BIODEGRADATION (Modified MITI Test)

## TEST SUBSTANCE

**Identity**: [2-(N-Ethylperfluorooctanesulfonamido) ethyl acrylate; may also be referred to as B1228, D-1, EtFOSEA, or FX-13. (2-Propenoic acid, 2-[ethyl[heptadecafluorooctyl)sulfonyl]amino]ethyl ester, CAS # 423-82-5)

**Remarks:** Material is an amber solid. Report lists sample as > 99%, as provided by sponsor. The purity/identity of the test substance cannot be substantiated. As presented in the report, the structural formula indicates that "purity" cannot be assigned as "99% or more". Lot number 4.

## METHOD

**Method**: OECD 301C
**Type**: Aerobic
**GLP**: Yes
**Year completed**: 1995
**Inoculum source:** Chemical Biotesting Center, Chemical Inspection and Testing Institute, Japan

**Remarks:** It appears that the inoculum was obtained from only one source, rather than the ten sources required in the method.

## RESULTS

**Test substance:** After 28-days, the percent biodegradability of the test substance was determined by three methods: Biochemical Oxygen Demand (BOD), Dissolved Organic Carbon (DOC), and Residual Test Substance Concentration. The results are as follows:

**Percent Biodegradability (of 3 replicates):**
**BOD:** 4, -1, and 6%
**DOC:** Not calculated; test substance not dissolved in solution
**Test Substance Concentration:** 5 and 1% (only two determined)

**Reference substance:** The aniline achieved 68% biodegradability in 28-days.

**Measurement of test substance:** The test substance concentration was only measured at the end of the study. Although this laboratory followed the

procedures as outlined in OECD 301C, the starting concentration should also be determined rather than relying on nominal concentrations. In addition, subsamples were removed from the bioreactors for pH and DOC determinations at the end of the study. The sample used for pH determinations and the remaining sample after centrifugation for DOC were returned to the bioreactor prior to sampling for the measurement of the test substance concentration. These samples should have been removed after the bioreactors were sampled for test substance concentration determination.

Calculations of theoretical oxygen demand should have made which account for possible oxygen consumption due to nitrification.

## CONCLUSIONS

This testing indicated that the test substance is not capable of readily biodegrading in aerobic environments.

## DATA QUALITY

**Reliability:** Klimisch ranking 3. There is no reference to the solubility information provided in the study. The quoted purity/identity of the test substance is not substantiated. Measurement of test substance concentration was only performed at the end of the study. The bioreactors were potentially contaminated by the practice of returning subsamples after pH and DOC determinations. The calculation of theoretical oxygen demand should have been performed using the correction for nitrification.

## REFERENCES

Study conducted at the request of Sumitomo 3M LTD by Mitsubishi Chemical Safety Institute Ltd., Yokohama, Japan

## OTHER

**Submitter:** 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

**Last changed**: 5/22/00

# M.S.I. REPORT No.1B363(2)G

Ready Biodegradability Test of D-1

Submitted to :

SUMITOMO 3M LIMITED

Prepared by :

Mitsubishi Chemical Safety Institute Ltd.

October 31, 1995

000504

# Ready Biodegradability Test of D-1
## (English version)

Study No. : 1B363(2)G

Study Title : Ready Biodegradability Test of D-1

Sponsor : SUMITOMO 3M LIMITED

This report was conducted in the Yokohama Laboratory of the Mitsubishi Chemical Safety Institute Ltd., 1000 Kamoshida-cho, Aoba-ku, Yokohama 227, Japan.

This report is an English version of the orginal, which was written in Japanese. The underesigned hereby declare that this version faithfully reflects the original report to the best of our knowledge.

**Mitsubishi Chemical Safety Institute Ltd.**

K. Yoshida

Date : October 31, 1995

Kikuo Yoshida, Ph.D.

Environmental Science Division of Yokohama Laboratory

**Approved by**

Jun Toriya

Date : October 31, 1995

Jun Toriya, B.Sc.

Head of Yokohama Laboratory

# COMPLIANCE WITH THE GLP STANDARDS

Study No. : 1B363(2)G

Study Title : Ready Biodegradability Test of D-1

Sponsor : SUMITOMO 3M LIMITED

To the best of our knowledge and belief, the study described in this report was conducted in compliance with the Good Laboratory Practice (GLP) standards applied to industrial chemicals under the Chemical Substances Control Law.

## Facility management

Sakae Koike, B.Sc. Sealed date :April 28, 1992

Facility management

Head of Yokohama Laboratory

## Study director

Kikuo Yoshida, Ph.D. Sealed date :April 28, 1992

Study Director

Environmental Science Division

# QUALITY ASSURANCE STATEMENT

Study No. : 1B363(2)G

Study Title : Ready Biodegradability Test of D-1

Sponsor : SUMITOMO 3M LIMITED

We hereby certify that the above study has been performed in accordance with the Good Laboratory Practice (GLP) standards applied to industrial chemicals under the Chemical Substances Control Law.

Dates of inspections and reportings to the Study Director and the Facility Management are as follows :

| | Date of Inspection | Date of Reporting |
|---|---|---|
| In-progress study : | March 18, 1992 | March 30, 1992 |
| | April 15, 1992 | April 15, 1992 |
| | April 16, 1992 | April 16, 1992 |
| Final report : | April 28, 1992 | April 28, 1992 |

## Quality Assurance Staff

Junko Katoh, Ph.D. Sealed date :April 28, 1992

Yumiko Miura, B.Sc. Sealed date :April 28, 1992

We, the undersigned declare that the work was performed according to the procedures herein described, and that this report provides a true and correct record of the results obtained.

## Experiment Staff

Ayumi Okuda, M.Sc. Sealed date :April 28, 1992
Environmental Science Division

Junko Koyasu, B.Sc. Sealed date :April 28, 1992
Environmental Science Division

Yukiko Mizoguchi Sealed date :April 28, 1992
Environmental Science Division

## Staff for cultivation of activated sludge

Yasuo Sato Sealed date :April 28, 1992
Environmental Science Division

CONTENTS

# EXPLANATORY REMARKS

Study No. : 1B363(2)G

Study Title : Ready Biodegradability Test of D-1

The ready biodegradability test of D-1 was originally started on January 29, 1992, and the BOD measurement of the test substance was initiated on February 13, 1992(Study No.1B363G). However, the degradability value of aniline based on the BOD measurements did not exceed 40%.

Therefore, the test was terminated on February 20, 1992 and the new test (Study No. 1B363(2)G) was started with the same test substance and the same test method after approval of the sponsor.

Some of data used in this report (IR chart, calibration curve and recovery) are those obtained in the former study (Study No. 1B363G).

# SUMMARY

## STUDY TITLE

Ready Biodegradability Test of D-1

## METHOD

The method described in the Order, which prescribes the procedure of testing new chemical substances as required by the Chemical Substances Control Law(Japanese Law 117, 1973). ("Ready biodegradability, Modified MITI test" in OECD Guidelines for Testing of Chemicals).

Test bottle : Bottle 1 : aniline + sludge + basal medium
Bottle 2 : sludge + basal medium
Bottles 3~5 : test substance + sludge + basal medium
Bottle 6 : test substance + water

Measurements : Biochemical oxygen demand (BOD) (for 28 days)
Dissolved organic carbon (DOC) (at day 28)
Residual test substance concentration (at day 28)

## RESULTS

Measured values (day 28)

| | Bottle No. | | | | Theoretical |
|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | value |
| BOD, mg | 1.0* | -0.3* | 1.6* | 0.0 | 27.7 |
| DOC, mg/l | 0.7* | 0.3* | 1.6* | 2.0 | 28.8 |
| Test substance, mg/l | 101.3 | 105.2 | 91.5 | 106.7 | 100.0 |

* value corrected with BOD or DOC value of Bottle 2.

Degradabilities (%)

| Bottle No. | 3 | 4 | 5 |
|---|---|---|---|
| BOD | 4 | -1 | 6 |
| DOC | NA* | NA* | NA* |
| Test substance | 5 | 1 | NA** |

* not calculated because the test substance was not dissolved in the test solution.
** not calculated because the test substance might be lost accidentally during condensation of the solution under vacuum.

## CONCLUSION

From the degradability results based on the BOD and the residual test substance concentration , it can be concluded that the test substance is not readily biodegradable and is not transformed under the conditions prescribed by the Modified MITI test.

## 1 STUDY TITLE

Ready Biodegradability Test of D-1

## 2 PURPOSE

This study was conducted to evaluate the ready biodegradability of the test substance for notification under the Chemical Substance Control Law of Japan.

## 3 TEST GUIDELINES

The test was conducted according to the method described in the Order, which prescribes the procedure of testing new chemical substances as required by the Chemical Substances Control Law (Japanese Law 117, 1973). This method is also known as the "Ready biodegradability, Modified MITI test" in the OECD Guidelines for the Testing of Chemicals No. 301C.

## 4 GLP COMPLIANCE

The test was conducted according to the Good Laboratory Practice (GLP) standards applied to industrial chemicals under the Chemical Substances Control Law.

## 5 STUDY PERIOD

March 17, 1992 to April 28, 1992

(BOD measurement : March 18, 1992 to April 15, 1992)

## 6 SPONSOR

SUMITOMO 3M LIMITED

8-8 Minamihashimoto 3-chome, Sagamihara 229, Japan

## 7 TESTING FACILITY

Yokohama Laboratory

Mitsubishi Chemical Safety Institute Ltd. (M.S.I.)

1000 Kamoshida-cho, Aoba-ku, Yokohama 227, Japan

Head Office:

Mitsubishi Chemical Safety Institute Ltd. (M.S.I.)

1-30 Shiba 2-chome, Minato-ku, Tokyo 105, Japan

## 8 STORAGE AND RETENTION OF TEST SUBSTANCE AND RECORDS

The following records, the raw data, and a small sample of the test substance will be stored in the archives of M.S.I. for 10 years after the submission of the final report. After this period, the sponsor will be contacted for the approval of the disposal of data. Data can be stored in the archives for a further specified period at the sponsor's request with an additional fee.

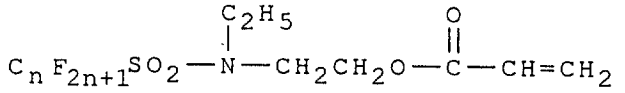
1) Protocol.
2) Final report.
3) Raw data.
4) Quality assurance reports.
5) Test substance (approx. 2 g).
6) Other documents required under the GLP standards.

9 **TEST SUBSTANCE**

1) Name* : D-1
(M.S.I. identification No. 1B363(2)G)

2) Chemical name* : 2-[N-Ethyl-N-perfuloroalkyl(C=1~8)sulfonyl-amino]ethylacrylate

3) Structural formula* :



(n=1~8, main component; n=8)

4) Elemental composition : C;28.8%, H; 1.9%, N; 2.3%, O; 9.8%, F;52.0% S; 5.2% (Measured by M.S.I.)

5) Physico-chemical properties

Solubility* : insoluble in water
insoluble in DMSO
>50% in acetone

Melting point* : 27~42℃

Boiling point* : ca. 150℃/1mmHg

8) Batch* : Lot No. 4

9) Purity* : >99% (n=1~7;ca.21 %(total), n=8;ca.78%)

10) Appearance : amber solid

11) Date of receipt : September 25, 1991

12) Supplied quantity* : 100 g

* provided by the sponsor.

## 10 CONFIRMATION OF IDENTITY AND STABILITY

### 10.1 IDENTITY

For the identification of the test substance, the infrared absorption spectrum of the test substance was measured. The measured spectrum was consistent with that supplied by the sponsor.

1)Apparatus : Perkin-Elmer model 1640 infrared spectrophotometer.

[Figure 1 (P-23)]

### 10.2 STABILITY

After the exposure period, the infrared absorption spectrum of the test substance that had been stored in a refrigerator during the course of the study was measured. The spectrum was consistent with that shown in Fig. 1, indicating that the test substance was stable under the storage condition.

1)Apparatus : Perkin-Elmer model 1640 infrared spectrophotometer.

[Figure 2 (P-24)]

## 11 TEST METHODS

The test was performed according to the method described in the Order, which prescribes the procedure of testing new chemical substances as required by the Chemical Substances Control Law (Japanese Law 117, 1973). This method is also known as the "Ready biodegradability, Modified MITI test" in OECD Guidelines for Testing of Chemicals No.301C (1981).

The test substance was exposed to the activated sludge in a closed-system oxygen consumption measuring apparatus. The biochemical oxygen demand (BOD) was measured over a 28 day period. After this period, the concentrations of the dissolved organic carbon (DOC) and the residual test substance in the test bottles were measured.

The biodegradability of the test substance was evaluated from these results.

### 11.1 ACTIVATED SLUDGE

1) Mixed liquor suspended solid (MLSS) : 3300 mg/l

2) Source : Chemical Biotesting Center, Chemical Inspection and Testing Institute, Japan

3) Date of receipt : January 23, 1992

### 11.2 EXPOSURE CONDITIONS

1) Temperature : 25 ± 1 ℃

2) Exposure period : 28 days

3) Test volume : 300 ml

4) Concentration :

| | | |
|---|---|---|
| test substance (bottles 3~6) | : | 100 mg/l |
| aniline*1 (bottle 1) | : | 100 mg/l |
| activated sludge (bottles 1~5) | : | 30 mg/l |

*1 Reference substance : Syowa Chemicals, Lot No.SC-2726

5) Test bottle contents :

Bottle 1 : Aniline + activated sludge + basal medium
29µl (30 mg) of aniline was added to the basal medium*2, then activated sludge was added.

Bottle 2 : Activated sludge + basal medium
Activated sludge was added to the basal medium*2.

Bottles 3~5 : Test substance + activated sludge + basal medium
30 mg of the test substance was added to the basal medium*2, then activated sludge was added.

Bottle 6 : Test substance + deionized water
30 mg of the test substance was added to 300 ml of deionized water (purified by Milli-Q).

*2 The total volume of the basal medium and the sludge was held fixed at 300 ml.

11.3 BOD MEASUREMENT

The BOD was measured for 28 days.

1)Apparatus : closed system oxygen consumption measuring apparatus Ohkura Electric Co., Model OM-2001 (M.S.I. ID No.B).

11.4 pH MEASUREMENT

After the exposure period, 20 ml of the test solution in each test bottle was transferred into a 20 ml glass beaker for pH measurement.

After pH measurement, the test solution was returned to each bottle for measurement of residual test substance concentration.

1)Apparatus : pH meter, Denki Kagaku Keiki, Model COM-10

11.5 DOC MEASUREMENT

The concentration of the DOC was measured as follows.

1)Apparatus : TOC analyzer, Shimadzu Co., model TOC-5000

2)Conditions :

| | |
|---|---|
| Furnace temperature | : 680°C(TC) |
| Air flow rate | : 150 ml/min. |
| Sensitivity | : × 5 |
| Injection volume | : 50 µl |

3)Calibration curve

The following standard solutions were injected into the TOC analyzer. The calibration curve was prepared by the data processor of the analyzer.

standard solutions

| | |
|---|---|
| TC(total carbon) | 20 and 50 mg C/l aqueous solutions of potassium biphthalate. |
| IC(inorganic carbon) | 0 mg C/l deionized water (purified by Milli-Q) and 10 mg C/l aqueous solution of sodium hydrogen carbonate. |

4)Measurement of DOC in the bottles

Ten ml of the test solution in each bottle was transferred into a 10 ml centrifuge tube and centrifuged at 3000 rpm for 10 minutes. Five ml of supernatant was used for the measurement of DOC.

The remaining supernatant and the precipitate were returned to each bottle for the measurement of the residual test substance concentration.

11.6 MEASUREMENT OF RESIDUAL TEST SUBSTANCE CONCENTRATION

The concentration of the residual test substance was measured by the gas chromatography (GC) as follows:

1) Apparatus

| | |
|---|---|
| GC | :Hewlett-Packard. Model 5890A (No.2) with FID detector |
| Integrator | :Hewlett-Packard. Model 3392A |

2) Conditions

| | |
|---|---|
| Column | :J & W. DB-17(50%Phenyl 50%methyl polysiloxane), 30 m × 0.25 mm i.d., 0.25μm (film thickness) |
| Temperature | :[Column oven]100℃(1min.)→10℃/min.→200℃(1min.) [Injector]250℃, [Detector]280℃ |
| Carrier gas | :He 30 ml/min. |
| Detector flow | :$H_2$ 40ml/min., air 400 ml/min. |
| Attenuation | :[GC] 1 V |
| Injection volume | :1 μl |

3) Calibration curve

The standard solutions of the test substance with 0, 250, 500, and 1000 mg/l in ethyl acetate were analyzed. The total peak area of test substance was calculated by adding the area of the peaks with retention time of about 5.6, 6.2 and 6.5 min. The total area was plotted against the concentration. The correlation coefficient was calculated to be 1.00 by the method described in Japanese Industrial Standards Z9041-1968.

[Figure 3 (P-25) and Figure 4 (P-26)]

4) Recovery test

Duplicate test bottles (identical to the test bottles 3~5 described in § 11.2) were kept in a closed-system oxygen consumption measuring apparatus at 25±1℃ for 30 minutes.

Each test solution was treated by the procedures described in § 11.6.6. The concentration of the test substance was measured by GC. The average recovery of the test substance was 97%.

[Table 3 (P-21) and Figure 5 (P-27)]

The concentration of the residual test substance measured for bottles 3 ~6 were corrected with the average recovery value.

5) Detection limit

The detection limit of the test substance was calculated to be 3 mg/l based on the minimum detectable peak area of 100000 μV・sec for the largest peak (R.T. 6.2 min.) in the GC chromatogram.

[Table 3 (P-21) and Figure 5 (P-27)]

6) Measurement of test substance in test bottles

The contents in each bottle was separately transferred into a 500 ml glass separatory funnel, which was then extracted twice with 100 ml of

ethyl acetate. The extract was filtered through a grass funnel containing 50 g sodium sulfate, anhydrous to remove water. Then filtrate was condensed to ca. 20 ml under vacuum (<50℃). The solution was brought up to 50 ml with ethyl acetate.

The ethyl acetate solution was then analyzed by GC. The concentration of the residual test substance was determined as described in §11.6.2.

## 11.7 EQUATIONS FOR CALCULATION OF DEGRADABILITY

Equations for calculation of degradability based on BOD, DOC, and residual test substance concentration are as follows:

1) Degradability based on BOD

Degradability (%) = (BODs- BODb)/ThOD × 100

where BODs : Oxygen consumption (mg) in bottles 1, 3, 4, and 5.
BODb : Oxygen consumption (mg) in bottle 2.
ThOD : Theoretical oxygen demand (mg) of aniline and the test substance.

**ThOD**

aniline : $C_6H_7N + 35/4O_2 \rightarrow 6CO_2 + 7/2H_2O + NO_2$
ThOD : 90.2 mg $O_2$/30 mg-aniline

test substance : $C+O_2 \rightarrow CO_2$, $H+1/4O_2 \rightarrow 1/2H_2O$, $N+O_2 \rightarrow NO_2$, $S+O_2 \rightarrow SO_2$, $F \rightarrow F$
ThOD : 27.7 mg $O_2$/30 mg-test substance

2) Degradability based on DOC

Degradability (%) = (1 - (DOCs-DOCb)/DOCc)×100

where DOCs : DOC (mg/l) in bottles 3, 4, and 5.
DOCb : DOC (mg/l) in bottle 2.
DOCc : DOC (mg/l) in bottle 6.

3) Degradability based on residual test substance concentration

Degradability (%) = (1 - Cs/Cc) × 100

where Cs : concentration (mg/l) in bottles 3, 4, and 5.
Cc : concentration (mg/l) in bottle 6.

## 12 RESULTS

There was no specific circumstances which might have affected the reliability of the test results.

### 12.1 OBSERVATION OF TEST BOTTLES AFTER EXPOSURE PERIOD

The solutions in the bottles were colorless except the bottle 1, in which the solution was cloudy.

Growth of the sludge was observed in bottle 1 in contrast with the bottle 2. No growth was observed in the bottles 3, 4 and 5.

### 12.2 pH MEASUREMENT

After 28 days of exposure, the pH was determined to be 7.4, 7.4, 7.5 and 7.4 for bottles 3, 4, 5 and 6, respectively.

[Table 1 (P-19)]

### 12.3 ACTIVITY OF SLUDGE

The degradability value of aniline based on the BOD measurements was 59% after 7 days. The activity of the sludge was thus shown to be satisfactory.

[Table 1 (P-19) and Figure 6 (P-28～31)]

### 12.4 DEGRADABILITY BASED ON BOD

BOD*[1] in bottles 3, 4, and 5 (as corrected with the value in bottle 2) were 1.0, -0.3 and 1.6 mg, respectively. BOD in bottle 6 was 0.0 mg.

(*[1] Maximum theoretical value = 27.7 mg)

The degree of degradability based on the BOD measurements were 4, -1 and 6% for bottles 3, 4, and 5, respectively.

[Table 1 (P-19) and Figure 6 (P-28～31)]

### 12.5 DEGRADABILITY BASED ON DOC

DOC*[2] in bottles 3, 4 and 5 (as corrected with the value in bottle 2) were 0.7, 0.3, and 1.6 mg/l, respectively. DOC in bottle 6 was 2.0 mg/l.

(*[2] Maximum theoretical value = 59.8 mg/l)

Degradability based on the DOC measurements was not calculated because the test substance was not dissolved in the test solution.

[Table 1 (P-19) and Table 2 (P-20)]

12.6 DEGRADABILITY BASED ON THE RESIDUAL TEST SUBSTANCE CONCENTRATION

The test substance*3 was detected at concentrations of 101.3, 105.2, 91.5 and 106.7 mg/l in bottles 3, 4, 5 and 6, respectively.

(*3 Initial concentration = 100 mg/l)

The degree of degradability based on the residual test substance concentration were 5 and 1 % for the bottle 3 and 4, respectively.

[Table 1 (P-19), Table 4 (P-22), and Figure 7 (P-32~33)]

During condensation of the filtrate of the bottle 5 under vacuum, a part of the filtrate spouted from an evaporating flask. The spouted filtrate was recovered and then condensed with the remaining filtrate. However, the residual test substance concentration in the bottle 5 was obviously lower than those in the bottle 3 and 4 and it would impair reliability of biodegradability of the test substance in the bottle 5. Therefore, the degree of degradability based on the residual test substance concentration was not calculated for the bottle 5.

## 13 CONCLUSIONS

From the degradability results based on the BOD and the residual test substance concentration, it can be concluded that the test substance is not readily biodegradable and is not transformed under the conditions prescribed by the Modified MITI test.

Table 1 Summary of the test results

Degradabilities were calculated by the equations shown in Sec. 11.7.

a) Degradability based on BOD

| Bottle No. | Test substance | ThOD mg | day 7 | | day 14 | | day 21 | | day 28 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | BOD mg | % Degra-dability | BOD mg | % Degra-dability | BOD mg | % Degra-dability | BOD mg | % Degra-dability |
| 1 | aniline | 90.2 | 56.2 | 59 | 63.7 | 65 | 66.3 | 67 | 67.4 | 68 |
| 2 | ------ | --- | 3.1 | --- | 5.0 | --- | 5.5 | --- | 5.8 | --- |
| 3 | D-1 | 27.7 | 3.0 | 0 | 5.0 | 0 | 6.1 | 2 | 6.8 | 4 |
| 4 | D-1 | 27.7 | 2.4 | -3 | 4.1 | -3 | 4.8 | -3 | 5.5 | -1 |
| 5 | D-1 | 27.7 | 3.5 | 1 | 5.7 | 3 | 6.7 | 4 | 7.4 | 6 |
| 6 | D-1 | 27.7 | 0.0 | --- | 0.0 | --- | 0.0 | --- | 0.0 | --- |

Where % degradability was calculated to be negative, this value is shown in brackets.

b) pH measurement

| Bottle No. | pH | |
|---|---|---|
| | day 0 | day 28 |
| 1 | --- | 8.3 |
| 2 | 6.9 | 7.4 |
| 3 | 6.9 | 7.4 |
| 4 | 6.9 | 7.4 |
| 5 | 6.9 | 7.5 |
| 6 | 6.8 | 7.4 |

c) Degradability based on DOC

| Bottle No. | Organic carbon added, mg/l | TC mg/l | IC mg/l | DOC mg/l |
|---|---|---|---|---|
| 2 | ------ | 1.7 | 0.0 | 1.7 |
| 3 | 28.8 | 2.5 | 0.1 | 2.4 |
| 4 | 28.8 | 2.1 | 0.1 | 2.0 |
| 5 | 28.8 | 3.3 | 0.0 | 3.3 |
| 6 | 28.8 | 2.0 | 0.0 | 2.0 |

d) Degradability based on residual test substance

| Bottle No. | Concentration mg/l | Degradability % |
|---|---|---|
| 2 | <3 | --- |
| 3 | 101.3 | 5 |
| 4 | 105.2 | 1 |
| 5 | 91.5 | NA* |
| 6 | 106.7 | --- |

* not calculated because the test substance might be lost accidentally during condensation of the solution under vacuum.

# Table 2 Results of DOC measurements

## Standard solution

TC 50.0 mg/L
vial :s1

| # | arer |
|---|---|
| 1 | 34637 |
| 2 | 34795 |
| 3 | 34640 |
| MN | 34690 |

TC 20.0 mg/L
vial :s2

| # | arer |
|---|---|
| 1 | 14107 |
| 2 | 13981 |
| 3 | 14048 |
| MN | 14045 |

IC 10.0 mg/L
vial :s3

| # | arer |
|---|---|
| 1 | 7185 |
| 2 | 7107 |
| 3 | 7187 |
| MN | 7159 |

IC 0.0 mg/L
vial :s4

| # | arer |
|---|---|
| 1 | 0 |
| 2 | 0 |
| 3 | 0 |
| MN | 0 |

## Bottle 2

TC

| # | arer | mg/L | RMK |
|---|---|---|---|
| 1 | 1178 | 1.71 | |
| 2 | 1157 | 1.68 | |
| 3 | 1135 | 1.65 | |
| MN | 1156 | 1.68 | |

IC

| # | arer | mg/L | RMK |
|---|---|---|---|
| 1 | 0 | 0.00 | |
| 2 | 0 | 0.00 | |
| 3 | 0 | 0.00 | |
| MN | 0 | 0.00 | |

## Bottle 3

TC

| # | arer | mg/L | RMK |
|---|---|---|---|
| 1 | 1743 | 2.53 | |
| 2 | 1743 | 2.53 | |
| 3 | 1713 | 2.49 | |
| MN | 1733 | 2.52 | |

IC

| # | arer | mg/L | RMK |
|---|---|---|---|
| 1 | 0 | 0.00 | |
| 2 | 0 | 0.00 | |
| 3 | 166 | 0.23 | |
| MN | 55 | 0.08 | |

## Bottle 4

TC

| # | arer | mg/L | RMK |
|---|---|---|---|
| 1 | 1470 | 2.14 | |
| 2 | 1418 | 2.06 | |
| 3 | 1374 | 2.00 | |
| MN | 1420 | 2.06 | |

IC

| # | arer | mg/L | RMK |
|---|---|---|---|
| 1 | 157 | 0.22 | |
| 2 | 0 | 0.00 | |
| 3 | 0 | 0.00 | |
| MN | 52 | 0.07 | |

## Bottle 5

TC

| # | arer | mg/L | RMK |
|---|---|---|---|
| 1 | 2340 | 3.40 | |
| 2 | 2302 | 3.35 | |
| 3 | 2215 | 3.22 | |
| MN | 2285 | 3.32 | |

IC

| # | arer | mg/L | RMK |
|---|---|---|---|
| 1 | 0 | 0.00 | |
| 2 | 0 | 0.00 | |
| 3 | 0 | 0.00 | |
| MN | 0 | 0.00 | |

## Bottle 6

TC

| # | arer | mg/L | RMK |
|---|---|---|---|
| 1 | 1442 | 2.10 | |
| 2 | 1326 | 1.93 | |
| 3 | 1354 | 1.97 | |
| MN | 1374 | 2.00 | |

IC

| # | arer | mg/L | RMK |
|---|---|---|---|
| 1 | 0 | 0.00 | |
| 2 | 0 | 0.00 | |
| 3 | 0 | 0.00 | |
| MN | 0 | 0.00 | |

| SPL # | TC.mg/L | RMK | IC.mg/L | RMK | TOC.mg/L | |
|---|---|---|---|---|---|---|
| 2 | 1.68 | | 0.00 | | 1.68 | <---- Bottle 2 |
| 3 | 2.52 | | 0.08 | | 2.44 | <---- Bottle 3 |
| 4 | 2.06 | | 0.07 | | 1.99 | <---- Bottle 4 |
| 5 | 3.32 | | 0.00 | | 3.32 | <---- Bottle 5 |
| 6 | 2.00 | | 0.00 | | 2.00 | <---- Bottle 6 |

Table 3 Calculation of recovery and detection limit

| | Peak area μV·sec (A) | Concentration in solution, mg/l: for GC analysis (B) | Concentration in solution, mg/l: in bottle (C) | Recovered mass mg (D) | Added mass mg (E) | Recovery % (F) | Detection limit mg/l (G) |
|---|---|---|---|---|---|---|---|
| Recovery test 1 | 4805210 | 578.8 | 96.5 | 29.0 | 30.0 | 97 | ———— |
| Recovery test 2 | 4815940 | 580.1 | 96.7 | 29.0 | 30.0 | 97 | ———— |
| Blank | <132800 | <16.0 | <2.7 | ———— | 0.0 | ———— | 3 |

Average recovery: 97 %

Standard solution Concentration (H): 500 mg/l
Peak area (I): 4150910 μV·sec
Volume of solution for GC analysis (J): 0.050 l
Volume of test solution for GC analysis (K): 0.300 l

Equation :B=H×A÷I C=B×J÷K D=C×K F=D÷E×100 G=C raise decimal fractions to unit.

Calculation of detection limit

| Retention time, min | 500 mg/l std. Peak area μV·sec | 500 mg/l std. Rate of peak area, % | Blank Peak area μV·sec |
|---|---|---|---|
| 5.6 | 298720 | 7.2 | ———— |
| 6.2 | 3127000 | 75.3 | <100000 |
| 6.5 | 725190 | 17.5 | ———— |
| Total | 4150910 | 100.0 | <132800 |

The minimum detectable peak area of the test substance was calculated by the following equation based on the minimum detectable peak area of 100000 μV·sec for the largest peak (retention time 6.2 min.) in GC chromatogram.

$$\text{Minimum detectable peak area } (\mu V\cdot sec) = 4150910\ (\mu V\cdot sec) \times \frac{100000\ (\mu V\cdot sec)}{3127000\ (\mu V\cdot sec)} = 132745\ (\mu V\cdot sec) < 132800\ (\mu V\cdot sec)$$

000524

Table 4 Residual test substance concentration in test bottles

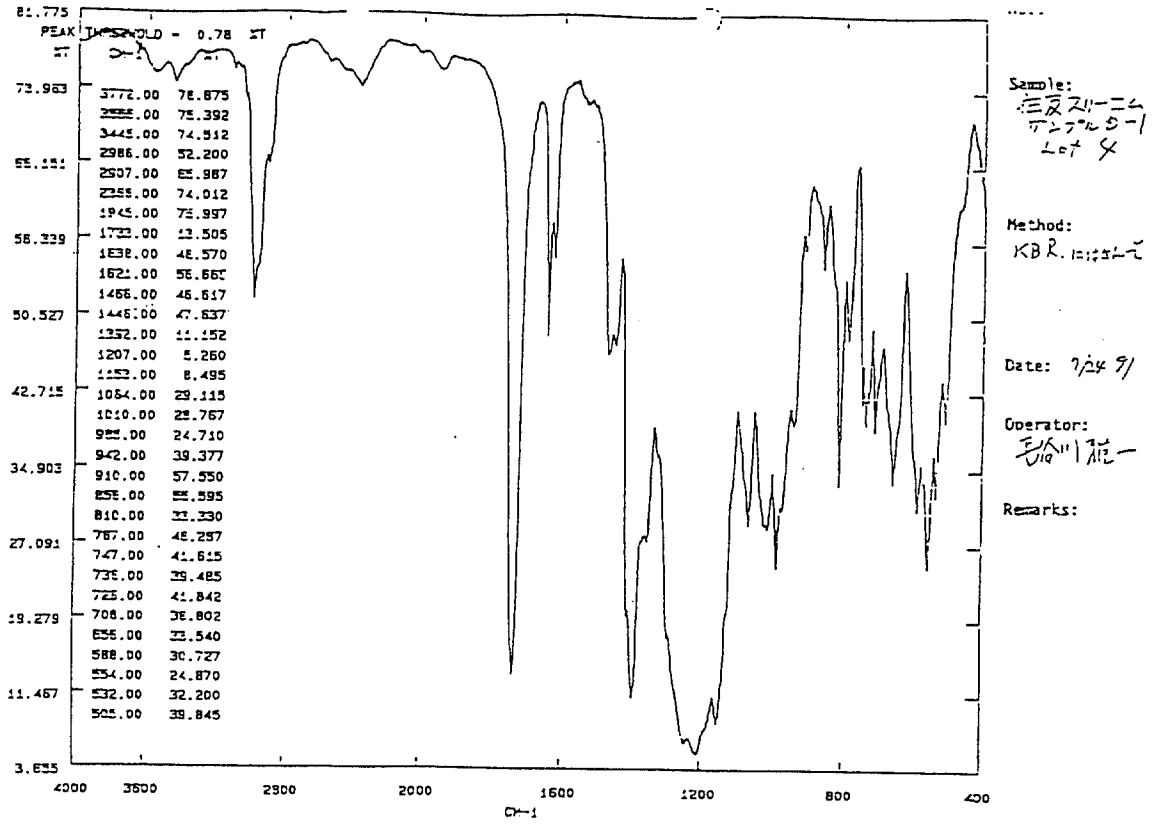
| Bottle No. | Peak area μV·sec (A) | Concentration in solution, mg/l | |
|---|---|---|---|
| | | for GC analysis (B) | in bottle (C) |
| 2 | <132800 | <15.2 | <3 |
| 3 | 5149880 | 589.3 | 101.3 |
| 4 | 5349150 | 612.1 | 105.2 |
| 5 | 4654900 | 532.7 | 91.5 |
| 6 | 5428120 | 621.2 | 106.7 |

| | | | |
|---|---|---|---|
| Standard solution | Concentration | (D): | 500 mg/l |
| | Peak area | (E): | 4369340 μV·sec |
| Volume of solution for GC analysis | | (F): | 0.050 l |
| Volume of test solution for GC analysis | | (G): | 0.300 l |
| Average recovery | | (H): | 97 % |

Equation :$B=A\times D\div E$ $C=A\times D\div E\times F\div G\div H\times 100$

Figure 1 Infrared absorption spectra of the test substance

Measure by the sponsor




Measure in M.S.I.

X: 4000.0, 400.00 cm-1; 0.07, 60.53 %T 92/01/13 09.31
4 scans; mode ratio; resol 4.00 cm-1; apod strong
183630 D-1 Lot No.4 A. Okuda
threshold 6.00%; band

| cm-1 | % | cm-1 | % | cm-1 | % | cm-1 | % |
|---|---|---|---|---|---|---|---|
| 2988.3 | 24.17 | 2364.1 | 50.05 | 1729.3 | 4.34 | 1639.4 | 19.44 |
| 1619.8 | 28.11 | 1468.8 | 18.77 | 1302.0 | 5.27 | 1202.0 | 2.18 |
| 1062.1 | 7.99 | 1017.3 | 7.50 | 984.3 | 7.19 | 858.1 | 36.16 |
| 808.1 | 9.87 | 784.1 | 22.25 | 747.4 | 18.34 | 653.7 | 11.30 |

16 peaks found

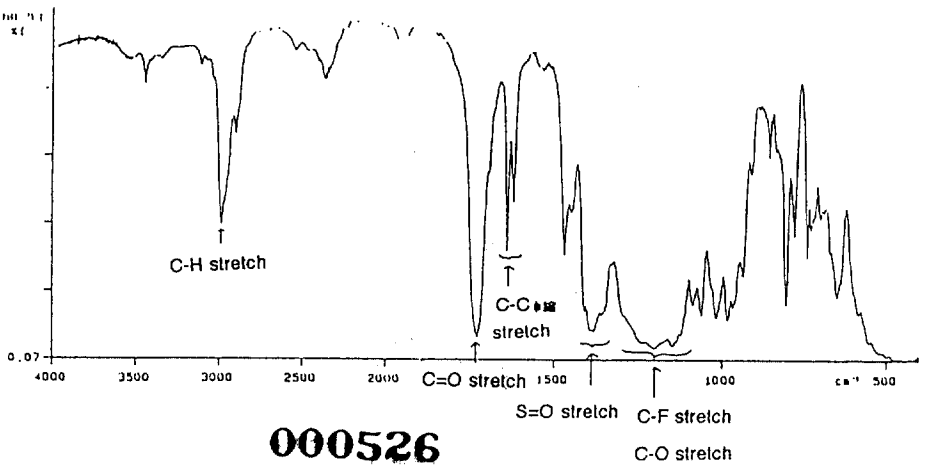


Figure 2 Infrared absorption spectrum of the test substance stored in a refrigerator

X: 4000.0, 400.00 cm-1; 0.14, 77.86 %T 92/04/16 16.39
4 scans; mode ratio; resol 4.00 cm-1; apod strong
18363(2)G O-1 Lot No.4 A. Okuda
threshold 5.00%; band

| cm-1 | % | cm-1 | % | cm-1 | % | cm-1 | % |
|---|---|---|---|---|---|---|---|
| 2987.2 | 51.57 | 2363.0 | 68.77 | 1731.1 | 3.85 | 1638.8 | 42.99 |
| 1619.8 | 54.81 | 1468.8 | 40.04 | 1392.9 | 5.38 | 1205.5 | 1.32 |
| 1062.3 | 20.58 | 1018.0 | 17.95 | 985.1 | 16.75 | 808.1 | 25.32 |
| 784.2 | 46.59 | 747.2 | 40.04 | 735.8 | 43.83 | 653.6 | 25.40 |

16 peaks found

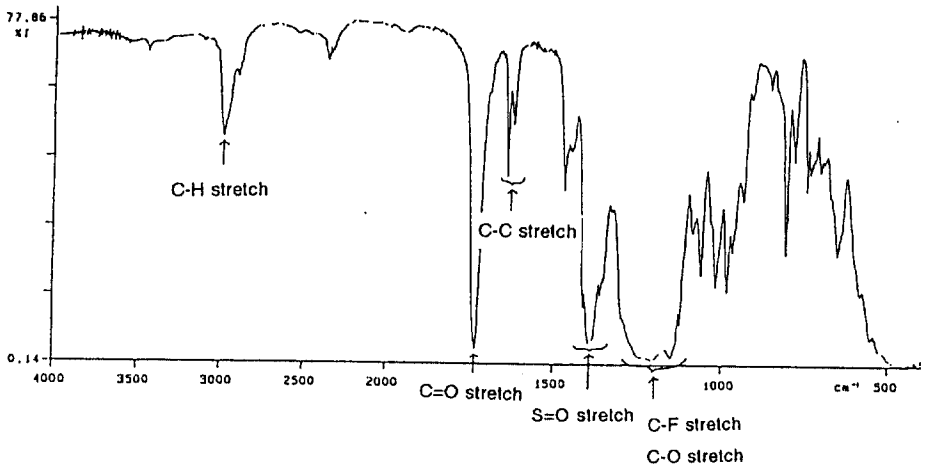


Figure 3 Calibration Curve of the Test Substance

Calibration Curve

| Concentration X(mg/l) | Peak Area Y(μV•sec) |
|---|---|
| 0 | 0 |
| 250 | 1.92785E+06 |
| 500 | 3.89009E+06 |
| 1000 | 8.12341E+06 |

$Y = -7.552 \times 10^4 + 8.139 \times 10^3 X$

r= 0.999707

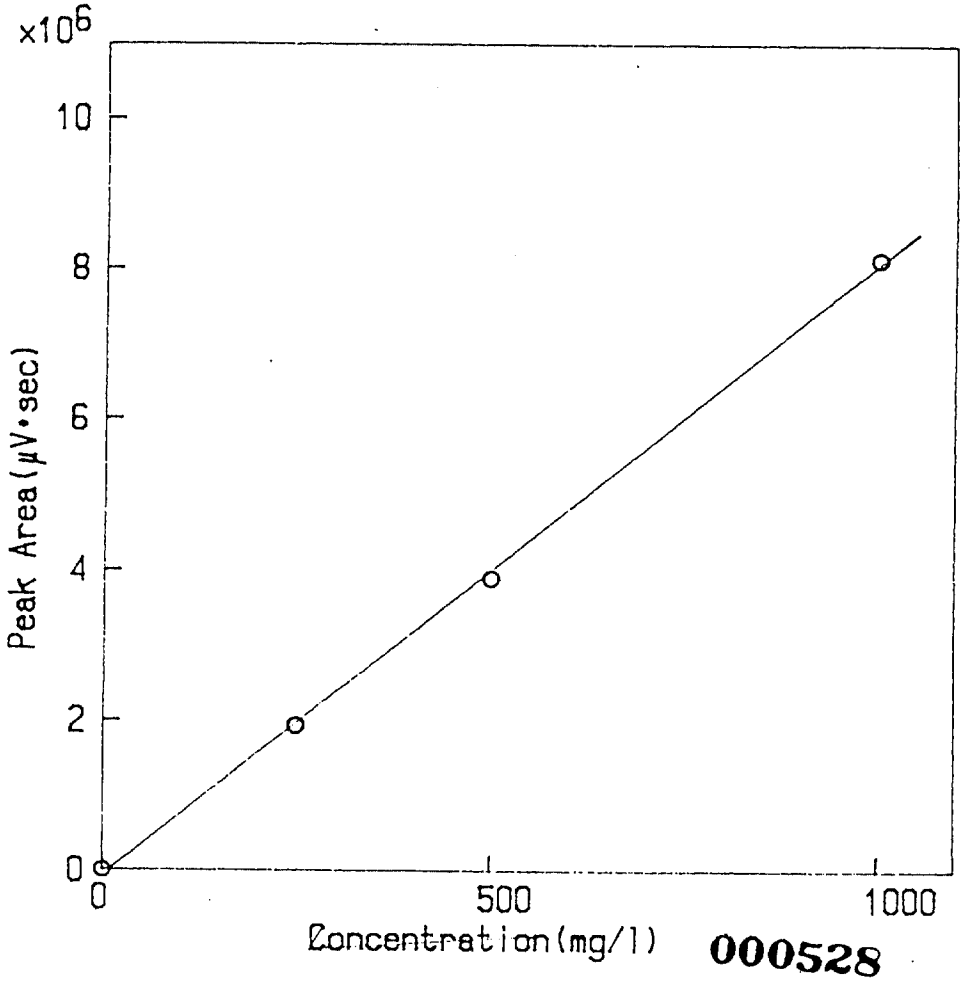
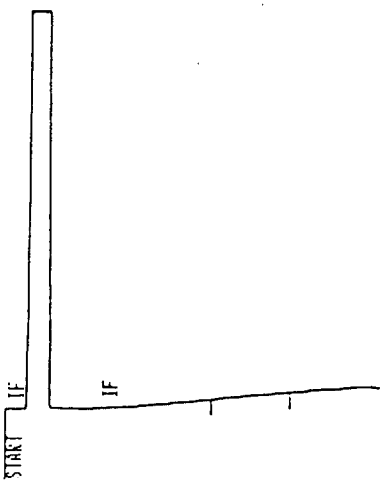


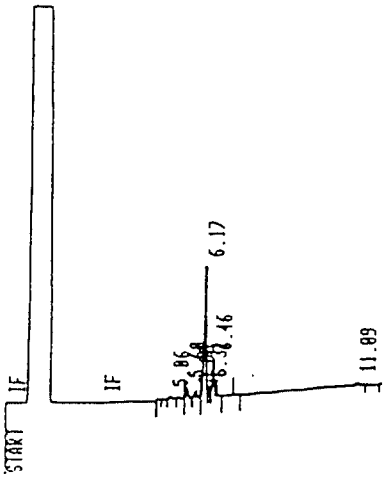
Figure 4 GC chromatograms of the test substance -- Calibration curve

0 mg/l in ethylacetate




NO RUN PEAKS STORED

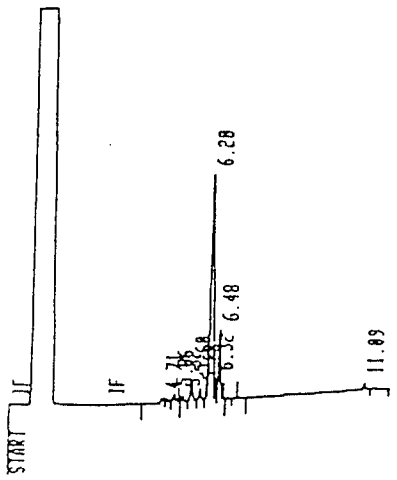
250 mg/l in ethylacetate




| AREA% RT | AREA TYPE | AR/HT | AREA% |
|---|---|---|---|
| 5.60 | 145420 D BP | 0.096 | 2.952 |
| 6.17 | 1432000 D BV | 0.078 | 29.070 |
| 6.46 | 350430 D VB | 0.069 | 7.114 |
| 11.09 | 2998400 BV | 1.670 | 60.865 |

total peak area:1927850μv·sec

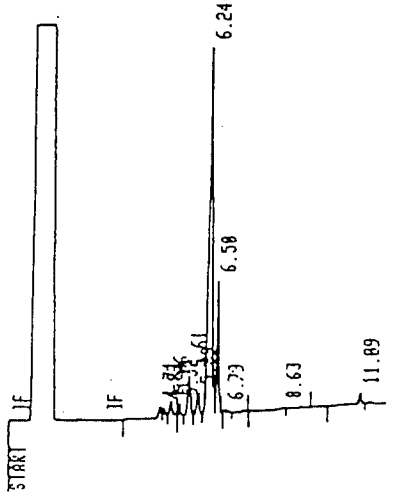
500 mg/l in ethylacetate 夜




| AREA% RT | AREA TYPE | AR/HT | AREA% |
|---|---|---|---|
| 5.60 | 291280 D PP | 0.098 | 4.136 |
| 5.88 | 156380 D PV | 0.090 | 2.217 |
| 6.20 | 2885100 D VV | 0.090 | 40.899 |
| 6.32 | 188178 D VV | 0.064 | 2.668 |
| 6.48 | 713210 D VB | 0.071 | 10.110 |
| 11.09 | 2819600 PV | 1.335 | 39.978 |

total peak area:3890090μv·sec

1000 mg/l in ethylacetate



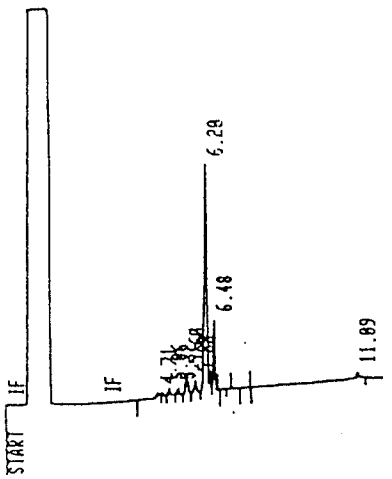

| AREA% RT | AREA TYPE | AR/HT | AREA% |
|---|---|---|---|
| 4.83 | 100220 D VV | 0.090 | 1.154 |
| 5.06 | 177070 D VB | 0.086 | 1.988 |
| 5.32 | 117440 D PP | 0.076 | 1.252 |
| 5.61 | 580310 PP | 0.103 | 6.188 |
| 5.89 | 318480 D PV | 0.097 | 3.396 |
| 6.24 | 6123600 VV | 0.118 | 65.293 |
| 6.58 | 1419500 D VB | 0.076 | 15.135 |
| 11.09 | 534090 D BV | 0.26. | 5.695 |

total peak area:8123410μv·sec

Figure 5 GC chromatograms of the test substance -- Rocovery and detection limit

Recovery and detection limit are shown in Table 3.

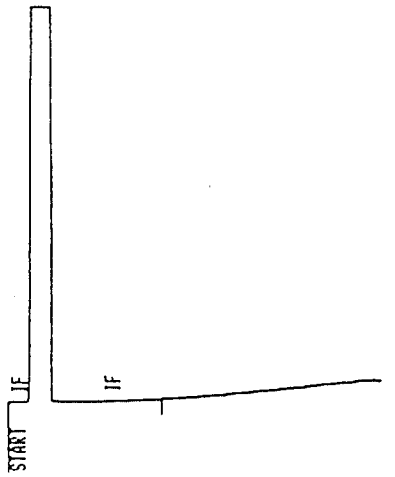
500 mg/l in ethylacetate



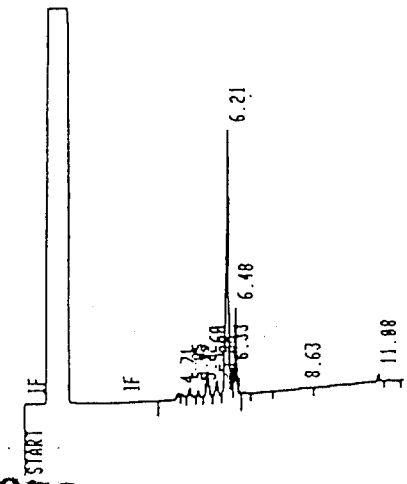

| AREA% RT | AREA TYPE | AR/HT | AREA% |
|---|---|---|---|
| 5.06 | 105990 D VV | 0.096 | 1.664 |
| 5.60 | 298720 D VP | 0.099 | 4.689 |
| 5.88 | 161710 D PV | 0.091 | 2.538 |
| 6.29 | 3127000 D VV | 0.092 | 49.079 |
| 6.48 | 725190 D VB | 0.071 | 11.382 |
| 11.09 | 1952700 PV | 1.091 | 30.648 |

total peak area:4150910μv·sec

Blank




Recovery test-1

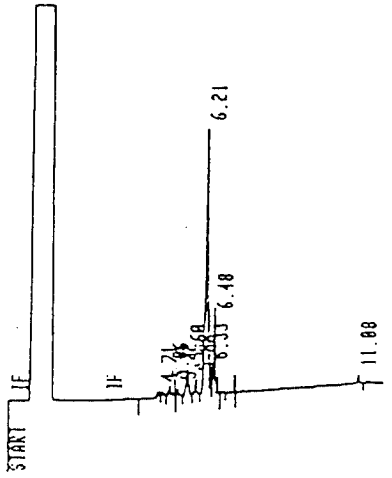



| AREA% RT | AREA TYPE | AR/HT | AREA% |
|---|---|---|---|
| 5.05 | 144480 D VV | 9.105 | 1.606 |
| 5.60 | 362910 D VP | 0.100 | 4.035 |
| 5.88 | 194960 D PV | 0.092 | 2.167 |
| 6.21 | 3565700 D VV | 0.095 | 39.641 |
| 6.33 | 232790 D VV | 0.064 | 2.588 |
| 6.48 | 876600 D VB | 0.072 | 9.745 |
| 8.63 | 618280 VV | 0.756 | 6.874 |
| 11.08 | 2999300 VV | 1.182 | 33.344 |

000530

total peak area:4805210μv·sec

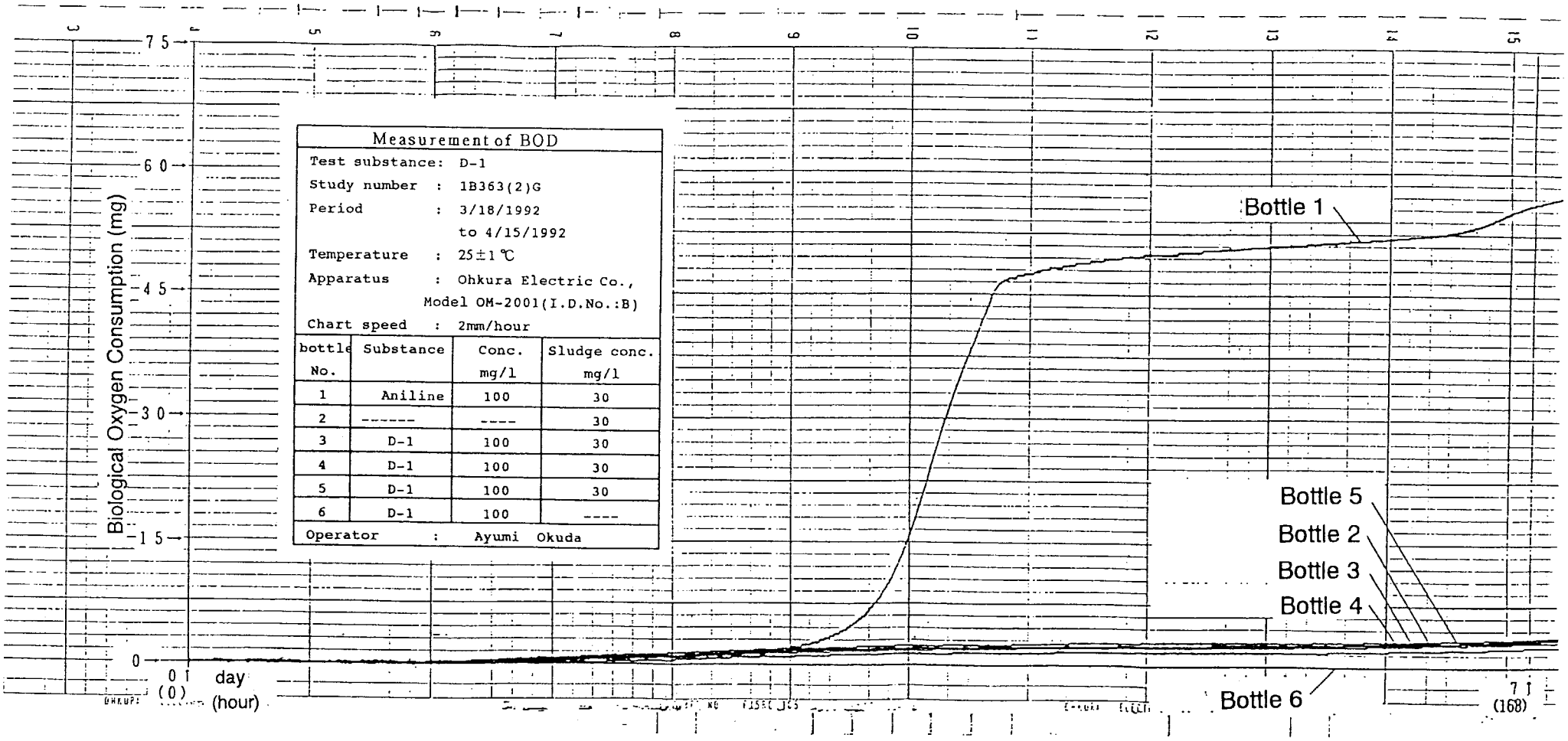
Recovery test-2 2




| AREA% RT | AREA TYPE | AR/HT | AREA% |
|---|---|---|---|
| 5.06 | 104890 D PB | 0.084 | 1.269 |
| 5.60 | 360210 D PP | 0.099 | 4.359 |
| 5.88 | 195250 D PV | 0.092 | 2.363 |
| 6.21 | 3529200 D VV | 0.096 | 43.315 |
| 6.33 | 232240 D VV | 0.064 | 2.810 |
| 6.48 | 876030 D VB | 0.073 | 10.600 |
| 11.08 | 2915900 BV | 1.250 | 35.284 |

total peak area:4815940μv·sec

Figure 6 BOD chart




000531

Figure 6 (Continued)

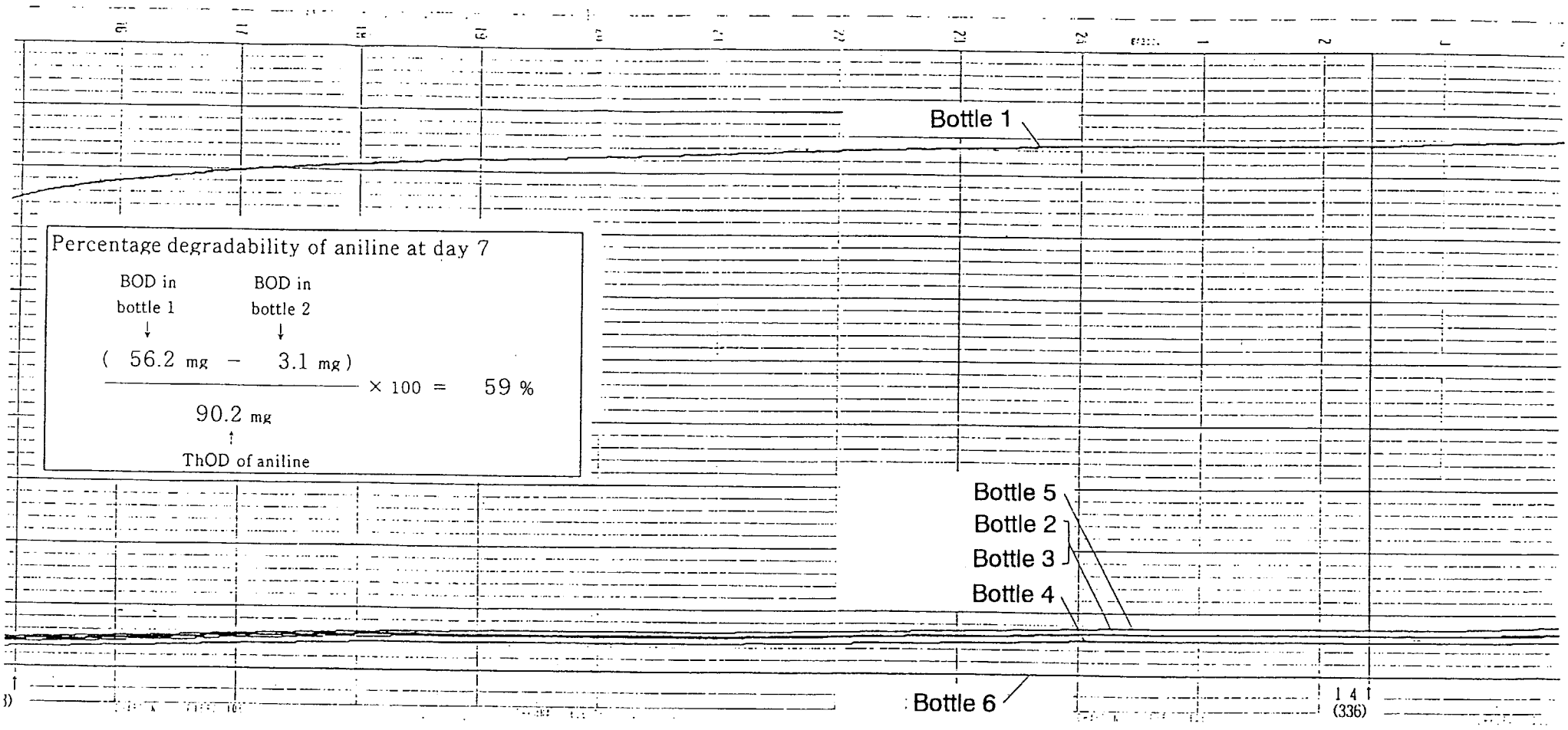


Figure 6 (Continued)

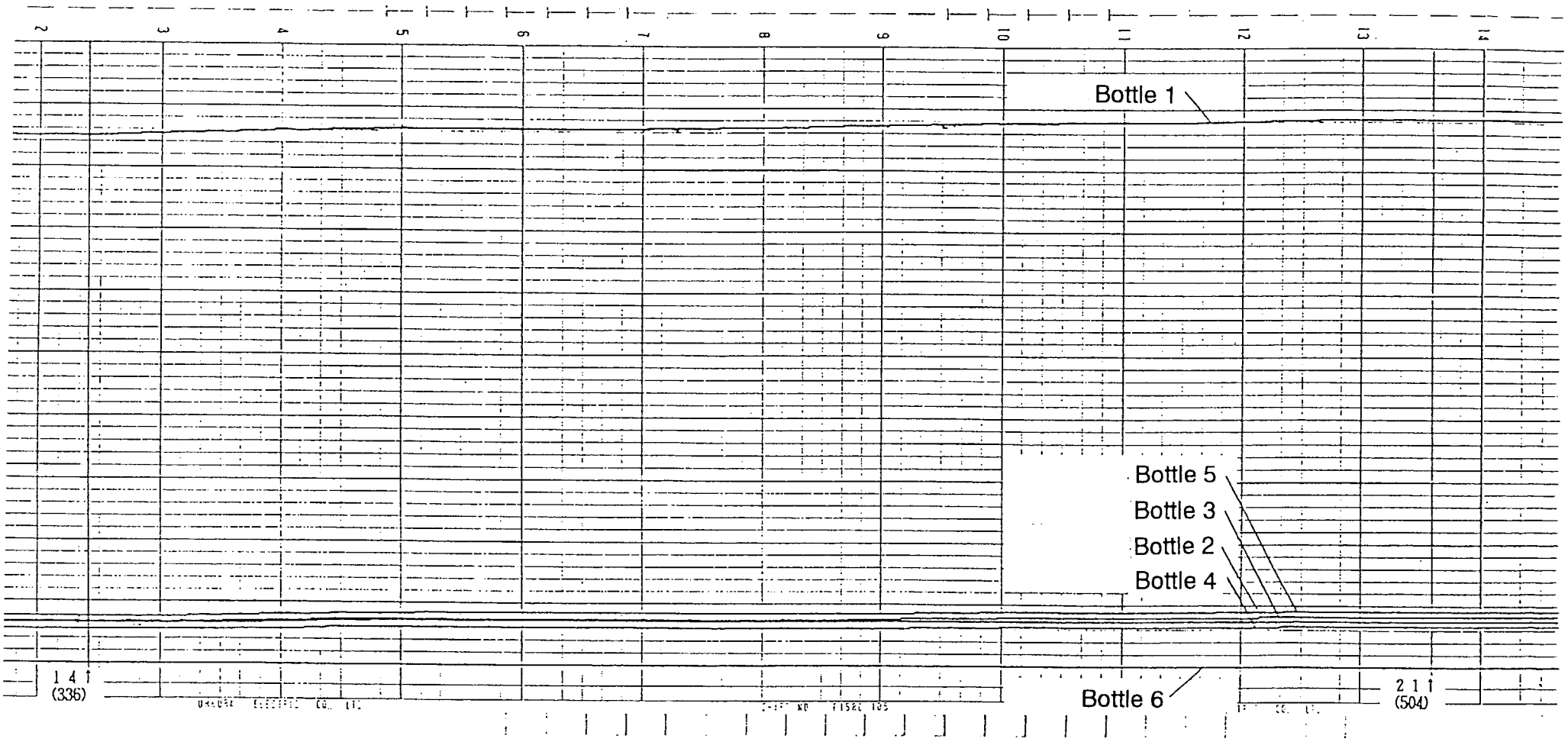


Figure 6 (Continued)

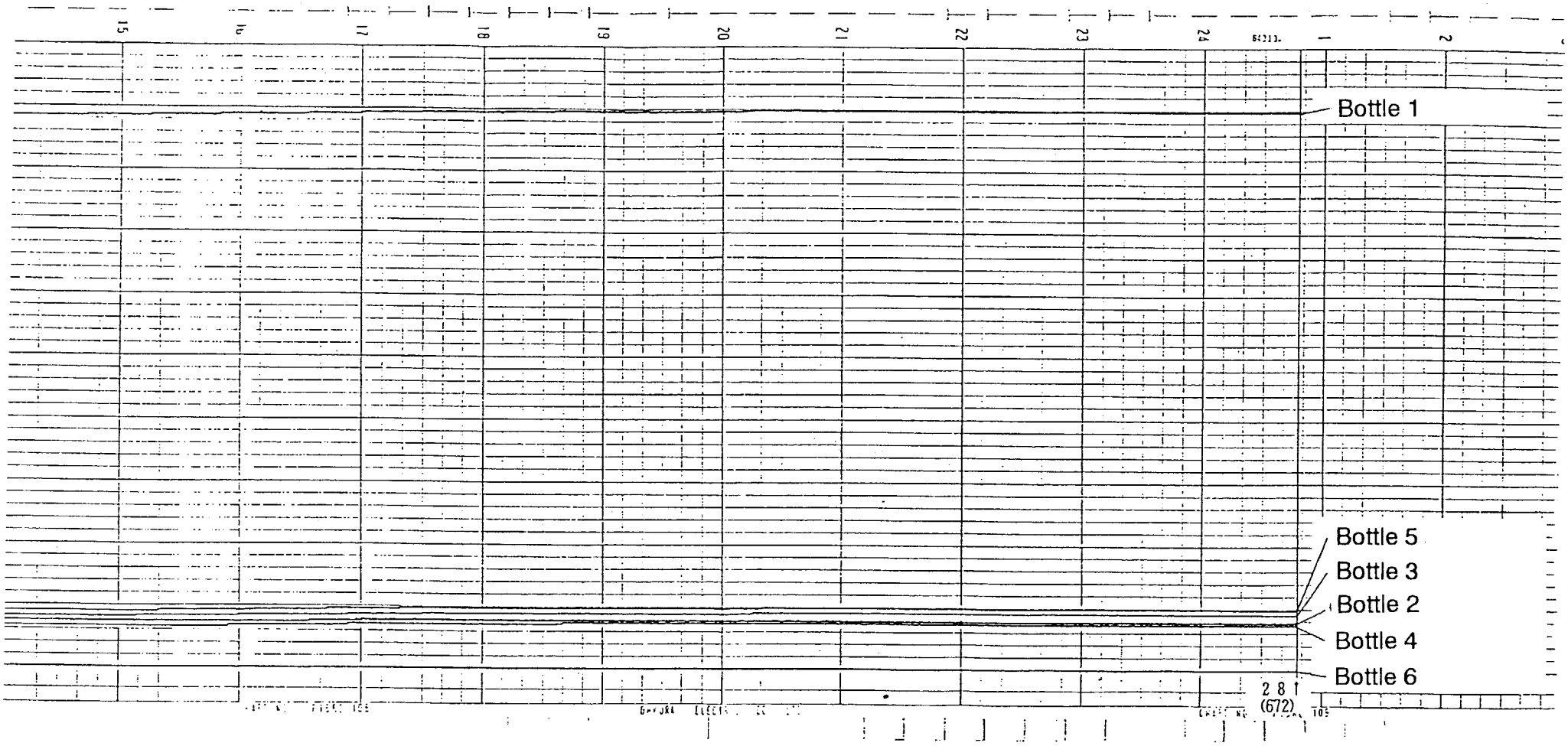
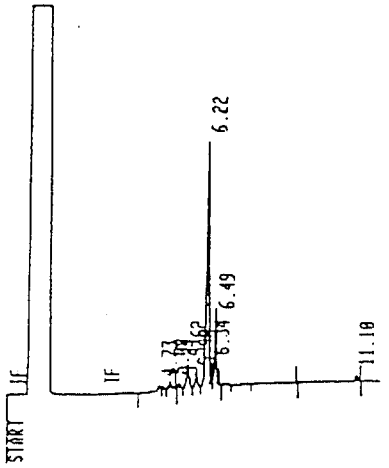


Figure 7 GC chromatograms of the test substance -- Measurement of residual test substance concentration

The concentrations of the residual test substance in the bottles are shown in Table 4.

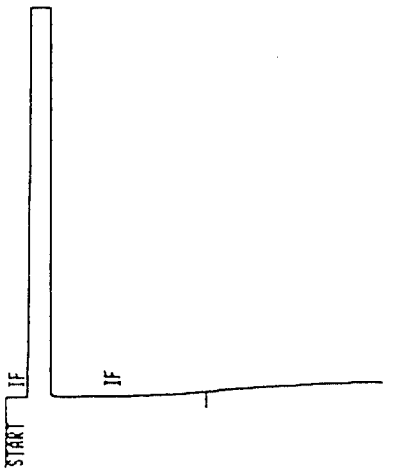
500 mg/l in ethylacetate




| AREA% RT | AREA TYPE | AR/HT | AREA% |
|---|---|---|---|
| 5.62 | 325870 D PP | 0.098 | 6.848 |
| 5.90 | 178020 D PV | 0.092 | 3.741 |
| 6.22 | 3241400 D VV | 0.092 | 68.114 |
| 6.34 | 211410 D VV | 0.064 | 4.443 |
| 6.49 | 802070 D VB | 0.072 | 16.855 |

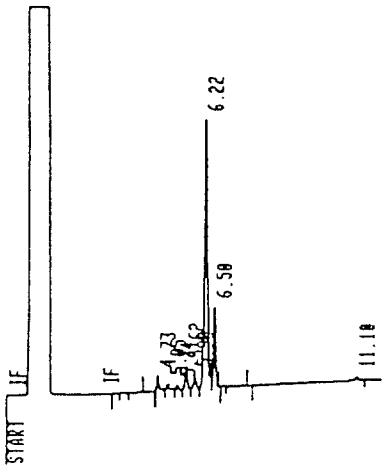
total peak area:4369340µv·sec

Bottle 2




NO RUN PEAKS STORED

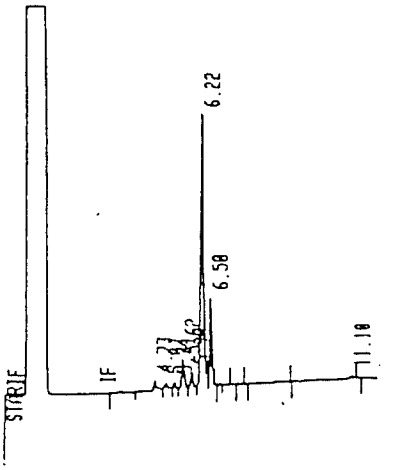
Bottle 3




| AREA% RT | AREA TYPE | AR/HT | AREA% |
|---|---|---|---|
| 4.73 | 215680 D PV | 0.096 | 3.106 |
| 5.05 | 121510 VV | 0.115 | 1.750 |
| 5.62 | 334190 D VP | 0.100 | 4.813 |
| 5.90 | 185690 D PV | 0.092 | 2.674 |
| 6.22 | 3979000 VV | 0.102 | 57.300 |
| 6.50 | 836690 D VB | 0.072 | 12.049 |
| 11.10 | 1271400 BV | 1.199 | 18.309 |

total peak area:5149880µv·sec

Bottle 4

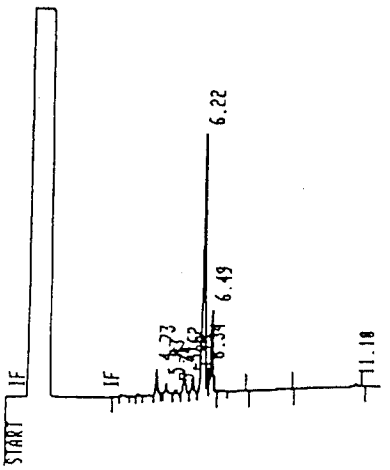



| AREA% RT | AREA TYPE | AR/HT | AREA% |
|---|---|---|---|
| 4.73 | 140400 D PV | 0.110 | 2.421 |
| 5.07 | 108370 D VV | 0.108 | 1.867 |
| 5.62 | 379960 D VP | 0.101 | 6.547 |
| 5.90 | 205250 D PV | 0.093 | 3.537 |
| 6.22 | 4053900 VV | 0.102 | 69.856 |
| 6.50 | 915290 D VB | 0.072 | 15.772 |

total peak area:5349150µv·sec

Figure 7 (Continued)

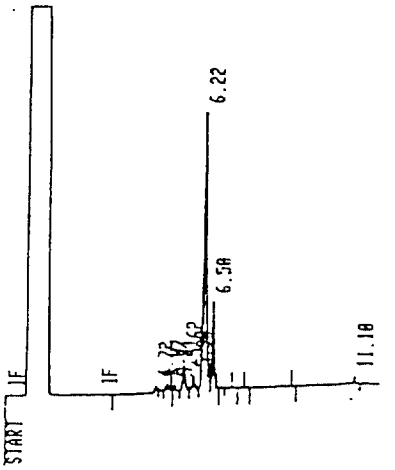
Bottle 5




| AREA% RT | AREA | TYPE | AR/HT | AREA% |
|---|---|---|---|---|
| 4.73 | 322600 | D PV | 0.085 | 5.810 |
| 5.03 | 164980 | D VV | 0.103 | 2.971 |
| 5.62 | 335320 | D VP | 0.101 | 6.039 |
| 5.90 | 183900 | D PV | 0.092 | 3.312 |
| 6.22 | 3472700 | D VV | 0.093 | 62.540 |
| 6.34 | 226410 | D VV | 0.064 | 4.077 |
| 6.49 | 846880 | D VB | 0.072 | 15.251 |

total peak area:4654900μv·sec

Bottle 6




| AREA% RT | AREA | TYPE | AR/HT | AREA% |
|---|---|---|---|---|
| 5.62 | 361480 | D PP | 0.100 | 5.850 |
| 5.90 | 200840 | D PV | 0.093 | 3.250 |
| 6.22 | 4159200 | VV | 0.103 | 67.306 |
| 6.50 | 907440 | D VB | 0.072 | 14.685 |
| 11.10 | 550590 | D BV | 0.418 | 8.910 |

total peak area:5428120μv·sec