Medium

The test medium consisted of a mixture of acclimated microbial inoculum, fresh raw sewage (settled) and supernatant of a fresh garden soil slurry.

Acclimation of the microorganisms was carried out in a minimum salts-vitamin medium supplemented with 25 mg/l each of yeast extract and vitamin-free casamino acid. During the acclimation period nonlabeled MC-680 was added in increments. On Day 1 and Day 7, 10 mg and 100 mg of the material was added, respectively. The contents were mixed well and aerated every other day, and held at 20°C in the dark. At various days during acclimation, test media were prepared using the acclimation culture to test the activity of the culture. After 18 days (9/9/75) the initial test series (1.0 and 0.01%) were set up.

At the start of the experiment (Day 0), fifty ml of supernatant of the acclimated microbial culture and 10 ml of settled fresh sewage were combined and diluted to 500 ml with the minimum salt and vitamin solution. Reagent water according to ASTM specifications was used for this preparation. Hydrogen ion concentration of acclimation culture and final test media were measured and revealed for both a pH of 7.0 at the start of the study.

B. Reaction Flask Contents

Erlenmeyer flasks (125 ml) were employed for all tests. The pre-weighed test material was placed first into the flasks, followed by addition of 30 ml of medium. In the case of the

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MC-680 (^{14}C -labeled) was dissolved in ethylacetate. A volume of this solution containing the equivalent of 30 micrograms was pipetted into the reaction flask. The solvent was evaporated prior to addition of medium. To improve dispersion of the test material, 2 drops of a surfactant (TWEEN-80) were added to each of the test flasks.

Evolved $^{14}\text{CO}_2$ was continuously absorbed in an alkali solution. A small plastic cup was suspended over the medium in the center of the Erlenmeyer flask; the cup contained 1.0 ml of 0.5 N KOH. A piece of pleated filter paper was placed into the alkali solution in such a way that half of the piece was projecting into the open space above (see Figure 1).

On September 9, 1975, two test groups (T-I: 0.01% and T-II: 1.0%) were started with four flasks each. The test material was weighed directly into the reaction flasks prior to adding 30 ml of the media. Table I presents the quantities of labeled and non-labeled MC-680 used in each test flask. Control flasks receiving the identical acclimated microorganism culture and ^{14}C -tagged D-glucose were also started. A third test group (T-III) containing ^{14}C -tagged MC-680 in the media at a concentration of 1 ppm (parts per million) was started on October 7, 1975. The test medium was prepared fresh for this series in the same manner as previously described. The acclimated inoculum was of the same origin as the one used in T-I and T-II. Duplicate positive control samples containing ^{14}C -glucose were also initiated on October 7 using a culture media identical to that prepared for test group T-III.

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A control flask receiving water and HgCl_2 (50 mg/l) instead of the microbial media, but containing ^{14}C -tagged MC-680 was included at this time. Table II presents the total radioactivity contained in each of the control flasks at the beginning of the experiment.

C. Operational Routines and Sampling Procedures

The reaction flasks were incubated in Dubnoff Shakers and held under continuous shaking. The rate of shaking was approximately 85 cycles per minute. The temperature of the reaction vessels varied with the ambient room conditions between 19 and 23° C. Except for brief periods of sampling, the flasks were kept in the dark throughout the study to prevent algae growth. Periodically, each flask was purged with a 70:30 O_2/N_2 mixture; this was done usually at sampling time and not less than once a week.

The KOH solution from the center cup was transferred quantitatively with several small rinses to a scintillation counting vial. The filter paper was added to the same vial and the contents were radioassayed in Instagel.

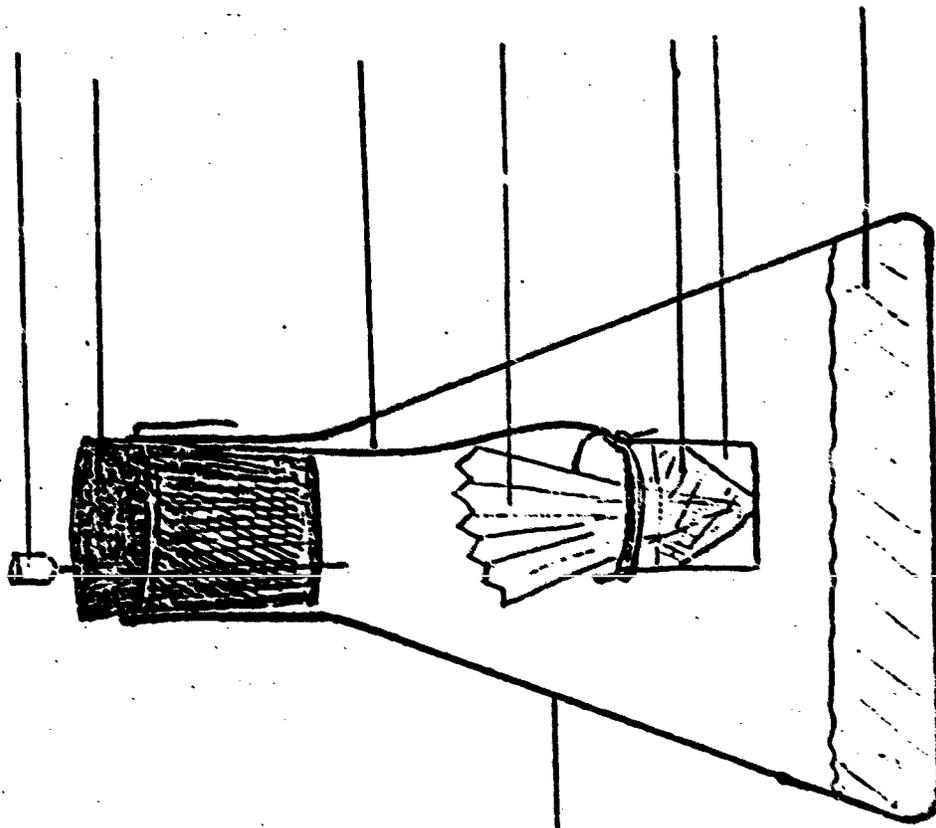
Counting was conducted in the Mark II counter (Nuclear Chicago). Efficiency corrections were made using the channels ratio method. Samples were dark adapted for at least 24 hours prior to radio assay and were routinely counted for 10 minutes.

During the early phase of incubation, the liberated $^{14}\text{CO}_2$ was monitored at short intervals. During the last 4 weeks samples were obtained once a week.

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FIGURE 1



Syringe needle (vent)
Rubber Stopper

Wire holding Koff-cup

Pleated Filter Paper

0.5 N KOH (1.0 ml)
Small Polystyrene Disposable Cup

30 ml Medium containing Test Material

Shake Flask System

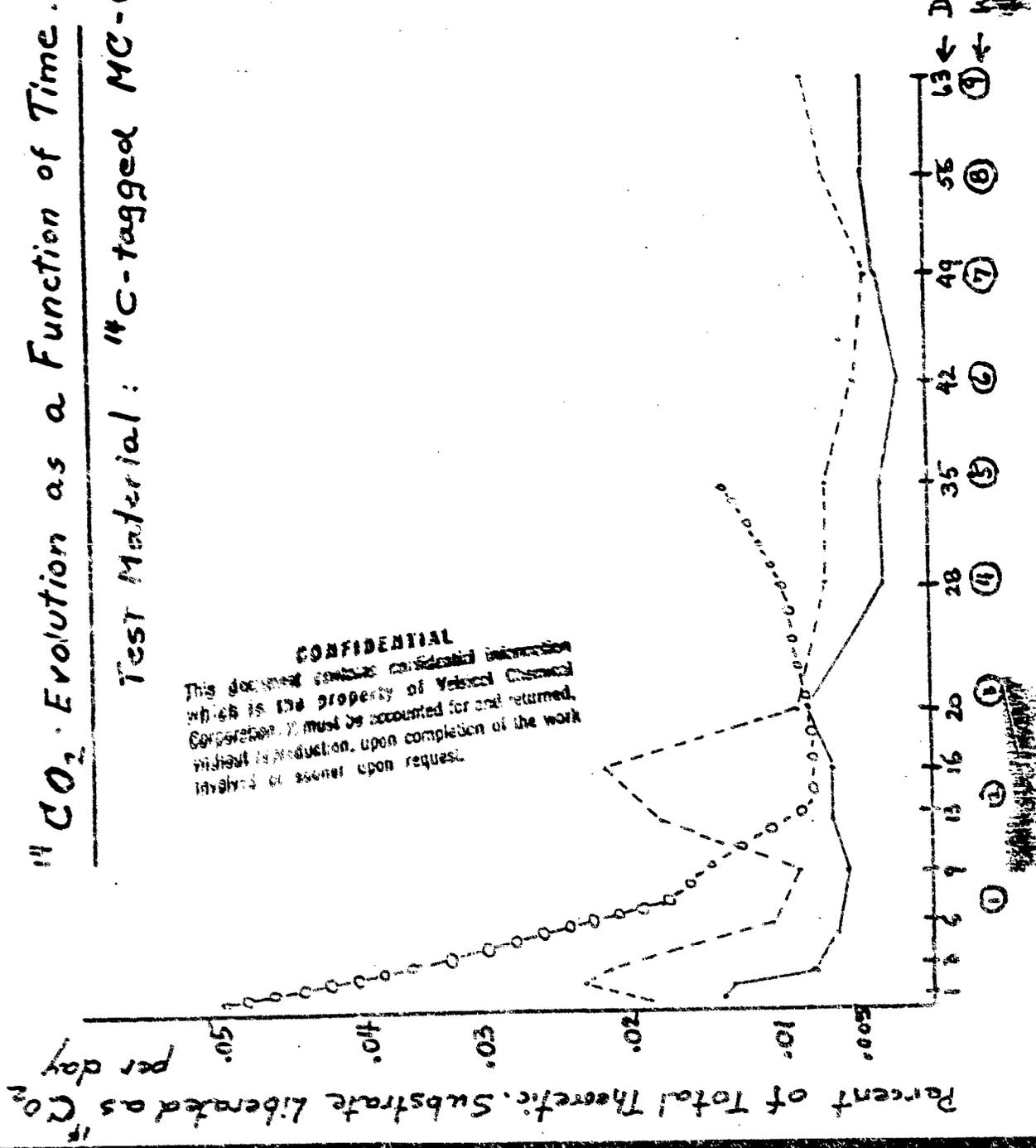
Shake Flask System

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FIGURE 2

¹⁴C₂ Evolution as a Function of Time.
 Test Material: ¹⁴C-tagged MC-680

TII ——— (1.0%)
 TII - - - - - (0.01%)
 TIII -o-o-o-o- (1 ppm)



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TABLE I

TEST MATERIAL: MC-680

Bio-degradation Study with ¹⁴C-tagged MC-680 - Status Report

Outline of Experiment

Test Samples

Concentration (percent)	Identification	Date Started	Replicate Number (Flasks)	MC-680 Added to Medium (30 ml)			Total Radioactivity* net dpm x 10 ⁶
				nonlabeled (mg)	¹⁴ C-tagged (mg)	Total Quantity (mg)	
1.0	T-II	9-9	1	289.42**	10.58**	300.00	798.58
			2	290.10	9.90	300.00	747.25
			3	289.89	10.11	300.00	763.10
			4	289.68	10.32	300.00	778.95
1.0 x 10 ⁻²	T-I	9-9	1	0	2.91**	2.91	219.65
			2	0	3.27	3.27	246.82
			3	0	3.41	3.41	257.39
			4	0	3.41	3.41	257.39
			5	0	2.75	2.75	207.57
1.0 x 10 ⁻⁴	T-III	10-7	1	0	31.46 (µg)	0.031	2.374
			2	0	31.46	0.031	2.374
			3	0	31.46	0.031	2.374
			4	0	31.46	0.031	2.374

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* Specific activity of ¹⁴C-tagged MC-680 equal 34 µc/mg
 ** Test material was weighed directly into flasks.

TABLE II
TEST MATERIAL: MC-680
Bio-degradation Study with ¹⁴C-tagged MC-680 - Status Report
Outline of Experiment - continued

¹⁴ C-tagged Material Added	Date Started	Sample Identification	Control Samples	
			Total Radioactivity ⁶ net dpm x 10 ⁶	Media (30 ml)
D-Glucose	9-9	G1 - 1	12.117	Acclimated microorganisms
		G1 - 2	12.176	
D-Glucose	10-7	G1 - 5	13.728	Acclimated microorganisms
		G1 - 6	12.762	
MC-680	10-7	C-H ₂ O	2.374	H ₂ O, diat. + HgCl ₂

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TABLE III a

TEST MATERIAL: MC-680

Bio-degradation Study with ¹⁴C-tagged MC-680 - Status Report

¹⁴C-Activity Respired as ¹⁴CO₂ - Test Group T-I (0.01 percent)

Test Day Number	Average ¹⁴ C-Activity per Sampling Period		Daily Rate of ¹⁴ CO ₂ Liberation		Percent of Total Theoretical Substrate ¹⁴ C-Activity
	net dpm*	Range net dpm	net dpm	net dpm	
1	47,759	37,114 - 52,369	47,759	47,759	0.019
2	57,769	37,553 - 102,859	57,769	57,769	0.023
3	52,605	39,372 - 71,755	52,605	52,605	0.022
6	78,793	62,506 - 92,927	26,264	26,264	0.011
9	67,790	61,767 - 73,126	22,597	22,597	0.009
13	170,143	102,380 - 232,422	42,536	42,536	0.018
16	161,195	78,914 - 261,006	53,732	53,732	0.022
20	86,330	56,946 - 134,133	21,583	21,583	0.009
28	133,222	66,423 - 228,427	16,653	16,653	0.007
35	117,152	82,728 - 187,110	16,736	16,736	0.007
42	88,482	67,042 - 117,250	12,640	12,640	0.005
49	73,720	48,555 - 107,073	10,531	10,531	0.004
Total	1,134,960	0.463 percent of initial radioactivity			

* net dpm* present mean of 4 reaction flasks.

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TABLE III b

TEST MATERIAL: MC-680

Biodegradation Study with ¹⁴C-Tagged MC-680 - Status Report

¹⁴C-Activity respired as ¹⁴CO₂ - Test Group T-I (0.01 percent)

Test Day Number	¹⁴ C-Activity per Sampling Period net dpm	Sample Replicate No. 5 (started: 10- ⁰⁷⁵)	
		Daily Rate of ¹⁴ CO ₂ Liberation	Theoretical Substrate ¹⁴ C-Activity
1	31,383		0.015
3	33,296		0.008
7	100,559		0.012
14	133,652		0.009
21	123,689		0.009
Total:	422,579 = 0.204 percent of initial radioactivity.		

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TABLE IV

TEST MATERIAL: MC-680

Biodegradation Study with ¹⁴C-Tagged MC-680 - Status Report

¹⁴C-Activity respired as ¹⁴CO₂ - Test Group T-II (1.0 percent)

Test Day Number	Average ¹⁴ C-Activity per Sampling Period net dpm*	Range net dpm	Daily Rate of ¹⁴ CO ₂ Liberation	
			net dpm	Percent of Total Theoretical Substrate ¹⁴ C-Activity
1	108,175	93,555 - 133,212	108,175	0.014
2	101,529	71,045 - 152,393	101,529	0.013
3	59,526	52,302 - 65,054	59,526	0.008
6	149,522	126,358 - 184,569	49,841	0.007
9	147,579	99,820 - 176,708	49,193	0.006
13	207,201	147,856 - 319,675	51,800	0.007
16	151,481	84,234 - 210,298	50,494	0.007
20	250,460	90,320 - 393,767	62,615	0.008
23	216,931	104,971 - 267,661	26,254	0.003
35	132,925	117,269 - 256,202	25,631	0.003
42	126,470	90,948 - 176,187	18,953	0.002
49		107,101 - 169,350	19,839	0.003
Total:	3,836,466			

* Values represent mean of 4 identical reaction flasks.

** This and subsequent values are mean of 3 flasks; one reaction flask produced extreme high values of ¹⁴CO₂ liberation.

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REPORT TO

MICHIGAN CHEMICAL CORPORATION

BIODEGRADABILITY STUDY WITH

¹⁴C-TAGGED MC-680

CAS# 37853-59-1

JUNE 11, 1976

1,2-Bis-(tribromophenoxy)

IBT NO. 632-67189

e thane

Industrial BIO-TEST Laboratories, Inc.

1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

June 11, 1976

Dr. F. A. Daniher, Manager
Research & Development
Michigan Chemical Corporation
1975 Green Road
Ann Arbor, Michigan 48105

Dear Dr. Daniher:

Re: IBT No. 632-07189 - Biodegradability Study with
¹⁴C-Tagged MC-680

We are submitting herewith our laboratory report
prepared in connection with the above study.

Very truly yours,

J. C. Calandra

J. C. Calandra
President

JCC:trm

0193

REPORT TO
MICHIGAN CHEMICAL CORPORATION
BIODEGRADABILITY STUDY WITH
¹⁴C-TAGGED MC-680

JUNE 11, 1976

IBT NO. 632-07189

I. Introduction

At the request of Michigan Chemical Corporation, a biodegradation study was conducted to determine the extent of degradation of the flame-retardant material MC-680.

A sample of ¹⁴C-labeled MC-680 with a specific activity of 34 microcuries (μc) per mg was prepared by Amersham/Searle Corporation. The test material was tested at 3 different concentrations using a simple shake-flask system. The rate of degradation was monitored by measuring the amount of ¹⁴CO₂ liberated.

This report describes the test procedures and presents the results obtained during 30 weeks of testing.

II. Summary

The purpose of this study was to obtain information on the rate of biodegradability of the fire-retardant MC-680 in an acclimated heterogeneous microbial system.

Uniformly ^{14}C -tagged MC-680 was exposed to microorganisms derived from fresh settled sewage and garden soil in a shake flask system. The extent of ultimate degradation was monitored by measuring the amount of $^{14}\text{CO}_2$ liberated due to degradation of the labeled test material by the microorganisms present. Each reaction vessel (Erlenmeyer flask) was equipped with a small center cup containing the KOH solution for absorption of the respired $^{14}\text{CO}_2$. The experiment was maintained for a period of 30 weeks for test groups I and II, and 26 weeks for test group III, not including previous microbial acclimation.

During an 18-day acclimation period microorganisms derived from sewage and garden soil were exposed to nonlabeled MC-680. The adapted bacterial seed organisms were then used to prepare the test media. Three different concentrations of ^{14}C -labeled test material in microbial media were prepared (1.0 and 0.01 percent, and 1.0 ppm). Control tests consisted of reaction flasks containing ^{14}C -tagged D-Glucose to demonstrate microbial activity of the culture toward a known biodegradable compound. In another control sample ^{14}C -MC-680 was suspended in distilled water (with addition of HgCl_2) eliminating the presence of viable microorganisms.

Incubation of tests T-I and T-II (0.01 and 1.0 percent) was terminated after 211 days or 30 weeks; from test group T-III (1 ppm) a final sample was obtained at 183 days (26 weeks).

Evolution of $^{14}\text{CO}_2$ from degradation of uniformly labeled $^{14}\text{C-MC-680}$ was evident in all test samples. The rate of degradation, however, is considered low. Following an initially accelerated $^{14}\text{CO}_2$ liberation, $^{14}\text{CO}_2$ absorption decreased to about 0.001 to 0.008 percent of the total ^{14}C -activity in the system in groups T-I and T-II. The test group containing 1.0 ppm of $^{14}\text{C-MC-680}$ in the medium revealed slightly higher recovery values (range: 0.003-0.014 percent).

Total ^{14}C -activity, liberated as $^{14}\text{CO}_2$, accumulated to 2.67×10^6 dpm in group T-I (0.01%) and to 4.64×10^6 dpm in group T-II (1.0%), representing to recoveries of 1.11 and 0.53 percent of the initially applied ^{14}C -activity (labeled MC-680). In the third test group (T-III) containing 1 part of $^{14}\text{C-MC-680}$ per million parts of media, the total ^{14}C -activity recovered during 183 days of incubation amounted to 1.41 percent of total ^{14}C applied. These results indicate that the rate of degradation was slightly favored in the lower concentration levels. Inhibition of growth rate of the microbial population in the presence of MC-680 at the 1.0 percent level may have been responsible for a reduced $^{14}\text{CO}_2$ evolution.

Table I presents a summary of the radiometric assays of tests T-I, T-II and T-III for each sample period; in addition, Figure 1 illustrates these data. Table II presents a summary of all control samples.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:

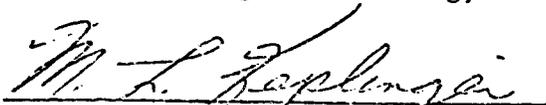


Irene A. Dressler, M. S.
Group Leader
Metabolic Studies

Report approved by:



Florence K. Kihoshita, Ph. D.
Technical Manager, Toxicology



M. L. Keplinger, Ph. D.
Manager, Toxicology

trm

TABLE I

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680Summary of Respired $^{14}\text{CO}_2$ Activity

Test Day Number	Average Daily Rate of $^{14}\text{CO}_2$ Evolution Expressed As Percent of Total Theoretical Substrate ^{14}C -Activity		
	Test Material Concentrations:		
	1.0 percent (T-II)	0.01 percent (T-I)	1 ppm (T-III)
1	0.014	0.019	0.050
2	0.013	0.024	N. A.
3	0.008	0.022	0.037
6	0.007	0.011	N. A.
7	N. A.	N. A.	0.018
9	0.006	0.009	N. A.
13	0.007	0.018	N. A.
14	N. A.	N. A.	0.008
16	0.007	0.022	N. A.
20	0.008	0.009	N. A.
21	N. A.	N. A.	0.008
28	0.003	0.007	0.010
35	0.003	0.007	0.014
42	0.002	0.005	0.012
49	0.003	0.004	0.010
56	0.003	0.007	N. A.
63	0.003	0.008	N. A.
70	0.002	0.004	0.005
77	0.002	0.004	N. A.
91	N. A.	N. A.	0.003
98	0.001	0.004	N. A.
119	0.001	0.006	0.008
147	0.003	0.003	N. A.
155	N. A.	N. A.	0.006
161	0.002	0.005	N. A.
183	0.002	0.003	0.006
211	0.001	0.002	N. A.

N. A. = Not analyzed.

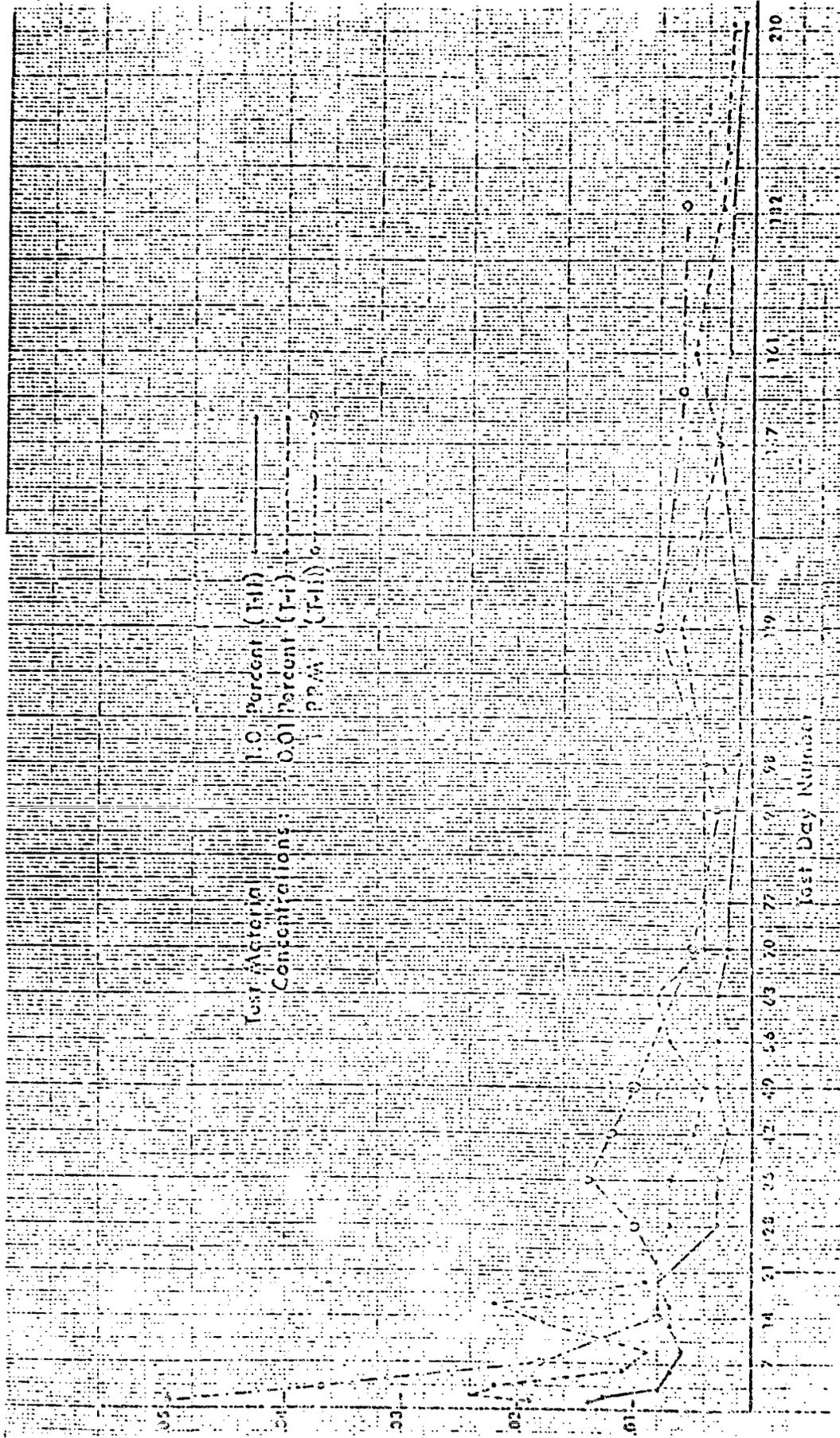


Figure 1. AVERAGE DAILY RATE OF $^{14}\text{CO}_2$ EVOLUTION TEST MATERIAL MC-680

TABLE II

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680Summary of Respired $^{14}\text{CO}_2$ Activity

Test Day Number	Average Daily Rate of $^{14}\text{CO}_2$ Evolution Expressed As Percent of Total Theoretical Substrate ^{14}C -Activity		
	Glucose 1 & 2	Glucose 3 & 4	H_2O - HgCl_2
1	27.29	20.86	0.000
2	4.81	-	-
3	6.18	7.80	0.002
6	5.78	-	-
7	-	7.28	0.000
9	5.53	-	-
13	4.94	-	-
14	-	2.98	0.000
16	3.35	-	-
20	2.54	-	-
21	-	1.03	0.001
28	1.49	0.59	0
35	5.49	0.64	<0.001
42	1.96	0.66	0.000
49	0.84	0.41	0.008
56	0.55	0	0
63	0.49	-	-
70	0.44	0.35	<0.001
77	0.55	-	-
91	-	0.23	0.001
98	1.01	-	-
119	0.16	0.13	0.001
147	0.12	-	-
155	-	0.14	0.002
161	-	-	-
183	0.09	0.13	0.001
211	0.07	-	-

III. Experimental Procedures

A. Determination of Toxic Concentration Levels

A screening test was initially conducted in which non-labeled MC-680 was used to examine the effects of the chemical on the biological oxidation rate of microorganisms. Oxygen utilization was determined manometrically at various concentration levels using the conventional Warburg Constant Volume Respirometer method. The biological seed culture was prepared from fresh sewage and top soil. The concentrations of MC-680 in the media ranged from 0.1 ppm to 10 percent.

The results of the manometric measurements indicated concentration-dependent toxicity at the 10 percent level. Amongst the remaining test levels, ranging from 0.1 ppm to 1 percent, oxygen uptake during the first 72 hours was similar to that observed in the control flask (microbial media without test material). A slight decrease of oxygen utilization was observed after 72 hours at the 1 percent test level indicating possible growth inhibition of the microbial population effected by the presence of the test material.

B. Microbial Acclimation

Acclimation of the microorganisms to the test compound was carried out in a minimum salts-vitamin medium supplemented with 25 mg/l each of yeast extract and vitamin-free casamino acid. During the acclimation period nonlabeled MC-680 was added in increments. On Day 1 and Day 7, 10 mg and 100 mg of the material were added, respectively. The contents were mixed well and aerated every other day, and held at 20°C in the dark. At

various intervals during acclimation, test media samples were prepared using the acclimation culture and tested for microbial activity. Radiocarbon-labeled glucose served as a readily biodegradable compound.

Following 18 days of acclimation, the first 2 tests were initiated with 0.01 and 1.0% of MC-680 (T-I and T-II, respectively). A third test group containing 1 ppm of ^{14}C MC-680 per microbial medium (T-III) was started 46 days after initiation of the acclimation culture. The original inoculum (acclimation media) was used in each test.

C. Reaction Flask Contents

The test medium was prepared as follows: 50 ml of supernatant of acclimated microbial culture and 10 ml of settled fresh sewage were combined and diluted to 500 ml with a minimum salt and vitamin solution. Reagent water according to ASTM specifications was used for this preparation. Hydrogen ion concentration of the final test media was adjusted to a pH value of 7.1 at the start of the study.

Thirty ml of the above solution was added to 125 ml-Erlenmeyer flasks containing the test material. Flask replication consisted of 4 for each concentration level. The test chemical was weighed directly into the flask or transferred quantitatively dissolved in ethylacetate (1.0 ppm). The solvent was completely evaporated prior to addition of medium. Table III presents the actual quantities applied to each reaction flask. Two drops of a surfactant (Tween-80) were added to aid dispersion of the suspended material in the 1 percent concentration tests.

TABLE III

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

Outline of Experiment

Test Samples

Concentration (percent)	Identification	Date Started	Replicate Number (Flasks)	MC-680 Added to Medium (30 ml)			Total Radioactivity* net dpm x 10 ⁶
				Nonlabeled (mg)	¹⁴ C-tagged (mg)	Total Quantity (mg)	
1.0	T-II	9-9	1	289.42**	10.58**	300.00	798.58
			2	290.10	9.90	300.00	747.25
			3	289.89	10.11	300.00	763.10
			4	289.68	10.32	300.00	778.95
1.0 x 10 ⁻²	T-I	9-9	1	0	2.91**	2.91	219.65
			2	0	3.27	3.27	246.82
			3	0	3.41	3.41	257.39
			4	0	3.41	3.41	257.39
			5	0	2.75	2.75	207.57
1.0 x 10 ⁻⁴	T-III	10-7	1	0	31.46 (µg)	0.031	2.374
			2	0	31.46	0.031	2.374
			3	0	31.46	0.031	2.374
			4	0	31.46	0.031	2.374

* Specific activity of ¹⁴C-MC-680 equals 34 µc/mg.

** Test material was weighed directly into flasks.

Duplicate control tests containing ^{14}C -D-glucose in place of the test compound MC-680 were initiated at each of the 2 starting dates. These flasks received microbial media and inoculum identical to that of the respective test sets and were included to demonstrate microbial activity of the culture towards a known degradable material.

A control flask receiving water and HgCl_2 (50 mg/l) instead of the microbial media, but containing ^{14}C -MC-680 was also included. Table IV presents the total radioactivity contained in each of the control flasks at the beginning of the experiment.

Evolved $^{14}\text{CO}_2$ was continuously absorbed in an alkali solution. A small plastic cup was suspended over the medium in the center of the Erlenmeyer flask; the cup contained 1.0 ml of 0.5 N KOH. A piece of pleated filter paper was placed into the alkali solution in such a way that half of the piece was projecting into the open space above (see Figure 2).

TABLE IV

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

Outline of Experiment - continued

Control Samples

14C-tagged Material Added	Date Started	Sample Identification	Total	
			Radioactivity net dpm x 10 ⁶	Media (30 ml)
D-Glucose	9-9	G1 - 1	12.117	Acclimated microorganisms
		G1 - 2	12.176	
D-Glucose	10-7	G1 - 5	13.728	Acclimated microorganisms
		G1 - 6	12.762	
MC-680	10-7	C-H ₂ O	2.374	H ₂ O, dist. + HgCl ₂

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Syringe needle: vent

Rubber stopper

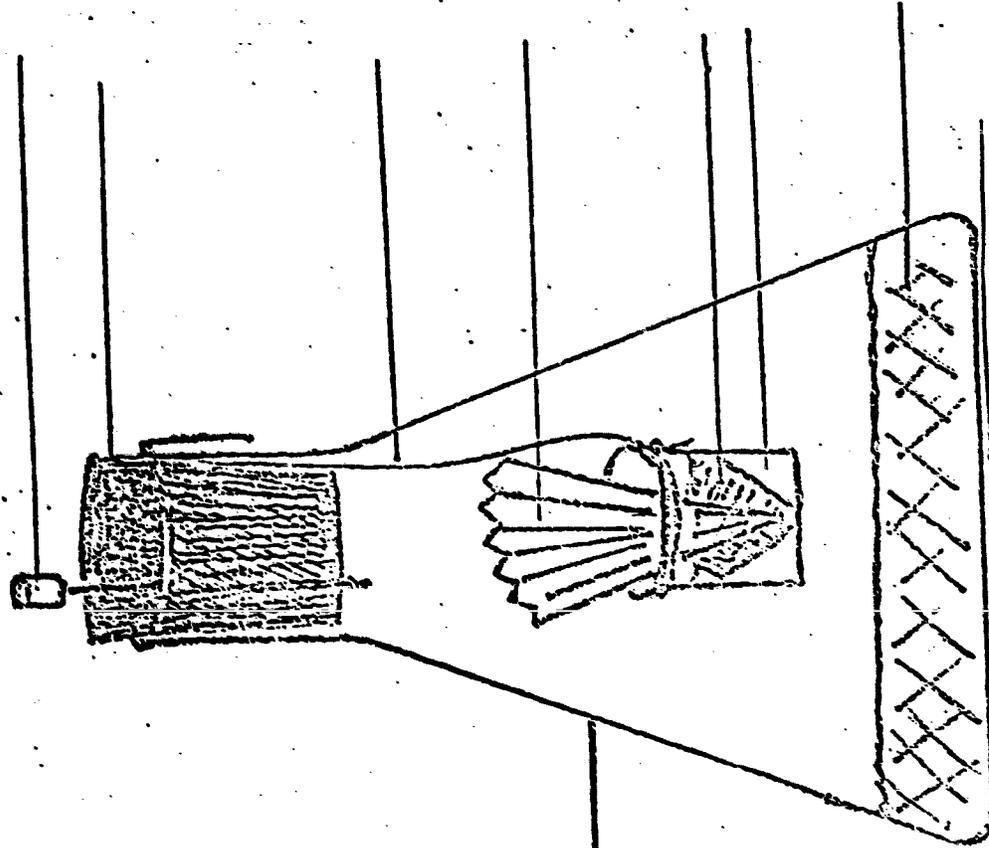
Wire holding KOH cup

Pleated filter paper

0.5 N KOH (1.0 ml)

Polystyrene cup

Medium containing
 ^{14}C -MC-680
(30 ml)



Erlenmeyer flask
(125 ml)

Figure 2. Shake-Flask System

C. Operational Routines and Sampling Procedures

The reaction flasks were incubated in Dubnoff Shakers with continuous shaking. The rate of shaking was approximately 85 cycles per minute. The temperature of the reaction vessels varied with the ambient room conditions between 19 and 23°C. Except for brief periods of sampling, the flasks were kept in the dark throughout the study to prevent algae growth. Periodically, each flask was purged with a 70:30 O₂/N₂ mixture; this was done usually at sampling time and not less than once a week.

The KOH solution from the center cup was transferred quantitatively with several small rinses to a scintillation counting vial. The filter paper was added to the same vial and the contents were radioassayed in Instagel.

During the early phase of the experiment, liberation of ¹⁴C₂O was monitored at shorter intervals; after 3 weeks of incubation, samples were obtained once a week. Total incubation time for tests T-I and T-II was 211 days (30 weeks); the T-III (1 ppm)-units were tested for a total of 183 days or 26 weeks.

D. Measurement of Radioactivity

A Searle Analytic Mark II liquid scintillation counter was employed for all radiometric determinations. Efficiency corrections were made using the channels ratio method¹. Quenching curves were prepared using toluene-¹⁴C standards (radioactivity: 2 x 10⁵ dpm) quenched by additions of variable amounts of carbon tetrachloride².

¹Bruno, G. A., and Christian, J. E., Anal. Chem. **35**, 1024 (1961).

²¹⁴C-Quenched Standards, Amersham/Searle Corporation, Arlington Heights, Illinois.

Counting efficiencies were in a range between 77 and 82 percent.

The samples were routinely counted for 10 minutes and occasionally for longer periods (100 minutes). The samples were counted in low potassium glass vials and allowed to dark adapt in the cooled counting chamber (4°C) for at least 24 hours prior to radioassay.

E. Sensitivity of Detection

Detection of 5 counts above background could be made with extended counting time (100 minutes), this represents 7 disintegrations per minute (dpm) or 92.7 picograms of ^{14}C -MC-680. For any particular sample, the sensitivity in terms of concentration would depend upon the volume of sample employed.

IV. Results

Tables V through VII present the data of the radiometric assays of liberated $^{14}\text{CO}_2$ in the flask. The results are computed as percent of theoretical $^{14}\text{CO}_2$ respiration from the total known substrate activity at each sampling interval.

Evolution of $^{14}\text{CO}_2$ arriving from universally radiocarbon-labeled MC-680 was evident in all tests throughout the study period. During the first two weeks of incubation, the rate of ultimate degradation was comparatively high in all test levels, with a high value of 0.05 percent theoretical CO_2 evolution on Day 1 in the 1 ppm concentration test. Following approximately 6 weeks of exposure, $^{14}\text{CO}_2$ -liberation appeared to have stabilized to an average daily rate ranging from 0.003 to 0.010 percent in the 3 test groups. Total percent recovery of ^{14}C -activity absorbed as $^{14}\text{CO}_2$ was highest in the 1 ppm ^{14}C -MC-680 group; lowest recovery was observed in the 1.0 percent group.

Tables VIII and IX present the data of radioassays of the control flasks containing ^{14}C -tagged D-glucose, and ^{14}C -MC-680 without viable microorganisms.

The positive ^{14}C -glucose control served to assure the viability and activity of the seed organisms used in the test flasks. Within 28 days of continuous incubation an average of approximately 71 percent of the initial radioactivity was completely degraded in flasks 1 and 2. Positive control flasks No. 5 and 6 were started 4 weeks later using the same acclimated

bacterial population in combination with fresh sewage microorganisms.

After 28 days, 65 percent of the original ^{14}C activity had been degraded to $^{14}\text{CO}_2$.

Only trace amounts of $^{14}\text{CO}_2$ were detected in flasks containing $^{14}\text{C-MC-680}$ in the absence of microorganisms.

TABLE V

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Test Group I (0.01 percent)

Test Day Number	Flask Number	Theoretical Substrate ¹⁴ C Activity (dpm)	Net spina	Net dpm ^b	Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
1	1	219,650,000	29,877	37,114	0.017	0.019
	2	246,820,000	41,555	51,557	0.021	
	3	257,390,000	42,262	52,369	0.020	
	4	257,390,000	40,296	49,995	0.019	
2	1	219,612,886	36,034	44,874	0.020	0.018
	2	246,768,443	82,699	102,859	0.042	
	3	257,337,631	30,305	37,553	0.015	
	4	257,340,005	36,860	45,789	0.018	
3	1	219,558,012	59,700	71,755	0.033	0.024
	2	246,665,564	42,994	51,800	0.021	
	3	257,300,078	32,679	39,372	0.015	
	4	257,294,216	39,419	47,493	0.018	
	1	219,496,257	56,168	71,279	0.032	0.022
	2	246,613,784	67,847	88,458	0.036	
	3	257,260,706	48,505	62,506	0.024	
	4	257,246,723	71,554	92,927	0.036	

TABLE V continued

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680

^{14}C Activity Respired as $^{14}\text{CO}_2$ - Test Group I (0.01 percent)

Test Day Number	Flask Number	Total		Net cpm ^a	Net cpm ^b	Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
		Theoretical Substrate ^{14}C Activity (dpm)	^{14}C Activity				
9	1	219,424,978	49,661	61,767	0.028		
	2	246,525,326	58,793	73,126	0.030		
	3	257,198,200	50,943	63,520	0.025		
	4	257,153,796	58,560	72,745	0.028		0.009
13	1	219,363,211	186,170	232,422	0.106		
	2	246,452,200	169,441	211,010	0.086		
	3	257,134,680	81,904	102,380	0.040		
	4	257,081,051	107,941	134,758	0.052		0.018
16	1	219,130,789	63,368	78,914	0.036		
	2	246,241,190	208,283	261,006	0.106		
	3	257,032,300	106,560	132,702	0.052		
	4	256,946,293	138,071	172,158	0.067		0.022
20	1	219,051,875	45,557	56,946	0.026		
	2	245,980,184	57,281	71,423	0.029		
	3	256,899,598	66,422	82,820	0.032		
	4	256,774,135	107,709	134,133	0.052		0.009

TABLE V continued

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Test Group I (0.01 percent)

Test Day Number	Flask Number	Theoretical Substrate ¹⁴ C Activity (dpm)	Total		Net ¹⁴ C Activity (cpma)	Net ¹⁴ C Activity (dpm)	Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
			Net ¹⁴ C Activity (cpma)	Net ¹⁴ C Activity (dpm)				
28	1	218,994,929	184,112	228,427	0.104			
	2	245,908,761	99,950	124,316	0.051			
	3	256,816,778	91,661	113,723	0.044			
	4	256,640,002	53,593	66,423	0.026		0.007	
35	1	218,766,502	149,875	187,110	0.086			
	2	245,784,445	66,348	82,728	0.034			
	3	256,703,055	84,239	104,905	0.041			
	4	256,573,579	75,374	93,866	0.037		0.007	
42	1	218,579,392	94,293	117,280	0.054			
	2	245,701,717	53,969	67,042	0.027			
	3	256,598,683	73,082	91,467	0.036			
	4	256,479,713	62,511	78,139	0.030		0.005	
49	1	218,462,112	38,990	48,555	0.022			
	2	245,634,675	60,766	75,768	0.031			
	3	256,506,683	82,232	107,073	0.042			
	4	256,401,574	50,914	63,484	0.025		0.004	

TABLE V continued

TEST MATERIAL: MC-680

Biodegradability Study With 14C-MC-680

14C Activity Respired as 14CO₂ - Test Group I (0.01 percent)

Test Day Number	Flask Number	Total Theoretical Substrate 14C Activity (dpm)	Net cpma	Net dpm	Rate of 14CO ₂ Liberation (Percent of Total 14C Activity)	Average Daily Rate of 14CO ₂ Liberation (Percent of Total 14C Activity)
56	1	218,413,557	62,900	78,137	0.036	
	2	245,558,907	97,039	120,545	0.049	
	3	256,399,610	129,484	160,850	0.063	
	4	256,338,090	80,044	99,310	0.039	0.007
63	1	218,335,420	93,432	115,921	0.053	
	2	245,438,362	139,810	173,247	0.071	
	3	256,238,760	115,825	144,240	0.056	
	4	256,238,780	108,175	134,546	0.052	0.008
70	1	218,219,499	45,460	56,262	0.026	
	2	245,265,115	69,058	85,574	0.035	
	3	256,094,520	69,184	85,624	0.033	
	4	256,104,234	60,319	74,652	0.029	0.004
77	1	218,163,237	48,365	59,932	0.027	
	2	245,179,541	46,752	57,933	0.024	
	3	256,008,896	81,794	101,734	0.040	
	4	256,029,582	63,759	78,910	0.031	0.004

TABLE V continued

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Test Group I (0.01 percent)

Test Day Number	Flask Number	Total		Net cpm ^a	Net dpm ^b	Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
		Theoretical ¹⁴ C Activity (dpm)	Substrate ¹⁴ C Activity				
98	1	218,103,305	180,782	180,782	224,853	0.103	
	2	245,121,608	159,950	159,950	198,696	0.081	
	3	255,907,162	185,135	185,135	230,842	0.090	0.004
	4	255,950,672	190,426	190,426	236,554	0.092	
119	1	217,878,452	121,753	121,753	156,696	0.072	
	2	244,922,912	150,326	150,326	191,987	0.078	
	3	255,676,320	274,700	274,700	350,383	0.137	0.006
	4	255,714,118	434,757	434,757	555,245	0.217	
147	1						
	2	244,730,925	168,869	168,869	214,573	0.088	
	3	255,325,937	(2,564,049)	(2,564,049)	(3,165,492)	(1.24)	
	4						0.003
161	1	217,721,756	126,372	126,372	161,395	0.074	
	2	244,516,352	127,338	127,338	163,463	0.067	
	3	252,160,445	157,928	157,928	202,213	0.080	
	4	255,158,873	92,903	92,903	120,653	0.047	0.005

TABLE V continued

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680 ^{14}C Activity Respired as $^{14}\text{CO}_2$ - Test Group I (0.01 percent)

Test Day Number	Flask Number	Theoretical ^{14}C Activity (dpm)	Total		Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
			Net cpm ^a	Net dpm ^b		
183	1	217,560,361	87,300	111,780	0.051	
	2	244,352,889	150,552	192,276	0.079	
	3	251,958,232	200,351	256,203	0.102	0.003
	4	255,038,220	104,992	134,605	0.053	
211	1	217,448,581	54,837	69,856	0.032	
	2	244,160,613	163,884	208,504	0.085	
	3	251,702,029	134,539	171,606	0.068	0.002
	4	254,903,615	72,605	92,964	0.036	

^a Net cpm is defined as sample count rate per minute (cpm) minus background count rate (50 cpm).

^b Net dpm is defined as net cpm corrected for counting efficiency to 100 percent (dpm).

TABLE VI

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680

^{14}C Activity Respired as $^{14}\text{CO}_2$ - Test Group II (1.0 percent)

Test Day Number	Flask Number	Total		Net cpm^a	Net dpm^b	Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
		Theoretical ^{14}C Activity (dpm)	Substrate ^{14}C Activity (dpm)				
1	1	798,580,000	75,499	75,499	93,555	0.012	0.014
	2	747,250,000	84,445	84,445	104,770	0.014	
	3	763,100,000	107,502	107,502	133,212	0.017	
	4	778,950,000	81,435	81,435	101,161	0.013	
2	1	798,486,445	57,191	57,191	71,045	0.009	0.013
	2	747,145,230	122,524	122,524	152,393	0.020	
	3	762,966,788	67,549	67,549	84,016	0.011	
	4	778,848,839	79,421	79,421	98,660	0.013	
3	1	798,415,400	43,515	43,515	52,302	0.007	0.008
	2	746,992,837	48,264	48,264	58,079	0.008	
	3	762,882,772	54,190	54,190	65,054	0.009	
	4	778,750,179	52,204	52,204	62,670	0.008	
6	1	798,363,098	101,370	101,370	126,554	0.016	0.007
	2	746,934,758	100,960	100,960	126,358	0.017	
	3	762,817,718	146,363	146,363	184,569	0.024	
	4	778,687,509	128,485	128,485	160,606	0.021	

TABLE VI continued

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Test Group II (1.0 percent)

Test Day Number	Flask Number	Total Theoretical Substrate ¹⁴ C Activity (dpm)	Net cpm ^a	Net dpm ^b	Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
9	1	798,236,544	80,355	99,820	0.013	0.006
	2	746,808,400	142,603	176,708	0.024	
	3	762,633,149	136,562	169,853	0.022	
	4	778,526,903	115,290	143,933	0.018	
13	1	798,136,724	118,433	147,856	0.019	0.007
	2	746,631,692		Sample Lost		
	3	762,463,296	123,103	154,071	0.020	
	4	778,382,970	257,019	319,675	0.041	
16	1	797,988,868	168,869	210,298	0.026	0.007
	2	746,511,692	135,451	169,102	0.023	
	3	762,309,225	113,975	142,291	0.019	
	4	778,063,295	67,556	84,234	0.011	
20	1	797,778,570	249,326	312,048	0.039	0.008
	2	746,342,590	315,407	393,767	0.053	
	3	762,166,934	164,153	205,706	0.027	
	4	777,979,061	72,346	90,320	0.012	

TABLE VI continued

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Test Group II (1.0 percent)

Test Day Number	Flask Number	Theoretical Substrate ¹⁴ C Activity (dpm)	Total		Net dpm	Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
			Net cpm	Net dpm			
28	1	797,466,522	215,467	267,661	0.034	0.003	
	2	745,948,823	(546,398)	(677,073)	(0.091)		
	3	761,961,228	205,711	257,461	0.034		
	4	777,588,741	85,761	104,971	0.013		
35	1	797,198,861	93,581	117,269	0.015	0.003	
	2	745,271,750	(444,394)	(552,729)	(0.074)		
	3	761,703,767	204,449	256,202	0.034		
	4	777,783,770	130,840	164,786	0.021		
42	1	797,081,592	103,792	110,885	0.016	0.003	
	2	744,719,021	(1,234,517)	(1,527,868)	(0.205)		
	3	761,447,565	140,597	176,187	0.023		
	4	777,618,984	72,758	90,948	0.012		
49	1	796,950,707	112,689	140,160	0.018	0.002	
	2	743,191,153	(3,846,100)	(4,650,665)	(0.626)		
	3	761,271,378	135,819	169,350	0.222		
	4	777,528,036	85,788	107,101	0.014		

TABLE VI continued

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Test Group II (1.0 percent)

Test Day Number	Flask Number	Total		Net cpm ^a	Net dpm ^b	Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
		Theoretical ¹⁴ C Activity (dpm)	¹⁴ C Activity (dpm)				
56	1	796,810,547	189,703	189,703	236,243	0.030	0.003
	2	738,540,488	(352,062)	(352,062)	(437,888)	(0.059)	
	3	761,102,028	96,884	96,884	120,653	0.016	
	4	777,420,935	133,998	133,998	166,457	0.021	
63	1	796,574,304	146,792	146,792	184,877	0.023	0.003
	2	738,102,600	(320,462)	(320,462)	(398,585)	(0.054)	
	3	760,981,375	136,004	136,004	169,793	0.022	
	4	777,254,478	115,290	115,290	143,574	0.018	
70	1	796,389,427	105,102	105,102	129,916	0.016	0.002
	2	737,704,015	(302,980)	(302,980)	(375,440)	(0.051)	
	3	760,811,582	100,858	100,858	125,134	0.016	
	4	777,110,904	96,482	96,482	119,556	0.015	
77	1	796,259,511	104,282	104,282	129,965	0.016	0.002
	2	737,328,575	(191,888)	(191,888)	(240,160)	(0.033)	
	3	760,686,448	92,022	92,022	114,171	0.015	
	4	776,991,348	136,376	136,376	168,991	0.022	

TABLE VI continued

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Test Group II (1.0 percent)

Test Day Number	Flask Number	Theoretical Substrate ¹⁴ C Activity (dpm)	Total		Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
			Net ¹⁴ C Activity (dpm)	Net ¹⁴ C Activity (cpm)		
56	1	796,810,547	189,703	236,243	0.030	0.003
	2	738,540,488	(352,062)	(437,888)	(0.059)	
	3	761,102,028	96,884	120,653	0.016	
	4	777,420,935	133,998	166,457	0.021	
63	1	796,574,304	146,792	184,877	0.023	0.003
	2	738,102,600	(320,462)	(398,585)	(0.054)	
	3	760,981,375	136,004	169,793	0.022	
	4	777,254,478	115,290	143,574	0.018	
70	1	796,389,427	105,102	129,916	0.016	0.003
	2	737,704,015	(302,380)	(375,440)	(0.051)	
	3	760,811,582	100,858	125,134	0.016	
	4	777,110,904	96,482	119,556	0.015	
77	1	796,259,511	104,882	129,965	0.016	0.002
	2	737,328,575	(191,888)	(240,160)	(0.033)	
	3	760,686,448	92,022	114,171	0.015	
	4	776,991,348	136,376	168,991	0.022	

TABLE VI continued

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680

^{14}C Activity Respired as $^{14}\text{CO}_2$ - Test Group II (1.0 percent)

Test Day Number	Flask Number	Total		Net cpm	Net cpm	Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
		Theoretical ^{14}C Activity (dpm)	Substrate ^{14}C Activity (dpm)				
98	1	796,129,546	173,260	215,230			
	2	737,088,415	(293,205)	(366,506)		0.027	
	3	760,572,277	182,765	226,756		(0.050)	
	4	776,822,357	170,598	211,398		0.030	
119	1	795,914,316	165,787	212,547		0.027	0.001
	2	736,721,909	98,325	126,058		0.017	
	3	760,345,521	156,200	200,000		0.026	
	4	776,610,959	136,004	174,141		0.022	
147	1			Contaminated Sample			0.001
	2			Contaminated Sample			
	3	760,145,521	438,546	559,370		0.074	
	4			Contaminated Sample			
161	1	795,701,769	226,707	290,650		0.037	0.003
	2	736,595,851	194,125	248,878		0.034	
	3	759,586,151	117,736	151,526		0.020	
	4	776,436,818	173,863	222,616		0.029	0.002

TABLE VI continued

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Test Group II (1.0 percent)

Test Day Number	Flask Number	Theoretical ¹⁴ C Activity (dpm)	Total		Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
			Net cpm ^a	Net dpm ^b		
183	1	795,411,119	153,560	196,368		
	2	736,346,973	(1,030,877)	(1,311,548)	0.025	
	3	759,434,625	282,436	360,250	(0.178)	
	4	776,214,202	197,578	252,657	0.047	
211	1	795,214,751	375,889	478,230	0.060	0.002
	2	735,035,425	300,250	382,972	0.052	
	3	759,074,375	132,275	168,503	0.022	
	4	775,961,545	108,175	137,978	0.018	0.001

^a Net cpm is defined as sample count rate per minute (cpm) minus background count rate (50 cpm).
^b Net dpm is defined as net cpm corrected for counting efficiency to 100 percent (dpm).

TABLE VII

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680 ^{14}C Activity Respired as $^{14}\text{CO}_2$ - Test Group III (1 ppm)

Test Day Number	Flask Number	Theoretical Substrate ^{14}C Activity (dpm)	Total		Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
			Net cpm	Net dpmb		
1	1	2,374,500	804	1,009	0.042	0.050
	2	2,374,500	939	1,180	-0.050	
	3	2,374,500	786	1,003	0.042	
	4	2,374,500	1,214	1,518	0.064	
3	1	2,373,491	1,020	1,278	0.054	0.037
	2	2,373,320	1,668	2,201	0.093	
	3	2,373,497	1,458	1,827	0.077	
	4	2,372,982	1,324	1,657	0.070	
7	1	2,372,213	1,280	1,602	0.068	0.018
	2	2,371,119	1,352	1,686	0.071	
	3	2,371,670	1,027	1,279	0.054	
	4	2,371,325	1,720	2,150	0.091	
14	1	2,370,611	902	1,128	0.048	0.008
	2	2,369,433	993	1,241	0.052	
	3	2,370,391	768	956	0.040	
	4	2,369,175	1,488	1,855	0.078	

TABL VII continued

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680

^{14}C Activity Respired as $^{14}\text{CO}_2$ - Test Group III (1 ppm)

Test Day Number	Flask Number	Theoretical Substrate ^{14}C Activity (dpm)	Total		Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
			Net cpm	Net dpm		
21	1	2,369,482	972	1,220	0.051	0.008
	2	2,368,192	864	1,084	0.046	
	3	2,369,435	920	1,150	0.049	
	4	2,367,320	1,543	1,919	0.081	
28	1	2,368,263	1,180	1,468	0.062	0.010
	2	2,367,108	1,239	1,537	0.065	
	3	2,368,285	1,079	1,340	0.057	
	4	2,365,401	1,675	2,091	0.088	
35	1	2,366,795	1,958	2,444	0.103	0.014
	2	2,365,571	2,170	2,712	0.115	
	3	2,366,945	1,701	2,129	0.090	
	4	2,363,310	1,397	1,740	0.074	
42	1	2,364,351	1,515	1,887	0.080	0.012
	2	2,362,859	2,118	2,638	0.112	
	3	2,364,816	1,349	1,674	0.071	
	4	2,361,570	1,359	1,684	0.071	

TABLE VII continued

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680

^{14}C Activity Respired as $^{14}\text{CO}_2$ - Test Group III (1 ppm)

Test Day Number	Flask Number	Total Theoretical ^{14}C Activity (dpm)	Net cpm^a	Net dpm^b	Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
49	1	2,362,464	1,320	1,646	0.070	
	2	2,360,221	1,354	1,676	0.071	
	3	2,363,142	1,450	1,810	0.077	
	4	2,359,886	1,405	1,751	0.074	0.010
70	1	2,360,818	1,994	2,499	0.106	
	2	2,358,545	2,147	3,919	0.166	
	3	2,361,332	1,758	2,198	0.093	
	4	2,359,135	1,655	2,098	0.089	0.005
91	1	2,358,319	943	1,276	0.054	
	2	2,354,626	1,103	1,436	0.061	
	3	2,359,134	499	657	0.028	
	4	2,356,037	1,418	2,052	0.087	0.003
119	1	2,357,043	5,496	7,028	0.298	
	2	2,353,190	3,565	4,541	0.193	
	3	2,358,477	3,868	4,934	0.209	
	4	2,353,985	4,183	5,342	0.227	0.008

TABLE VII continued

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680

^{14}C Activity Respired as $^{14}\text{CO}_2$ - Test Group III (1 ppm)

Test Day Number	Flask Number	Theoretical Substrate ^{14}C Activity (dpm)	Total		Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
			Net cpm ^a	Net dpm ^b		
155	1	2,350,015	4,394	5,633	0.240	0.006
	2	2,348,649	2,928	3,739	0.159	
	3	2,353,543	5,188	6,617	0.281	
	4	2,348,643	4,420	5,740	0.244	
183	1	2,344,382	2,220	2,839	0.121	0.006
	2	2,344,910	3,318	4,253	0.181	
	3	2,346,926	2,104	2,694	0.115	
	4	2,342,903	3,963	5,061	0.216	

^a Net cpm is defined as sample count rate per minute (cpm) minus background count rate (50 cpm).
^b Net dpm is defined as net cpm corrected for counting efficiency to 100 percent (dpm).

TABLE VIII

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Control Group - Glucose

Test Day Number	Flask Number	Total		Net cpm ^a	Net dpm ^b	Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
		Theoretical ¹⁴ C Activity (dpm)	¹⁴ C Activity				
1	1	12,117,000	2,631,551	3,224,940	26.62	27.29	
	2	12,175,000	2,777,750	3,404,105	27.96		
2	5	13,728,000	2,325,529	2,853,410	20.78	20.86	
	6	12,761,550	2,173,861	2,673,876	20.95		
3	1	8,892,060	337,812	418,602	4.71	4.80	
	2	8,770,895	345,995	429,274	4.89		
6	1	8,473,458	429,135	515,787	6.09	6.18	
	2	8,341,621	434,733	522,516	6.26		
6	5	10,874,590	1,428,520	1,763,605	16.22	7.80	
	6	10,087,674	1,219,461	1,509,234	14.96		
6	1	7,957,671	1,075,218	1,329,070	16.70	5.78	
	2	7,819,105	1,136,312	1,404,588	17.96		

TABLE VIII continued

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680

^{14}C Activity Respired as $^{14}\text{CO}_2$ - Control Group - Glucose

Test Day Number	Flask Number	Theoretical Substrate ^{14}C Activity (dpm)	Total		Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
			Net cpm	Net dpm		
7	5	9,110,985	2,272,725	2,795,480	30.68	7.28
	6	8,578,440	1,923,025	2,368,257	27.61	
9	1	6,628,601	854,650	1,059,046	15.98	5.53
	2	6,414,517	892,806	1,104,958	17.23	
13	1	5,569,555	833,282	1,032,568	18.54	4.94
	2	5,309,559	900,850	1,114,913	21.00	
14	5	6,315,505	1,111,060	1,373,374	21.75	2.98
	6	6,210,183	999,949	1,237,561	19.93	

TABLE VIII continued

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Control Group - Glucose

Test Day Number	Flask Number	Total Theoretical ¹⁴ C Activity (dpm)	Net cpm ^a	Net dpm ^b	Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
16	1	4,536,987	304,878	378,730	8.35	3.35
	2	4,194,646	396,775	492,888	11.75	
20	1	4,158,257	263,802	328,521	7.90	2.54
	2	3,701,758	368,953	458,897	12.40	
21	5	4,942,131	295,808	367,007	7.43	1.03
	6	4,972,622	281,640	349,429	7.03	
28	1	3,829,736	326,744	405,393	10.58	1.49
	2	3,242,861	346,020	429,305	13.24	
	5	4,575,124	157,679	195,632	4.28	0.59
	6	4,623,193	147,879	183,245	3.96	

TABLE VIII continued

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680 ^{14}C Activity Respired as $^{14}\text{CO}_2$ - Control Group - Glucose

Test Day Number	Flask Number	Theoretical ^{14}C Activity (dpm)	Total		Net dpm ^b	Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
			Net cpm ^a	Net dpm ^b			
35	1	8,954,343c	2,941,124	5,604,319	40.25	5.49	
	2	9,313,556c	2,777,725	3,408,252	36.60		
42	5	4,379,492	172,662	214,487	4.90	0.64	
	6	4,439,948	147,009	182,620	4.11		
49	1	5,350,024	577,984	717,102	13.40	1.96	
	2	5,905,304	666,616	826,042	13.99		
49	5	4,165,005	107,477	133,016	3.19	0.66	
	6	4,257,328	206,135	255,110	5.99		
56	1	4,632,922	226,707	281,624	6.08	0.84	
	2	5,079,262	230,897	286,475	5.64		
56	5	4,031,989	83,844	104,025	2.58	0.41	
	6	4,002,210	101,991	126,380	3.16		
56	1	4,351,298	144,250	179,193	4.12	0.55	
	2	4,792,789	139,226	172,737	3.60		

TABLE VIII continued

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Control Group - Glucose

Test Day Number	Flask Number	Total Theoretical Substrate ¹⁴ C Activity		Net cpm ^a	Net dpm ^b	Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
		(dpm)	(cpm)				
63	1	4,172,105	130,158	161,888	161,888	3.88	0.49
	2	4,620,052	109,240	135,702	135,702	2.94	
70	1	4,010,217	129,652	160,659	160,659	4.01	0.44
	2	4,484,350	79,806	98,892	98,892	2.20	
77	5	3,927,964	234,692	291,543	291,543	7.42	0.35
	6	3,875,827	222,667	276,605	276,605	7.14	
91	1	3,849,558	144,459	178,786	178,786	4.64	0.55
	2	4,385,458	106,220	131,460	131,460	3.00	
91	5	3,636,421	(24,262) ^d	(31,550)	(31,550)	(0.87)	0.23
	6	3,599,222	137,881	175,645	175,645	4.88	

TABLE VIII continued

TEST MATERIAL: MC-680

Biodegradability Study With ¹⁴C-MC-680

¹⁴C Activity Respired as ¹⁴CO₂ - Control Group - Glucose

Test Day Number	Flask Number	Theoretical ¹⁴ C Activity (dpm)	Total		Net dpm ^b	Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)	Average Daily Rate of ¹⁴ CO ₂ Liberation (Percent of Total ¹⁴ C Activity)
			Substrate Net cpm ^a	Net dpm ^b			
98	1	3,670,772	216,400	269,825	7.35	1.01	
	2	4,253,998	230,365	286,524	6.74		
119	1	3,400,947	137,501	174,938	5.14	0.16	
	2	3,957,474	49,127	62,582	1.58		
147	5	3,604,871	(3,032) ^d	(3,867)	(0.11)	0.13	
	6	3,423,577	97,907	124,722	3.64		
155	1	3,226,009	90,603	115,271	3.57	0.12	
	2	3,904,892	93,408	118,991	3.13		
155	5	3,601,004	(7,760) ^d	(9,911)	(0.28)	0.14	
	6	3,298,855	129,652	165,584	5.02		

TABLE VIII continued

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680

^{14}C Activity Respired as $^{14}\text{CO}_2$ - Control Group - Glucose

Test Day Number	Flask Number	Theoretical ^{14}C Activity (dpm)	Total		Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
			Net cpm ^a	Net dpm ^b		
193	1	3,110,738	103,470	132,146	4.25	0.09
	2	3,785,901	69,269	88,693	2.34	
211	5	3,591,093	(18,373) ^d	(23,555)	(0.66)	0.13
	6	3,133,271	86,514	111,058	3.54	
211	1	2,978,592	60,782	77,627	2.61	0.07
	2	3,697,208	39,101	49,874	1.35	

a Net cpm is defined as sample count rate per minute (cpm) minus background count rate (50 cpm).

b Net dpm is defined as net cpm corrected for counting efficiency to 100 percent (dpm).

c On Test Day 29 additional ^{14}C -D-Glucose was added to medium (Flask 1: 5.53×10^6 dpm; Flask 2: 6.50×10^6 dpm).

d Media in Flask 5 was accidentally contaminated with KOK solution. These data have not been included in the remaining average daily $^{14}\text{CO}_2$ liberation rates.

TABLE IX

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680

^{14}C Activity Respired as $^{14}\text{CO}_2$ - Control Group ($\text{H}_2\text{O}-\text{HgCl}_2$)

Test Day Number	Total Theoretical Substrate ^{14}C Activity (dpm)		Net cpm ^a	Net dpm ^b	Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
1	2,374,500	7ifb	0	0	0.000	0.000
3	2,374,500	54	70	70	0.003	0.002
7	2,374,430	7ifb	0	0	0.000	0.000
14	2,374,430	0	0	0	0.000	0.000
21	2,374,430	75	98	98	0.004	0.001
35	2,374,332	23	30	30	0.001	<0.001
42	2,374,302	4ifb	0	0	0.000	0.000
49	2,374,302	1,110	1,386	1,386	0.058	0.008
70	2,372,916	112	144	144	0.006	<0.001
91	2,372,772	255	435	435	0.018	0.001

ifb = indistinguishable from background count rate

TABLE IX continued

TEST MATERIAL: MC-680

Biodegradability Study With ^{14}C -MC-680

^{14}C Activity Respired as $^{14}\text{CO}_2$ - Control Group (H_2O - HgCl_2)

Test Day Number	Total Theoretical Substrate ^{14}C Activity (dpm)	Net cpm ^a	Net dpm ^b	Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)	Average Daily Rate of $^{14}\text{CO}_2$ Liberation (Percent of Total ^{14}C Activity)
119	2,372,337	689	882	0.037	0.001
155	2,371,455	1,307	1,671	0.070	0.002
182	2,369,784	457	589	0.025	0.001

^a Net cpm is defined as sample count rate per minute (cpm) minus background count rate (50 cpm) minus background count rate (50 cpm).

^b Net dpm is defined as net cpm corrected for counting efficiency to 100 percent (dpm).

CN-10-0680

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VELSICOL CHEMICAL CORPORATION

LABORATORY REPORT

1,2-Bis-(tribromophenoxy)
ethane
CAS# 3753-59-1

SUBJECT: PARTITION COEFFICIENTS OF MC-680,
CHLORENDIC ANHYDRIDE AND CHLORENDIC
ACID

UNITERM NO: _____

PROJECT NO: 482348 - 484028

REPORT NO: (2) - 1

AUTHOR: C. C. Yu and Y. H. Atallah

SECTION: Environmental Sciences

LOAN OUT

DATE TYPED: December 16, 1977

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PERIOD COVERED: September, 1977

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REFERENCES: Cited at end of text.

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OBJECT: To determine the partition coefficients of MC-680, chlorendic anhydride and chlorendic acid in n-octanol/water and 1,2-dichlorobenzene/water systems.

SUMMARY: ¹⁴C MC-680, ¹⁴C chlorendic anhydride and ¹⁴C chlorendic acid were used to determine the partition coefficients. In the n-octanol/water system, the partition coefficients of MC-680 and chlorendic acid were 1373 and 2.2 respectively. In the 1,2-dichlorobenzene/water system, the partition coefficient of chlorendic anhydride was 0.49.

CCY/YHA:sst

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INTRODUCTION

The partition coefficient of a given chemical in an organic/water system may serve as an indicator for the bioaccumulation potential of the chemical in aquatic environments (1). Therefore, the partition coefficients of MC-680 and chlorendic anhydride were determined to assist in the evaluation of the environmental fate of these two chemicals.

MATERIALS AND METHODS

Chemicals -

^{14}C MC-680 1,2-bis(2,4,6-tribromophenoxy - UL - ^{14}C) ethane, with a specific activity of 23 mCi/mM and ^{14}C chlorendic anhydride UL - ^{14}C , with a specific activity of 4 mCi/mM were prepared by Pathfinder Laboratories, Inc., St. Louis, Mo. Radiochemical purities of the ^{14}C chemicals were greater than 98%. ^{14}C chlorendic acid was prepared by spotting ^{14}C chlorendic anhydride on silica gel TLC plate, developed in benzene/dioxane/acetic acid (76:21:3) and then eluted with acetone (Reference 2).

Methods -

Chlorendic anhydride has been shown to hydrolyze rapidly to chlorendic acid in an aqueous solution (2). Therefore chlorendic acid was used in the n-octanol/water system. Chlorendic anhydride also reacted with alcohols to form monoalkyl chlorendate (2). Thus the n-octanol/water system had to be modified. Since 1,2-dichlorobenzene and n-octanol have similar dielectric constants, 1,2-dichlorobenzene was selected to replace n-octanol.

The partition coefficients of MC-680 and chlorendic acid were studied in the n-octanol/water system while chlorendic anhydride was studied in the 1,2-dichlorobenzene/water system. The methods described by Leo, Hansch and Elkins (1971) (Reference 3) were followed. Each chemical was studied at 2 concentrations. A suitable amount of ^{14}C chemical (between 0.2 μCi and 0.7 μCi) was delivered to a 15 ml centrifuge tube, the solvent was evaporated under N_2 , and then 2 ml of n-octanol or 1,2-dichlorobenzene was added to dissolve the ^{14}C chemical. Five ml of water was added to the tube and then the tube was covered with a parafilm. The tube was inverted 200 x in about 5 mins. and then was centrifuged at 3,000 ppm for 15 mins. (International Equipment Co., Model CS). After centrifugation, 0.1 ml of the organic phase and 1 ml of the water phase was counted in Aquasol (New England Nuclear). The tube inversion and sample taking was repeated one more time. Duplicate samples were counted using Searle Mark III Liquid Scintillation System Model 6880. An external standard pulse method was used to determine sample quenching. Counting efficiency, usually between 85 - 88%, was determined by an on-line computation program.

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RESULTS AND DISCUSSION

Concentrations of MC-680 in the n-octanol and the water phases were determined and the partition coefficients were calculated (Table 1). At lower concentrations, the equilibrium between n-octanol and water phases was reached during first 200 x inversion of tube. However at higher concentrations, the radioactivity in the aqueous phase of the second sampling increased slightly over that of the first sampling. Therefore, equilibrium was probably not reached during the second 200 x inversions of the tube. The average partition coefficient of the 4 determinations was 1373.

Concentrations of chlorendic acid in the n-octanol and the aqueous phases were determined and the partition coefficients were calculated (Table 2). The equilibrium was not reached during the first 200 x inversions of the tube, especially at the higher concentration. At second sampling the concentration in the n-octanol phase decreased, while the concentration in the aqueous phase increased. The partition coefficient of chlorendic acid was also affected by its concentration. The partition coefficients were larger at the higher concentration (i.e. 0.88 at the lower concentration and 3.55 at the higher concentration). Thus at higher concentrations the true equilibrium might not have been reached even after the second 200 x inversions of the tube (Table 2). The average partition coefficient in 4 determinations was 2.21.

For chlorendic anhydride in the 1,2-dichlorobenzene/water system, the equilibrium between the organic and the aqueous phases was reached during the first 200 x inversions of the tube. Apparently the partition coefficients were not affected by the difference in concentrations. The average partition coefficient of 4 determinations was 0.49. Since chlorendic anhydride was gradually hydrolyzed to chlorendic acid in aqueous solutions with a half-life of about 1 hour (Reference 2) the partition coefficient determination in 1,2-dichlorobenzene/water system for chlorendic anhydride might have actually represented that for a mixture of chlorendic anhydride and chlorendic acid.

When MC-680 and chlorendic acid were compared, it was apparent that MC-680 was more lipophilic than chlorendic acid. Thus MC-680 might tend to bioaccumulate in the aquatic environment more than chlorendic acid. On the other hand, no bioaccumulation of chlorendic acid or chlorendic anhydride in aquatic organisms is expected.

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References Cited

1. Brock, N., D. R. Branson, and G. E. Blau. 1974. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ. Sci. and Tech., 8 : 1113
2. Yu, C. C., Y. H. Atallah. 1977. Hydrolysis of chlorendic anhydride in aqueous solutions and stability on TLC plates. VCC project #482348, Report #1.
3. Leo, A., C. Hansch, and D. Elkins. 1971. Partition coefficients and their uses. Chem. Rev., 71 : 537 - 8.

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Table 1. Partition coefficient of MC-680
in n-octanol/water system.^{1/}

	1st Sampling			2nd Sampling			Average Partition Coefficient	
	Concentration (ppm)		Partition Coefficient ^{2/}	Concentration (ppm)		Partition Coefficient		
	<u>n-octanol</u>	<u>water</u>		<u>n-octanol</u>	<u>water</u>			
IA	3.127	0.00228	1371	3.158	0.00254	1243		
IB	3.201	0.00240	<u>1334</u>	3.209	0.00228	<u>1407</u>		
Average			1353			1325	1339	
<hr/>								
IIA	12.790	0.00883	1449	12.587	0.00925	1361		
IIB	12.893	0.00836	<u>1553</u>	12.800	0.1011	<u>1266</u>		
Average			1501			1313	1407	
<hr/>								
Average of I and II							1373	

^{1/} Each value in the table represents an average of 2 assays.

^{2/} Partition coefficient = concentration in n-octanol phase/concentration in aqueous phase.

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Table 2. Partition coefficient of chlorendic acid and chlorendic anhydride in n-octanol/water and 1,2-dichlorobenzene/water systems.

Compound	1st Sampling			2nd Sampling			Average Partition Coefficient
	Concentration (ppm)		Partition Coefficient ^{2/}	Concentration (ppm)		Partition Coefficient	
	n-octanol	water		n-octanol	water		
Chlorendic acid							
A	4.63	4.62	1.00	3.18	5.30	0.60	
B	4.89	4.29	<u>1.14</u>	3.80	4.94	<u>0.77</u>	
Average			1.07			0.68	0.88
IA	27.60	6.40	4.31	25.65	8.13	3.16	
IB	25.41	7.11	<u>3.57</u>	23.62	7.47	<u>3.16</u>	
Average			3.94			3.16	3.55
Average of I and II							2.21
	<u>1,2-Cl₂ benzene</u>	<u>water</u>		<u>1,2-Cl₂ benzene</u>	<u>water</u>		
Chlorendic anhydride ^{3/}							
A	1.92	3.72	0.52	2.22	3.88	0.57	
B	1.93	3.60	<u>0.54</u>	2.31	3.86	<u>0.60</u>	
Average			0.53			0.58	0.56
IA	6.23	11.78	0.53	7.20	12.00	0.60	
IB	3.71	12.85	<u>0.29</u>	3.17	12.93	<u>0.25</u>	
Average			0.41			0.42	0.42
Average of I and II							0.49

Each value in the table represents an average of 2 assays.

Partition coefficient = concentration in organic phase/concentration in aqueous phase.

Chlorendic anhydride was used in 1,2-dichlorobenzene/water system. It has been determined that chlorendic anhydride was gradually hydrolyzed to chlorendic acid in aqueous solutions (2).

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CN-10-0935

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VELSICOL CHEMICAL CORPORATION

LABORATORY REPORT

SUBJECT: WATER SOLUBILITY OF SEVERAL
FLAME RETARDANTS AND
INDUSTRIAL CHEMICALS
 AUTHOR: Ching C. Yu and Y. H. Atallah

PROJECT NO: 428038, 428048,
428148, 484028,
484048, 484058 & 4624
 REPORT NO: 1, 1, 1, 2, 1, 1, and
respectively.
 SECTION: Environmental Sciences

DATE TYPED: April 12, 1978
 PERIOD COVERED: February to April, 1978
 WORK DONE BY: C. C. Yu and P. L. Thomas
 SUPERVISOR: Y. H. Atallah
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REFERENCES: Notebook No. 3583
Pages 16, 17 and 22
Notebook No. 3615, Pages 44,
45, 48 - 50, 55 - 63, 84 - 95.

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OBJECT: To determine the water solubility of CBE, BP-4A, MC-935A, 2,4,6-tribromophenol, MC-680, MC-948 and PHT-4.
*↳ 1,2-Bis(tribromophenoxy)ethane
 CAS# 37853-59-1*

SUMMARY: Excess amounts of ¹⁴C labeled compounds in distilled water was shaken in water bath at 35°C overnight. After centrifugation at 15°C, 25°C or 35°C and at 12,000 x G for 1 hour, water solubility was determined by radioassay. The average solubility (ppm) of duplicate experiments at 15°C, 25°C and 35°C respectively were as follows: CBE (145, 116 and 101), BP-4A (0.72, 4.16 and 1.77), MC-935A (3.13, 1.61 and 2.26), 2,4,6-tribromophenol (996, 969 and 884), MC-680 (0.16, 0.20 and 0.08), MC-948 (0.91, 0.52 and 1.28) and PHT-4 (149, 241 and 242).

RECEIVED APR 25 1978

SIGNATURE: Ching C. Yu

INTRODUCTION

Water solubility of a given chemical may be related to its volatility, adsorption and leachability in soil, and bioaccumulation in aquatic organisms. Thus the water solubility of a compound is one of the principal factors which determine the fate of this compound in the environment. This study reports on the water solubility of several flame retardents and industrial chemicals as part of the chemical hazard assessment program.

MATERIALS AND METHODSChemicals

¹⁴C MC-680 was synthesized by Pathfinder Labs, St. Louis, Mo. Other ¹⁴C chemicals were synthesized by Midwest Research Institute, Kansas City, Mo. Radiochemical purity of all ¹⁴C compounds was greater than 98%. The labeling position and specific activity were as follows:

CBE: m-chloromethylbenzoate-(phenyl-UL-¹⁴C), 10.2 mCi/mM
 BP-4A: tetrabromobisphenol A-(phenyl-UL-¹⁴C), 9.32 mCi/mM
 MC-935A: poly(dibromophenylene) oxide-(phenyl-UL-¹⁴C) 48 μ Ci/mg
 2,4,6-Tribromophenol: phenyl-UL-¹⁴C, 12.1 mCi/mM
 MC-680: 1,2-bis(2,4,6-tribromophenoxy) ethane-(phenyl-UL-¹⁴C),
 23 mCi/mM
 PHT-4: tetrabromophthalic anhydride-(phenyl-UL-¹⁴C), 11.6 mCi/mM
 MC-948: bis(tribromoneopentyl) pentaerythritol cyclic diphosphate-
 (pentaerythritol UL-¹⁴C), 7.8 mCi/mM

Method

The ¹⁴C labeled materials were diluted with respective reference standards to achieve a suitable specific activity (Table 1). Appropriate amounts of the diluted ¹⁴C chemicals in solution (Table 1) were placed in polyallomer centrifuge tubes (Beckman cat. no. 326814). A total of 6 tubes were prepared for each compound. A gentle stream of nitrogen was used to remove the solvent from the tubes. Twenty ml of distilled water was added to each tube and then caps (Beckman cat. no. 338906) were placed

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over each tube and tightened. The tubes were placed in a water bath and shaken overnight at 35°C. Then the 6 tubes were centrifuged at 12,000 x G (Beckman Model L5-50 Preparative Ultracentrifuge, Rotor Type 50.2 Ti fixed angle, operated at 11,500 rpm) for 1 hour; 2 tubes at 15°C, 2 tubes at 25°C and the remaining 2 tubes at 35°C. After centrifugation, duplicate 2 ml of the solution were taken for radioassay.

Radioassay

All liquid samples were counted in Handifluor scintillation solution (Mallinckrodt, Inc.). A Mark III Liquid Scintillation System, Model 6880 (Searle Analytic, Inc.) was used for radiocarbon counting. Sample quenching was determined by an external standard pulse method. Counting efficiency, usually between 70 - 85%, was determined by an on-line computation program.

RESULTS AND DISCUSSION

The water solubility of CBE was obtained after 1 and 2 hours of centrifugation at 3 different temperatures (Table 2). The results showed that no significant difference in solubility was observed between the first and second centrifugation at any of the 3 temperatures. Therefore the water solubility of the other compounds was determined after 1 hour of centrifugation.

All tested compounds had very low water solubility, yet there was enough differences indicating varying degrees of hydrophobicity (Table 1). Among these compounds, 2,4,6-tribromophenol had the highest water solubility while MC-680 had the lowest water solubility.

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TABLE 1. Experimental parameters and water solubility of several flame retardants and industrial chemicals. ^{1/}

Solvent	Specific Activity (dpm/µg)	Amount (µg/tube)	Compounds					
			DB-4A Acetone	MC-935A Chloroform	2,4,6-Br ₃ -phenol Acetone	MC-680 Toluene	MC-248 Acetone	PHT-4 ^{2/} Acetone
		18.5	4503	276	12.2	5119	493	10.1
		30,004	113	3,007.5	30,004.5	107	1,025.5	50,009
Water Solubility (ppm) ^{2/}								
15°C	I	140	0.73	2.06	1182	0.26	1.01	147
	II	150	0.70	4.19	810	0.06	0.81	151
Average		145	0.72	3.13	996	0.16	0.91	149
25°C	I	119	3.66	0.80	792	0.19	0.49	166
	II	113	4.66	2.42	1146	0.21	0.55	311
Average		116	4.16	4.61	969	0.20	0.52	241
35°C	I	81	1.16	2.43	1082	0.09	1.17	230
	II	121	2.37	2.13	685	0.06	1.39	245
Average		101	1.77	2.28	884	0.08	1.28	241

^{1/}After 1 hour of centrifugation at 12,000 x G.

^{2/}Each value in the table represents the average of duplicate analyses.

^{3/}TLC analysis indicated that about 50% of the dissolved PHT-4 was converted to tetrabromophthalic acid.

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TABLE 2. Water solubility determination of CBE after 1 and 2 hours of centrifugation.^{1/}

Temperature	CBE Water Solubility (ppm)	
	After 1st hr. centrifugation	After 2nd hr. centrifugation
15°C	I	140
	II	<u>150</u>
	Average	145
25°C	I	119
	II	<u>113</u>
	Average	116
35°C	I	81
	II	<u>121</u>
	Average	101

^{1/}At 12,000 x G.

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TECHNICAL REPORT #88

SOLUBILITY OF MC-680 IN VARIOUS CLASS SOLVENTS

1,2-Eis-(tribromophenoxy)ethane

AUTHOR: J. DILL

DATE: DECEMBER 9, 1974

**cc: A. F. Kerst
F. A. Daniher
L. H. Hahn
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T. L. Smith
C. File.**

Technical Report #88

DATE: December 9, 1974

SUBJECT: Solubility of MC-680 in Various Class Solvents

Author: J. Dill

I. Purpose

The purpose of this investigation was to provide information pertaining to the solubility of FireMaster[®] MC-680 [bis(2,4,6-tribromophenoxy)ethane] in various class solvents.

II. Background

FireMaster MC-680 is obtaining line product status which requires thorough knowledge of its physical and chemical properties. One important property is its solubility in solvents, such information being useful for further testing, purification, mixing, cleaning up, etc. Preliminary studies indicate that this compound shows low solubility in most all common solvents at ambient temperature. This work describes experiments and the data obtained for solubility of MC-680 in class solvents at ambient and elevated temperatures.

III. Summary

The solubility of MC-680 in various class solvents was determined at ambient and elevated temperature. The data obtained from the experimental are presented in three forms:

Table I	The Solvents	Listed alphabetically
Table II	The Solvents	Listed by their dipole moments
Table III	The Solvents	Listed according to functional groups (class solvents)

All values stated in these tables were reported as gm per 100 ml at X°C (temperature).

There were conclusions drawn from the tables. It was found that, at ambient temperature, most of the solvents used with a dipole moment of less than 1.75 μ seemed to be the better solvents. With this information from Table II, Table III showed that the halogenated aliphatics and aromatics, aromatics and the cyclic ethers solvents were the better solvents.

At the elevated temperatures, o-dichlorobenzene was the best (102.8 gms/159°C). The solubility of MC-680 was dictated by the class of solvent and then by the individual test temperatures of the solvent.

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IV. Experimental

In the determination of the solubility of MC-680 in class solvents, the experiments were run at ambient and elevated temperatures.

The determinations at ambient temperature were conducted in an open air environment. In a 250 ml round bottom flask equipped with a magnetic stirrer and thermometer, 100 ml of solvent was added and allowed to stir until room temperature was obtained. The compound (MC-680) was then added in ~0.1 gm increments until the saturation point was obtained, allowing 10 minutes of stirring per addition of each increment. The temperature of the solution was monitored for possible increases due to the Δ Heat of solution.

The determinations at the elevated temperatures were conducted in a partially restricted environment. In a 250 ml round bottom flask equipped with a heating mantle, magnetic stirrer, thermometer, and a water cooled condenser, 100 ml of solvent was added and allowed to come to equilibrium 20°C below its boiling point. This predetermined temperature was maintained in a $\pm 1^\circ\text{C}$ range by the use of a thermo-watch. In a separate experiment, the approximate solubility of the compound was found by addition of MC-680 to a beaker containing 100 ml of solvent heated to 20°C below its boiling point. This predetermined amount of the MC-680 was then added to the round bottom flask. Additional amounts of MC-680 were then added in increments ranging from 10 gms initially to 0.1 gm for the final addition. The additions were directly proportional to the rate of dissolution versus time (maximum allowed stirring time - 10 minutes).

The results of the experiment appear in Tables I, II and III.

V. References

- 1) MCC Research Notebook No. 838, pg 102-103, 110-118, 127.
- 2) Organic Solvents, 3rd Edition, J. A. Riddick & W. B. Bunger, Wiley-Interscience Publishing Co., Copyright 1970.
- 3) MCC Technical Report #33.
- 4) MCC IOM to L. Hahn from J. Dill, October 30, 1974.

Joseph A. Dill
J. DILL

JD:pr

Attachments

TABLE I
 Solubility of MC-680
 (Listed Alphabetically)

<u>Solvent</u>	<u>Temperatures</u>	
	<u>Ambient</u> (gms at °C) <i>g./100 ml.</i>	<u>Elevated</u> (gms at °C)
Acetone	<0.06/22	<0.1/48
Benzene	<0.37/26.5	1.8/60
Carbon tetrachloride	<0.22/27.2	1.2/56.5
Cottonseed oil	<0.05/26	5.9/141
o-Dichlorobenzene	<0.51/26	102.8/159
Diglyme [bis-(2-methoxyethyl)ether]	<0.05/24	18.5/142
Dioxane	0.27/26	7.9/90*
N,N'-Dimethylformamide	<0.05/26	12.7/133
Dimethylsulfoxide	<0.05/26	43.7/168
Ethyl acetate	<0.06/25.5	<0.1/60*
n-Hexane	<0.05/24	Insoluble/50*
Methanol	<0.06/26	<0.1/50*
Methylene chloride	<0.33/26.5	<0.1/32*
Octanol	<0.05/26	52.0/175
Perchloroethylene	<0.4/28	11.5/100
n-Propanol	<0.05/26	9.12/77
Propylene glycol	<0.05/26.4	2.4/169
THF	<1.00/27	2.2/46
Xylene (mixed)	<0.35/26	18.7/141

* These values taken from Technical Report #33.

TABLE II

Solubility of MC-680
(Listed by Dipole Moment)

Solvents	μ in Debye Units (10^{-18} esu)	Solubility at Different Temp.	
		Ambient (gms at °C)	Elevated (gms at °C)
Cottonseed oil	--	<0.05/26	5.9/141
Benzene	0	<0.37/26.5	1.8/60*
Carbon tetrachloride	0	<0.22/27.2	1.2/56.5
Perchloroethylene	0	<0.4/28	11.5/100
n-Hexane	0.085	<0.05/24	Insoluble/50*
Xylene (mixed)**	0.28	<0.35/26	18.7/141
1,4-Dioxane	0.45	0.27/26	7.9/90*
Methylene chloride	1.14	<0.33/26.5	0.1/32*
THF	1.75	<1.00/27	2.2/46
Octanol	1.76	<0.05/26	52.0/175
Ethyl acetate	1.88	<0.06/25.5	<0.1/60*
Diglyme	1.97	<0.05/24	18.5/142
Propylene glycol	2.25	<0.05/26.4	2.4/169
o-Dichlorobenzene	2.27	<0.51/26	102.8/159
Acetone	2.69	<0.06/22	<0.1/48*
Methanol	2.87	<0.06/26	<0.1/50*
n-Propanol	3.09	<0.05/26	0.12/77
DMF	3.86	<0.05/26	12.7/133
DMSO	3.90	<0.35/26	18.7/141

* These values were taken from Technical Report #33.

** The dipole moment for xylene (mixed) is a calculated value. The mixture of xylenes, according to Organic Solvents, pg 613-614, are as follows: 40% meta, 20% ortho, 20% para, and contains ethyl benzene (assumed to be 20%).

Stated: μ meta = 0.30 μ ortho = 0.45 μ para = 0 μ ethyl benzene = 0.37

Calculate μ mixed xylenes = 0.284

TABLE III

Solubility of MC-680
(Listed by Class of Solvent*)

Solvent Classes and Solvents	Temperature	
	Ambient (gms at °C)	Elevated (gms at °C)
Aliphatic n-Hexane	≤0.05/24	Insoluble/50*
Aromatic Benzene	≤0.37/26.5	1.8/60*
Xylenes (mixed)	≤0.35/26.0	18.7/141
Carbonyls Acetone	≤0.06/22	<0.1/60*
Cyclic Ethers 1,4-Dioxane	≤0.27/26	7.9/90*
Tetrahydrofuran	≤1.00/27	2.2/46
Esters Ethyl acetate	≤0.06/25.5	<0.1/60*
Ether Diglyme	≤0.05/24	18.5/142
Halo-aliphatic Carbon tetrachloride	≤0.22/27.2	1.2/56.5
Methylene chloride	≤0.33/26.5	0.1/32*
Perchloroethylene	≤0.40/28	11.5/100
Halo Aromatic o-Dichlorobenzene	≤0.51/26	102.8/159
Hydroxy Methanol	<0.06/26	<0.1/50*
Octanol	≤0.05/26	52/175
n-Propanol	≤0.05/26	0.12/77
Propylene glycol	≤0.05/26.4	2.4/169
Nitrogen Containing N,N'-dimethylformamide	≤0.05/26	12.7/133
Oils Cottonseed Oil	<0.05/26	5.9/141
Sulfur Containing Dimethylsulfoxide	≤0.05/26	43.7/168

*These values were taken from Technical Report #33.

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VELSICOL CHEMICAL CORPORATION

LABORATORY REPORT

SUBJECT: Photolysis of Firemaster-680. UNITERM NO: _____
 PROJECT NO: 484028
 RETURN TO VAULT FILE REPORT NO: 4
 AUTHOR: Ching C. Yu SECTION: Environmental Sciences

LOAN OUT

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 REFERENCES: Notebook 3615, pages 16 - 18
Notebook 3583, pages 27.

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OBJECT: To determine the stability of Firemaster (FM)-680 [1,2-bis(2,4,6-tribromophenoxy) ethane] on solid surface upon exposure to ultraviolet (UV) radiation.
 CAS# 37853-59-1

SUMMARY: ¹⁴C FM-680 applied to silica gel surfaces was irradiated with UV light. It was found that FM-680 was degraded rapidly and followed a biphasic curve. The initial degradation half-life was about 0.4 day. After 1 day of exposure, the degradation then entered the second phase with a half-life of about 1.7 days.

At least 4 degradation products were detected by TLC analysis. One of the products was identified as 2-(2',4',6'-tribromophenoxy) ethanol which comprised 0.5 to 6% of the applied radiocarbon. This and other degradation products appeared to be transitory.

SIGNATURE: Ching C. Yu

INTRODUCTION

Chemicals in the environment are subjected to sunlight irradiation. Photodegradability of a chemical is an important factor in determining the persistency of that chemical in the environment.

This paper reports the photolysis of Firemaster (FM)-680 on a solid surface when irradiated with UV light.

MATERIALS AND METHODS

Chemicals

^{14}C FM-680 [1,2-bis(2,4,6-tribromophenoxy) ethane-phenyl $\text{UL-}^{14}\text{C}$], with specific activity of 23 mCi/mole and radiochemical purity greater than 98%, was prepared by Pathfinder Labs, Inc., St. Louis, Missouri.

Method

A silica gel G plate without fluorescence indicator (20 x 20 cm, 0.25 mm thickness, Macherey-Nagel and Co., distributed by Brinkmann Instruments, Inc.) was evenly marked with 9 pencil marks on one side of the plate. Five μl of ^{14}C FM-680 in a toluene solution (0.74 $\mu\text{g}/\mu\text{l}$) was applied to each mark. The plate was then exposed to UV light in a Chromato-Vue TLC Viewing Box (Ultra-Violet Products, Inc., San Gabriel, California). Each spot was exposed for a predetermined length of time and then each was covered with aluminum foil. After all of the spots had been exposed the plate was developed in a solvent which consisted of methylene chloride/n-propanol (95:5) and then subjected to radioautography using Kodak No-screen X-ray film.

The resolved radioactive spots were scraped from the plate and placed in scintillation vials. Two ml of methanol were added to each vial, shaken for 1 hour and then 10 ml of Handifluor counting solution (Mallinckrodt, Inc.) were added for radioassay.

Instrumentation

A Mark III Liquid Scintillation System, Model 6880 (Searle Analytic, Inc.) was used for radiocarbon counting. Sample quenching was determined by the external standard pulse method. Counting efficiency, usually between 70 - 85%, was determined by an on-line computation program.

Mass spectra were obtained by direct inlet probe in either the electronic impact (EI, 70 eV) or chemical ionization (CI, methane) mode (Hewlett Packard Model 5982A Quadrupole MS). Data output from the MS was monitored with a Hewlett Packard Model 5934A Dual Disc Data System.

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RESULTS AND DISCUSSION

On a silica gel surface, FM-680 was degraded rapidly when irradiated with UV light (Table 1). The disappearance of parent FM-680 from the silica gel surface followed a biphasic curve (Figure 1). Initially, FM-680 decreased rapidly with a half-life of about 0.4 day. However, after 1 day of UV exposure, the rate of FM-680 degradation decreased. The half-life of the second phase was determined to be 1.7 days. Total radiocarbon recovery steadily decreased. After 10 days of exposure, 37% of applied ^{14}C was recovered. Some of the FM-680 and degradation products were probably volatilized from the plate surface.

According to TLC analysis, at least 4 degradation products were detected (Table 1). Unknown 3 (at the origin of TLC plate) is probably the polymerized product(s) which gradually increased with time and reached a maximum (48%) after 2 days of exposure, and then gradually decreased as the exposure time increased.

Other degradation products were produced in small amounts (0.7% to 6%) and appeared to be transitory. One of the products (R_f 0.54) was identified as 2-(2',4',6'-tribromophenoxy) ethanol. The MS spectra of this compound are shown in Figures 2 and 3.

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Table 1 Photolysis of FM-680 on silica gel plate when irradiated with UV light.

Product	μg ^{2/}	UV exposure time (day) and % of applied radiocarbon ¹						
		0	0.08	0.63	1	2	6	10
FM-680	0.87	73.05	71.44	26.40	13.45	8.29	1.39	0.39
2-(2',4',6'-Tribromophenoxy)-ethanol	0.54	3.67	5.15	6.00	5.31	4.00	1.29	0.47
Unknown 1	0.46	2.24	2.63	2.14	1.30	0.98	0.43	0.72
Unknown 2	0.23	2.89	3.31	2.08	3.92	3.06	1.74	1.03
Unknown 3	0.00	7.12	12.27	41.94	47.18	47.67	37.66	33.91
		<u>55.97</u>	<u>94.20</u>	<u>81.56</u>	<u>71.16</u>	<u>64.01</u>	<u>42.57</u>	<u>36.52</u>

1/ 3.7 μg of ¹⁴C-FM-680 was applied for each treatment.

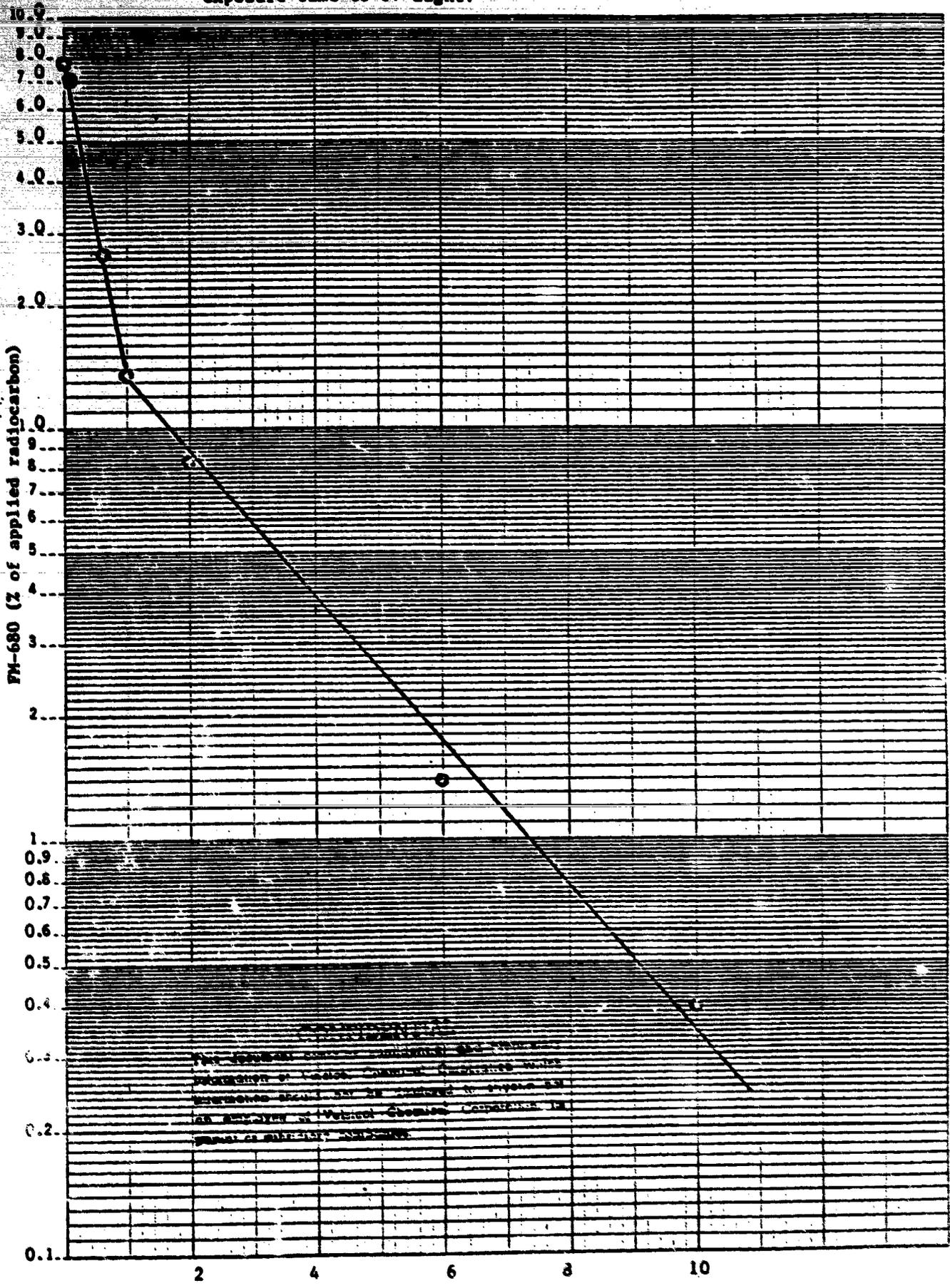
2/ On silica gel G, developed with methylene chloride: n-propanol (95:5).

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Figure 1 Plot of ^{14}C -FM-680 remaining on silica gel plate Versus exposure time to UV light.

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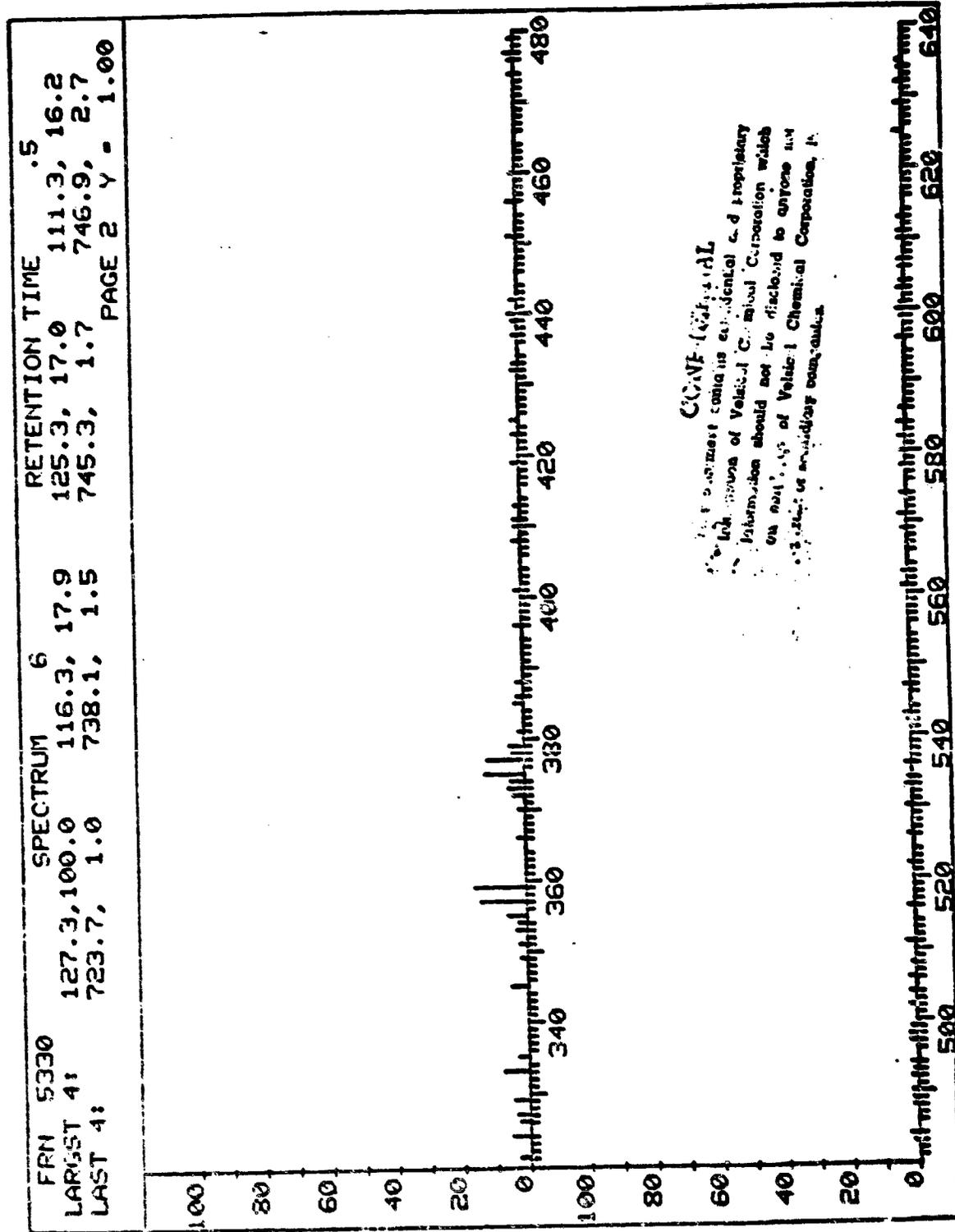
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Figure 2 Mass spectrum of 2-(2',4',6'-tribromophenoxy) ethanol obtained from photolysis of FM-680 on silica gel surface. (CI mode).



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SUBJECT: Rate of Hydrolysis Studies

ORIGINAL RECORD

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→ 1,2-Bis (tribromophenoxy)ethane
CAS# 37253-59-1

The rate of hydrolysis for VC 935A, VC 680, VC 984, and LV-T23P in water at 100°C has been determined. The tests were conducted using 25 parts of sample and 75 parts of water heated at reflux temperature, or approximately 100°C. Periodically, the pH of the reaction mixtures were measured using a pH meter. The tests were terminated after 7 days or when the pH of the reaction mixture reached the value of 1.0 units.

The pH of the water used in all the tests initially measured 6.3 units. All pH values listed at 0.0 hours were measured after the reaction mixtures had stirred for 0.5 hours at ambient temperature.

Table I lists the pH values observed for VC 935A and VC 680 taken at various intervals over a 190 hour test period. The results showed the pH units ranged from 4.0 to 2.8 (net change 1.2 units) for VC 935A and the pH units ranged from 7.6 to 8.9 (net change 1.3 units) for VC 680. Also in the case of VC 680, the maximum pH value of 8.9 units was observed at 6 hours. After that time period, the pH values gradually dropped to 8.4 units.

Table II lists the pH values observed for VC 984 and LV-T23P taken at various intervals over a 165 hour test period. The results showed the pH units ranged from 3.0 to 1.8 (net change 1.2 units) for VC 984 and the pH units ranged from 8.5 to 1.7 (net change 6.8 units) for LV-T23P

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TABLE I

<u>Time</u>	<u>VC-935A (DL-8)</u> <u>pH (units)</u>	<u>VC-680 (7018E - 2)</u> <u>pH (units)</u>
0.0	4.0	7.6
1.7	3.4	8.8
6.0	3.2	8.9
22.0	3.0	8.4
25.0	3.1	8.7
30.0	3.0	8.4
118.0	3.0	8.4
142.0	2.9	8.5
166.0	2.8	8.4
190.0	<u>2.8</u>	<u>8.4</u>
net change	1.2	1.3

TABLE II

<u>Time</u>	<u>VC 984 (974-140)</u> <u>pH (Units)</u>	<u>LV-T23P (2780)</u> <u>pH (Units)</u>
0.0	3.0	8.5
1.0	3.0	6.7
2.5	3.0	5.3
5.0	2.8	3.4
21.0	2.6	2.6
26.0	2.5	2.6
45.0	2.4	2.3
69.0	2.1	2.0
93.0	2.0	1.9
165.0	<u>1.8</u>	<u>1.7</u>
net change	1.2	6.8

Figure 3 Mass spectrum of 2-(2',4',6'-tribromophenoxy) ethanol obtained from photolysis of FM-680 on silica gel surface.. (EI mode).

