

BIOACCUMULATION

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 states that sample purity is 99% or more based on information supplied 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 101.

METHOD

Method/guideline followed: MITI described in "Chemical Substances Control Law" (Japanese Law No. 117, 1974). OECD 305C.

GLP (Y/N): Yes

Year: 1995

Exposure period: 8-weeks

Exposure concentrations: 0.01 and 0.1 mg/L

Test species: *Cyprinus carpio*

RESULTS**Bioconcentration Factors:**

	<u>Week 2</u>	<u>Week 4</u>	<u>Week 6</u>	<u>Week 8</u>
0.01 mg/L exposure:	0.4	0.6	0.6	0.5
duplicate	0.4	0.7	0.4	0.5
0.1 mg/L exposure	3	4	2	2
duplicate	3	3	2	2

CONCLUSIONS

Neither mortality nor abnormalities in appearance or behavior were observed in the treated and control fish during the 8-week test period.

The test substance is not bioaccumulative in carp under the prescribed test conditions.

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

DATA QUALITY

Reliability: Klimisch ranking 3.

The purity/identity of the test substance cannot be substantiated. The extraction/analytical methodology has not been validated therefore it is not possible to know if the test compound did not bioconcentrate in the fish or simply was not extractable by the methodology of the experiment. The report states that the chromatographic peaks eluting from "1 to 3 minutes" are considered to be metabolites. While this statement does not change the conclusions of the report, this assumption is cannot be justified.

OTHER

Last changed: 5/22/00

M. S. I. REPORT No. 2B121G

Bioaccumulation Study of Sample D-1

with Carp (Cyprinus carpio)

Submitted to :

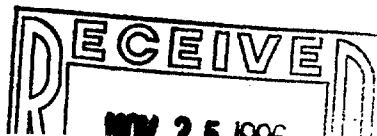
SUMITOMO 3M LTD.

Prepared by :

Mitsubishi Chemical Safety Institute Ltd.

Nov. 30, 1995

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TRANSLATION OF THE TEST REPORT

Study No. 2B121G
Study Title Bioaccumulation Study of Sample D-1 with Carp (Cyprinus
carpio)
Study Period From May 18, 1992 to Sept. 24, 1992
Sponsor SUMITOMO 3M LTD.

This test was conducted in Yokohama Laboratory, Mitsubishi Chemical Safety Institute Ltd. (M.S.I.), 1000 Kamoshida-cho, Aoba-ku, Yokohama 227, JAPAN.

The original test report was written in Japanese and this report was translated into English. The persons, the undersigned, hereby declare that this report reflects faithfully the original report as much as possible to our knowledge.

Translator from original Japanese version

Tadayoshi Shigeoka Nov. 30, 1995.
Tadayoshi SHIGEOKA, Ph.D. Date
Chief Research Scientist of Environmental Sciences Division

Shoko Tanimoto Nov. 30, 1995
Shoko TANIMOTO, B.Sc. Date
Scientist of Environmental Sciences Division

Approved by

Jun Toriya Nov. 30, 1995
Jun TORIYA, B.Sc. Date
Head of Yokohama Laboratory

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARD

Study No. 2B121G
Study Title Bioaccumulation Study of Sample D-1 with Carp (Cyprinus
carpio)
Sponsor SUMITOMO 3M LTD.

To the best of our knowledge and belief, the study described in this report was conducted in compliance with the Good Laboratory Practice (GLP) regulations recognized by Basic Industries Bureau, Japanese Ministry of International Trade and Industry (MITI).

Yokohama Laboratory,

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

* The company's name was changed on October 1, 1994.
(Former name : Mitsubishi-kasei Institute of
Toxicological and Environmental Sciences)

Test Facility Management

Sakae KOIKE, B.Sc.

Sealed Date : Sept. 24, 1992

Head of Yokohama Laboratory

Study Management

Tadayoshi SHIGEOKA, Ph.D.

Sealed Date : Sept. 24, 1992

Chief Research Scientist of Environmental Sciences Division

Study Director

Ayao NISHIKAWA

Sealed Date : Sept. 24, 1992

Scientist of Environmental Sciences Division

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

Experimental Scientist

Yasuo SATO

Sealed Date : Sept. 24, 1992

Scientist of Environmental Sciences Division

Naomi SAEKI, B.Sc.

Sealed Date : Sept. 24, 1992

Scientist of Environmental Sciences Division

QUALITY ASSURANCE STATEMENT

Yokohama Laboratory,

Mitsubishi Chemical Safety Institute Ltd.

Study Title Bioaccumulation Study of Sample D-1 with Carp (Cyprinus carpio)

Study No. 2B121G

We hereby certify that the above study was performed in compliance with the Good Laboratory Practice (GLP) regulations recognized by the Basic Industries Bureau, Japanese Ministry of International Trade and Industry (MITI).

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

	Dates of Inspection	Dates of Reporting
In-progress study	May 26, 1992	May 26, 1992
	June 23, 1992	June 23, 1992
	July 21, 1992	July 22, 1992
Final report	Sept. 24, 1992	Sept. 24, 1992

Quality Assurance Staffs

Junko KATOH, Ph.D. Sealed Date : Sept. 24, 1992

Yumiko MIURA, B.Sc. Sealed Date : Sept. 24, 1992

Fumio YAMAUCHI, Ph.D. Sealed Date : Sept. 24, 1992

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Summary

Sponsor : SUMITOMO 3M LTD.

Study Title : Bioaccumulation study of Sample D-1 with carp
(Cyprinus carpio)

Study No. : 2B121G

Test Substance : Sample D-1

Test Period : From May 18, 1992 to Sept. 24, 1992

Testing Method : Testing method of Ministry of International Trade and Industry (MITI) described in "Chemical Substances Control Law" (Japanese Law No.117, 1974).

(OECD testing guideline 305C)

Supply of test water : Continuous flow-through diluter system

flow rate : 1600 L/ day

Nominal concentration of Sample D-1 in test water :

High exposure level ; 0.1 μ g/mL

Low exposure level ; 0.01 μ g/mL

Exposure period : 8 weeks

Analytical method : GC analysis after pre-treatment

Results : Bioconcentration factors (BCFs) obtained in this study were as follows:

Bioconcentration factors (BCFs)					
level \ exposure period (weeks)	2	4	6	8	
High exposure level, sample fish No.1	0.4	0.6	0.6	0.5	
No.2	0.4	0.7	0.4	0.5	
Low exposure level, sample fish No.1	3	4	2	2	
No.2	3	3	2	2	

Conclusion : On the basis of the above results, it can be concluded that the test substance is not bioaccumulative under the prescribed test conditions.

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1. Introduction

1.1 Study Title

Bioaccumulation Study of Sample D-1 with Carp (Cyprinus carpio)

1.2 Objective

The objective of this study was to evaluate bioaccumulation potential of D-1 for application of a new chemical substance in Japan.

1.3 Testing Method

Testing method of Ministry of International Trade and Industry (MITI) described in "Chemical Substances Control Law" (Japanese Law No.117, 1974). (OECD testing guideline 305C)

1.4 GLP Compliance

The study was conducted in accordance with the Good Laboratory Practice (GLP) regulations recognized by Basic Industries Bureau, Japanese Ministry of International Trade and Industry (MITI).

1.5 Study Period

From May 18, 1992 to Sept. 24, 1992

(exposure period : From May 26, 1992 to July 21, 1992)

1.6 Storage and retention of records and sample

Following records, raw data, and sample will be retained in the archives of MITES for 10 years after submission of the final report. After this period, the sponsor will be contacted for approval of the disposal of any data. Data will be retained in the archives for a further specified period at the sponsor's request (additional fee).

1) The protocol and its ammendment record.

2) The final report.

3) Raw data.

4) Quality assurance reports.

5) Test substance (ca. 2g)

6) Other documents required by MITI GLP regulations.

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2. Test substance

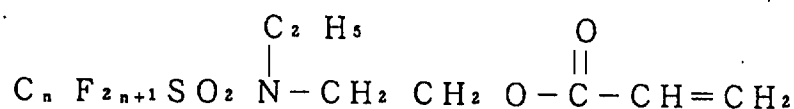
2.1 Chemical name, Structural formula, Molecular formula and weight.

Chemical name *:

2-[N-Ethyl-N-perfluoroalkyl(C= 1~8)sulfonylamino]ethylacrylate

[Abbreviated name: Samle D-1, identification No.2B121G]

Structural formula *:



$n = 1 \sim 8$, $n = 8$: ca. 78%, $n = 1 \sim 7$: ca. 21%

Molecular formula : $\text{C}_{15}\text{H}_{12}\text{O}_4 \text{NSF}_{17}$ ($n = 8$)

Molecular weight * : 625 ($n = 8$)

(* Data from the sponsor)

2.2 Physico-chemical properties

Appearance : amber like wax

Melting point * : $27 \sim 42^\circ\text{C}$

Boiling point * : $\text{ca. } 150^\circ\text{C}$ (1mmHg)

Solubility :

water : < 0.1% (25°C)

methanol : > 5 % (25°C)

acetonitrile : > 5 % (25°C)

acetone : > 50 %

n-hexane : > 5 % (25°C)

THF : > 5 % (25°C)

DMSO : insoluble

Infrared spectrum* : shown in Appendix-1

(* Data from the sponsor)

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2.3 Source

(1) Supplier : SUMITOMO 3M LTD.

(2) Supplied quantity* : 50 g

(3) Date of receipt : April 1, 1992.

(4) Lot. No. * : 101

(5) Purity * : > 99 %

Impurity * : Phenothiazine 60 ± 20 ppm

Hydroquinone monomethylether 170 ± 35 ppm

(* provided by the sponsor)

2.4 Confirmation of test substance

Before starting the study, infrared (IR) spectrum of Sample D-1 was measured. The spectrum (Fig.1(p.28)) was compared with the IR data provided by the sponsor (Appendix-1) and was confirmed the identity. And it also corresponded to the structural formula shown by the sponsor.

2.5 Stability of test substance under storage conditions

At the termination of the study, IR spectrum of Sample D-1 was measured and the spectrum (Fig.2(p.28)) was compared with the IR spectrum obtained at the start of this study (Fig.1). Test substance was confirmed to be stable under storage conditions stored in the refrigerator during the test period of ca.2.5 months.

3. Materials

3.1 Apparatus for bioaccumulation test

The flow-through test system is shown in Fig.3 (p.29).

[For test aquarium]

Dilution water supply pump : Yamazen (FMI) Co., QD-2CSC
Feed solution supply pump : Yamazen (FMI) Co., QG6-RH00STY

[For control aquarium]

Dilution water supply pump : Yamazen (FMI) Co., RP-D-2CKC
Feed solution supply pump : Tokyo Rika Kikai Co., GMW-8

3.2 Apparatus for analysis

Infrared spectrophotometer : Parkin Elmer Co., Model 1640
Homogenizer : Nihon Seiki Kaisha Ltd., AM-8
Shaker : Taiyo Kagaku Kogyo Co., SR-II
Rotary evaporator : Buchi Ltd., RE-111
Aspirator : Yamato Kagaku Co., BP-51
Electronic balance : Mettler Ltd., PL1200
Gas chromatograph : Shimadzu Ltd., GC-14A

3.3 Reagents

Acetonitrile : Kokusan Chemical Co., Guaranteed Reagent
Acetone : Junsei Chemical Co., Guaranteed Reagent
Chloroform : Junsei Chemical Co., Guaranteed Reagent
Ethyl Acetate : Kokusan Chemical Co., Guaranteed Reagent
Tetrahydrofuran (THF) : Kishida Chemical Co., Guaranteed Reagent
n-Hexane : Kishida Chemical Co., Guaranteed Reagent
Methanol : Junsei Chemical Co., Guaranteed Reagent
Sodium chloride : Kishida Chemical Co., Guaranteed Reagent
Sodium sulfate, anhydrous : Kokusan Chemical Co., Guaranteed Reagent

4. Analytical methods

4.1 Gas chromatographic(GC) conditions

Column : Shimadzu Ltd., Wide Bore column, CBP20-W25-100 ϕ 0.53mm $\times$ 25m

Temperature : Column ; 130°C

Injector ; 200°C

Detector ; 240°C

Flow rate of N₂ : 20mL/min

Range : 10¹

Attenuation : 7

Chart speed : 5mm/min

Injection volume : 3 μ L

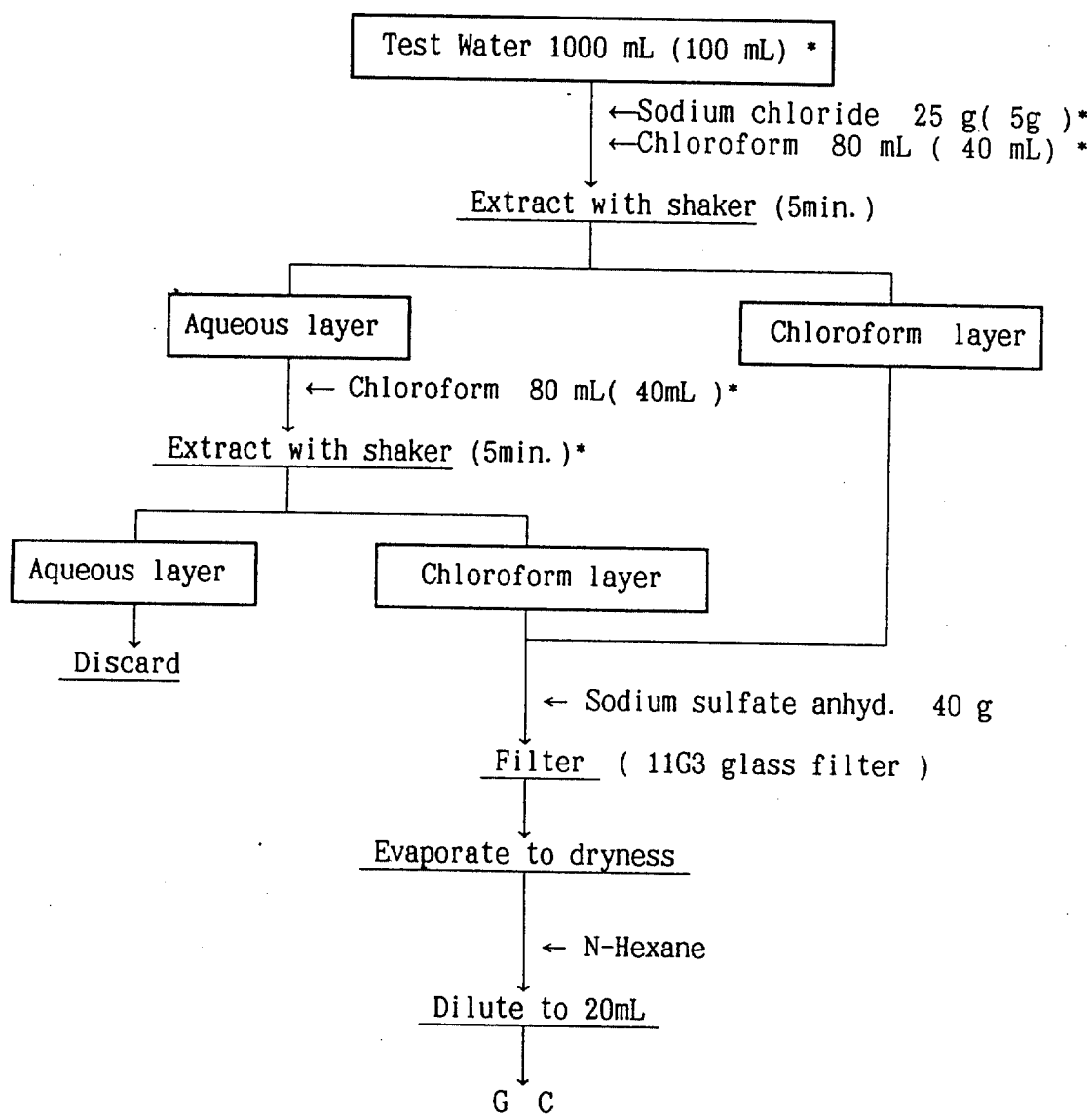
4.2 Calibration curve

Standard solution of 1000 μ g/mL was prepared by dissolving 100 mg of D-1 in 100 mL of n-hexane and this standard stock solution was diluted with n-hexane to prepare 0.25, 0.50, 1.00 μ g/mL n-hexane solutions of D-1. These solutions were injected into GC described above. The GC peak areas(μ V $\cdot$ sec) were plotted against the concentrations of D-1. The GC chromatograms and the calibration curve thus obtained are shown in Fig.4(p.30), and Fig.5(p.31), respectively. Quantity of D-1 was calculated from the total area of peaks at 3.9 and 4.5 min. on the chromatogram. The calibration curve is straight line through the origin and its correlation coefficient is 1.000.

D-1 was determined with the ratio of area on GC chromatograms against the standard solution (0.5 μ g/mL) per measurement.

4.3 Analytical method of test substance in test water

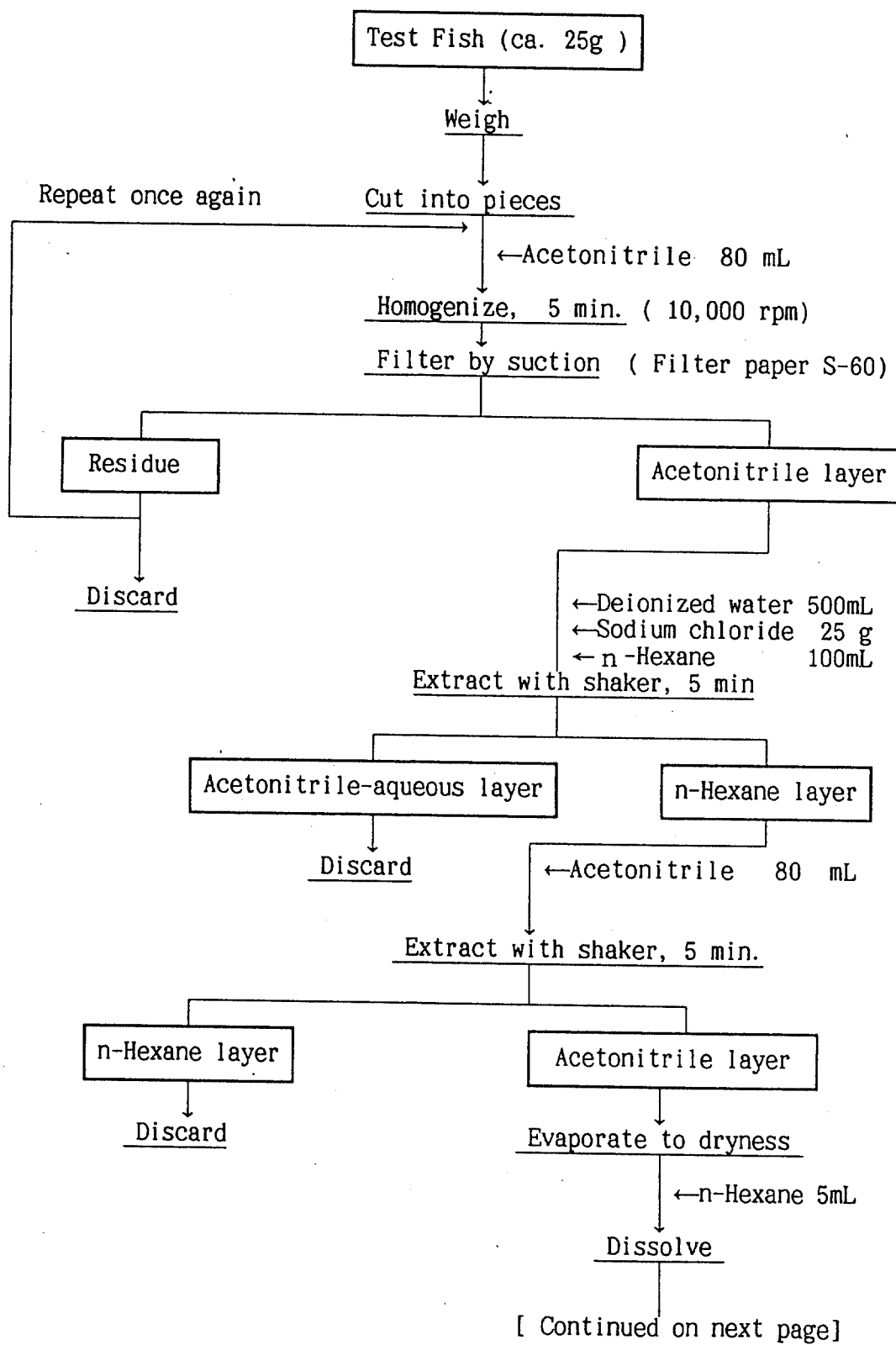
Analysis was performed according to the following procedure.



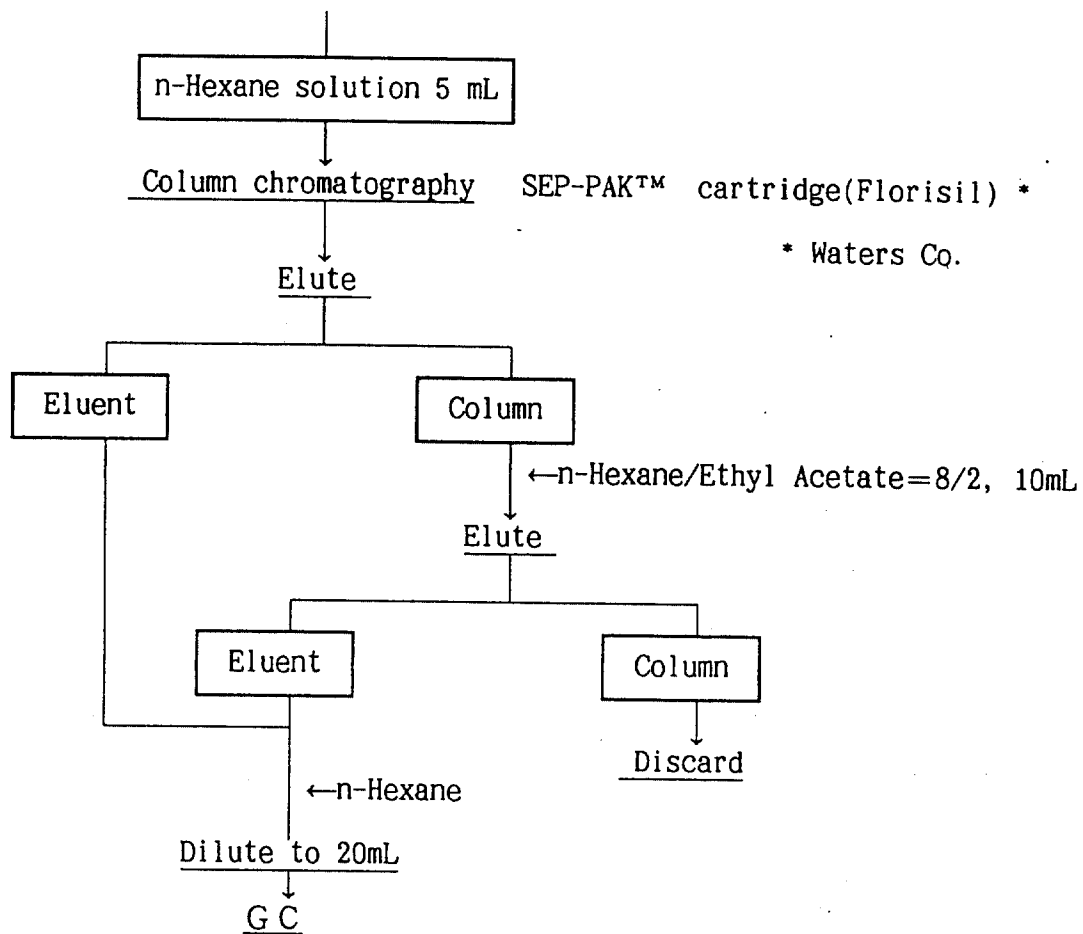
() * High exposure level

4.4 Analytical method of test substance in test fish

Analysis was performed according to the following procedure.



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4.5 Blank test

(1) Water

The blank tests were performed twice according to the procedure shown in 4.3, using 1000 mL of the test water in the absence of D-1 (Control aquarium). There was no peak which disturbed the analysis of D-1 in GC chromatograms from water (Fig. 6 (p. 32)).

(2) Fish

The blank tests were performed according to the procedure shown in 4.4, using the each two untreated test fish with D-1 (Control aquarium), at the beginning and the termination of the exposure period. There was no peak which disturbed the analysis of D-1 in GC chromatograms from fish (Fig. 7 (p. 33, 34)).

4.6 Recovery test

(1) Water

Two mL of acetone solution of D-1 ($5 \mu\text{g/mL}$) was added to 1000 mL of the test water in the absence of D-1. D-1 disperse solution of $0.01 \mu\text{g/mL}$ (the same as Low exposure level concentration in the test water) in the test water was thus prepared and the recovery tests were performed twice according to the procedure shown in 4.3. As shown in Table 1 (p. 35) and Fig. 8 (p. 36), the mean value of the recovery ratios was 94.6%. Analytical values of the test water were corrected by this value.

(2) Fish

Two mL of acetone solution of D-1 ($5 \mu\text{g/mL}$) were added to the untreated test fish with D-1 and the recovery test was performed twice according to the procedure shown in 4.4. In case that a fish body weight is 25g, the concentration of D-1 in fish would be $0.3 \mu\text{g/g}$ and correspond to 30 times of that in the test water. As shown in Table 2 (p. 35) and Fig. 9 (p. 37), the mean value of the recovery ratios was 99.2%. Analytical values of the test fish were corrected by this value.

4.7 Detection limit

There was no peak which disturbed the analysis of D-1 in GC chromatograms from blank water and blank fish. Therefore, the area of the minimum detectable peaks, which can be recognized visually, was set to be 30,000 $\mu V \cdot Sec.$. This value corresponds to ca. 0.02 $\mu g/mL$ (D-1) on the calibration curve. Detection limit is calculated according to following calculation formula and conditions. The results are as follows;

Fish	:	0.02 $\mu g/g$
Water	:	High exposure level 0.004 $\mu g/mL$
		Low exposure level 0.0004 $\mu g/mL$

It would be possible to calculate bioconcentration factors (BCFs) of following values in the case of fish weight 20g.

High exposure level (0.1 $\mu g/mL$)	:	> 0.2 times
Low exposure level (0.01 $\mu g/mL$)	:	> 2 times

.....

[Calculation formula]

Detection limit = Concentration in the final solution $\times$ Volume of the final solution $\div$ Amount of sample

[Conditions]

Fish	:	Volume of the final solution	20 mL
		Amount of sample (fish weight)	20 g
Water	:		
High exposure level	;	Volume of the final solution	20 mL
		Amount of sample	100 mL
Low exposure level	;	Volume of the final solution	20 mL
		Amount of sample	1000 mL

5. Acute toxicity to fish

The static acute toxicity test of the test substance to fish was conducted according to the Japanese Industrial Standard Method "JIS K 0102-1986, Industrial Waste Water Testing Method, 71. Acute toxicity study using fish".

The result obtained in this test were used as a reference for determining the nominal concentrations to be used in the bioaccumulation test because the nominal concentrations of high and low exposure levels should be lower than 1/100 and 1/1000 of 48hr-LC50, respectively.

5.1 Preparation of test solutions

Four mL of 400mg/mL THF solution of HCO-20 was mixed with 4mL of 50mg/mL THF solution of D-1. After evaporation of THF in the resultant solution by blowing N₂ gas, it was mixed with HCO-20 solution (6.4g in 100mL water) and dispersed. This dispersed solution was diluted to 2L with dechlorinated water (Yokohama municipal tap water was treated with activated charcoal) to prepare 100 μ g/mL solution of D-1.

[control level]

Eight g of HCO-20 was dissolved with 100 mL of deionized water and diluted to 2L with dechlorinated water.

5.2 Test conditions

(1) Test fish : Orange killifish (Oryzias latipes)

Source ; Miyazawa Fish Farm(1323, Suenaga, Takatsu-ku,
Kawasaki)

Date of receipt ; March 3, 1992.

Lot No. ; 92-H-0303

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Body length ; approx. 2 cm
Body weight ; approx. 0.2 g

(2) Acclimation :

Aquarium No. ; C-5
Water temperature ; 25 ± 2 °C
Period ; Over one week
Feed ; Tetramin ® (Tetra Werke, Germany)
Fish were fed once a day except holidays and
not fed 48 hours prior to the test.

Percentage of mortal fish during one week before test ; less than 5%

(3) Test conditions :

The number of fish ; 10 per one aquarium
Volume of water ; 2 L
Temperature ; $24.4 \sim 25.4$ °C
Duration ; 48 hours
Dissolved oxygen ; $4.2 \sim 7.4$ ppm
Aeration ; Test water was aerated during the test period.
Renewal of test water ; non

5.3 Results

The 48hr-LC₅₀ value of test substance was more than 100 μ g/mL
as shown in Table-3 (p 35).

6. Stability of test substance under the test conditions

6.1 Stability in water

(1) Preparation of test solution

The feed solution of high exposure level (concentration of D-1: 4.0mg/mL) was prepared by the procedure in 7.2(1). Then, 2.5mL of this solution was diluted to 1000mL with deionized water. It (concentration of D-1: 10 μ g/mL) was used for the stability test of D-1 in water.

(2) Method of measurement

The solution prepared by the procedure shown in 6.1 was left under room conditions, and 5mL of this solution was withdrawn and diluted with 100 mL of water on days 0, 1, and 2. Each of them was pre-treated by the procedure in Sec.4.3 and diluted to 100 mL with n-hexane. The resultant solution was analyzed by GC and variation of the test substance concentration with time was evaluated.

(3) Results

Typical GC chromatograms are shown in Fig.10(p.38) and the variation of concentration is shown in Fig.11(p.39). Based on the result that test substance remained 104% (9.5 μ g/mL $\rightarrow$ 9.9 μ g/mL) of initial concentrations after 2 days, it is concluded that D-1 is stable in water.

6.2 Stability in feed solution

(1) Preparation of test solution

The feed solution of low exposure level (concentration of D-1: 400 μ g/mL) was prepared by the procedure in 7.2(1).

000567 (2) Method of measurement

The test solution prepared by (1) was left under the room conditions.

At days 0, 3, 7, and 14, 2.5mL of the test solution was withdrawn and was diluted to 50 mL with acetone. Then each of 2.5mL of these solution was withdrawn and was diluted to 100mL with acetone, and analyzed by GC. Variation of the test substance concentration with time was evaluated.

(3) Results

Typical GC chromatograms are shown in Fig.12(p.40) and the variation of concentration is shown in Fig.13(p.41). Based on the result that test substance remained 99% ($394 \mu\text{g/mL} \rightarrow 390 \mu\text{g/mL}$) of initial concentrations after 14 days, it is concluded that D-1 is stable in feed stock solution.

7. Bioaccumulation test method

The study was performed in accordance with the testing method of Ministry of International Trade and Industry (MITI) described in "Chemical Substances Control Law" (Japanese Law No.117, 1974).

7.1 Test fish

Carp(Cyprinus carpio) were purchased from Sankyo Suisan Co. (1-1 Ichigayatamachi, Shinjuku-ku, Tokyo) on December 6, 1991. Prior to the initiation of the test, these fish were acclimatized over a week under the test condition (water temperature 25 ± 2 °C, fed with commercial pellets once a day) and the mean body length of them is ca.11 cm. Mortal fish were less than 5 % during the acclimation of a week prior to the introduction of fish into the test system.

7.2 Methods and conditions

(1) Preparation of feed solution

• High exposure level

The stock solution was prepared by dissolving 2.0 g of D-1 and 80 g of HCO-20 in 500mL of THF. (Conc. of D-1 : 4.0 mg/mL, Conc. of HCO-20: 160 mg /mL)

• Low exposure level

Fifty mL of the feed solution of high exposure level was diluted to 500 mL with THF (Conc. of D-1 : 0.40 mg /mL, conc. of HCO-20 : 16 mg/mL).

• Control aquarium

The 160g of HCO-20 was dissolved in 1L of THF(stock solution). The 370mL of this solution was diluted to 11 L with deionized water. (Conc. of HCO-20 : 5.4 mg /mL).

The feed solutions were renewed every 7 -10 days.

(2) Preparation of test solution

The feed solution prepared by the procedure shown in 7.2(1) was supplied to mixing tube by feed solution supply pump and dilution water (dechlorinated tap water) was also supplied to the same tube. Then these were mixed and the concentration of test substance reached nominal value. The test solution in mixing tube was supplied to an aquarium. Dilution water is the tap water of Yokohama city which was dechlorinated with activated charcoal. (Fig.3 (p.29))

(3) Conditions

Flow rate of feed solution : 40m L/day for high and low exposure levels
and 1.2 L/day for control level

Flow rate of dilution water : 1600 L/day (The turnover rate for each
aquarium : 20 times/ day)

Test fish : Carp (Cyprinus carpio, Lot.No. 91-K-1206, aquarium No. B5)

- Body weight : 31~40 g
- Body length : 11~ 12 cm
- Fat content of fish : 3.7 % (n=4, 3.3~ 4.3%)
- Water temperature : 24.2~24.7°C
- Dissolved oxygen : 4.9 ~ 8.2 mg/L
- Feeding : Fish were fed with commercial pellets
(Minipet® (Kyorin Co. Ltd.)) once a day except holidays.

• Nominal concentration of D-1 in water :

High exposure level ; 0.1 μ g/mL (conc. of HCO-20, 4mg/mL)

Low exposure level ; 0.01 μ g/mL (conc. of HCO-20, 0.4mg/mL)

Control ; 0 μ g/mL (conc. of HCO-20, 4mg/mL)

• Population density (at the beginning of the exposure period) :

High exposure level ; 15 fish / 80L test water

Low exposure level ; 15 fish / 80L test water

Control ; 12 fish / 80L test water

• Exposure period : 8 weeks

(4) Introduction of fish, biological observation and environmental control

The test system was operated for 4 days prior to the introduction of fish to confirm the stability of the concentration of D-1 in water. After that, fish were introduced into the test system. Fish were observed for mortality, behavior and some abnormalities twice a week. Dissolved oxygen and temperature of test water were monitored twice a week. These results were recorded.

(5) Analysis of D-1 in test water

Aliquot of test water was withdrawn twice a week and was analyzed by the procedure shown in 4.3.

(6) Analysis of D-1 in test fish

Three fish were collected in 2, 4, 6 and 8 week after exposure. Two of them were analyzed by the procedure shown in 4.4.

* Flow rate of dilution water was 800 L/day in first 3 days , but because of concentration decrease of D-1 due to uptake by fish, it was changed to 1600 L/day after the day for each aquaria.

8. Results and Discussion

8.1 Circumstances that may have affected the reliability of the test results.

There was no significant matter that may have affected the reliability of the test results during the test period of 8 weeks.

8.2 Biological observations of test fish

Neither mortality nor abnormality in appearance or behavior was observed in treated and control fish during the test period of 8 weeks.

8.3 Concentrations of test substance in test water

The results are shown in Table 4, 5(p.42, 43) and Fig.14, 15(p.44). (Appendix-2) The mean values during the exposure period were $0.092 \mu\text{g/mL}$ (high exposure level) and $0.0082 \mu\text{g/mL}$ (low exposure level).

The decreases of D-1 concentration in both levels were observed at 3 days from the initiation of test. This phenomena will be attributable to uptake of D-1 by fish. Therefore, flow rates of the feed solution for both aquaria were increased by a factor of two, and as a result, each concentration is approached to each nominal value. The coefficients of variation are 8.4 % (high exposure level) and 13.5 % (low exposure level), respectively.

8.4 Concentrations of test substance in test fish and bioconcentration factors(BCFs)

The results are shown in Table 6, 7 (p.45, 46) and Fig.16 ~18 (p.47~55). Concentrations of D-1 in fish are $0.032 \sim 0.062 \mu\text{g/g}$ (high exposure level) and $0.013 \sim 0.034 \mu\text{g/g}$ (low exposure level). The BCFs are $0.4 \sim 0.7$ (high exposure level) and $2 \sim 4$ (low exposure level).

Peaks observed at the retention time from 1 min. to 3min. will be considered as metabolites of D-1.

On the basis of the above results, it can be concluded that sample D-1 is not bioaccumulative in carp under the prescribed test conditions.

000573

Fig.1 Infrared spectrum of D-1 (before starting the study)

X: 4000.0, 400.00 cm-1; 0.06, 65.92 %T 92/05/20 11:00
 4 scans; mode ratio; resol 4.00 cm-1; apod strong
 2B121G O-1 Lot.No.101 N.Saeki
 threshold 3.00%; band

cm-1	%	cm-1	%	cm-1	%	cm-1	%
2986.8	46.13	1730.3	13.67	1638.4	46.05	1619.8	53.28
1468.6	46.61	1392.0	16.90	1202.5	6.36	1148.9	7.71
1062.0	29.33	1017.7	28.37	984.6	27.24	857.9	57.01
808.0	35.17	784.1	48.20	746.7	44.54	735.7	45.63
706.4	44.98	653.5	33.43				

18 peaks found

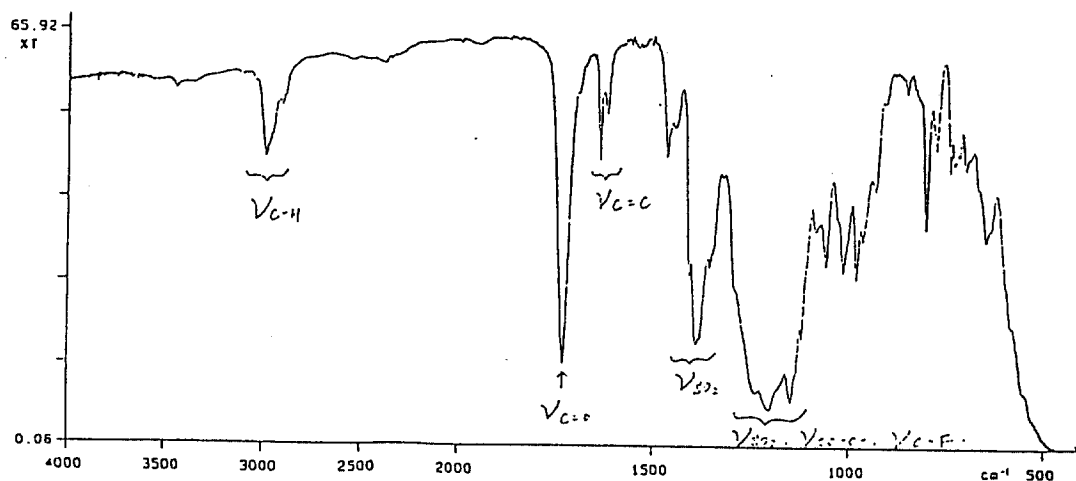
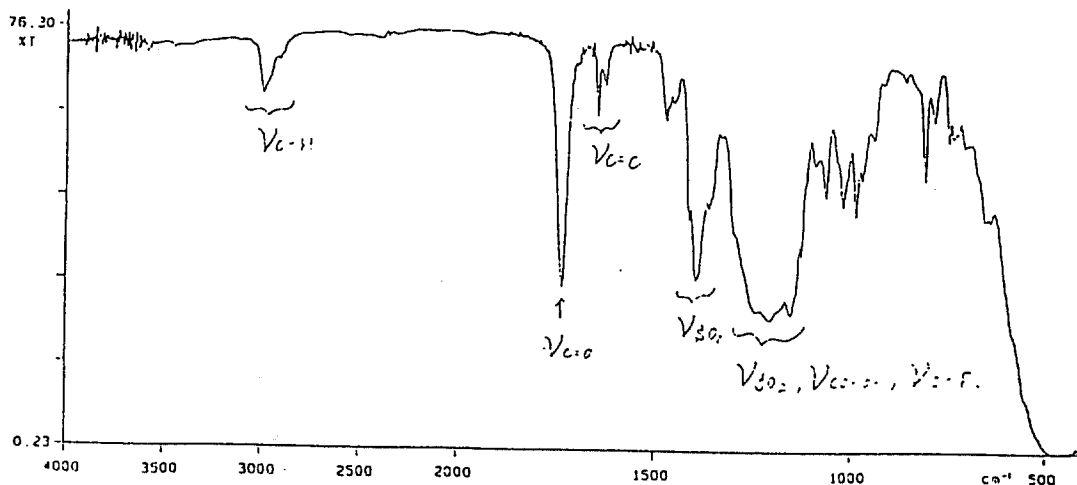


Fig.2 Infrared spectrum of D-1 (at the termination of the study)

X: 4000.0, 400.00 cm-1; 0.23, 76.30 %T 92/08/07 09:57
 4 scans; mode ratio; resol 4.00 cm-1; apod strong
 2B121G O-1 Lot.No.101 N. Saeki
 threshold 3.00%; band

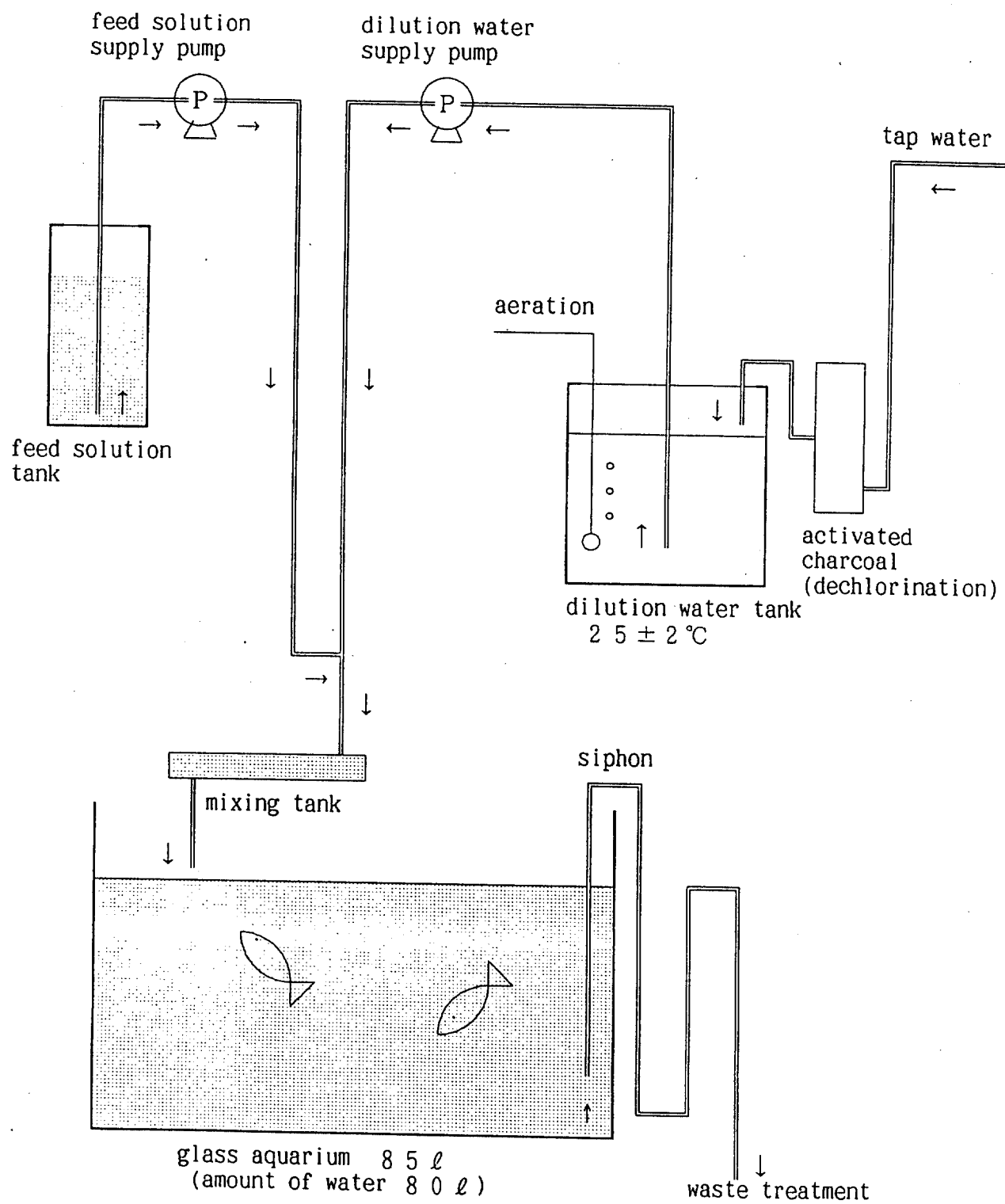
cm-1	%	cm-1	%	cm-1	%	cm-1	%
3854.2	70.59	3751.9	71.78	3676.2	71.50	3650.1	71.29
3630.0	71.45	3587.7	71.30	2987.1	64.35	1731.7	29.76
1638.4	60.89	1467.8	60.04	1392.9	31.11	1202.3	23.91
1062.4	46.04	1018.3	44.44	985.2	42.65	808.2	49.13
784.3	59.95	746.9	56.19	735.7	56.80		

19 peaks found



000574

Fig. 3 Bioconcentration test system



000575

Fig. 4 Gas chromatograms of D-1(Standard solution)
for calibration curve

(n-hexane only)

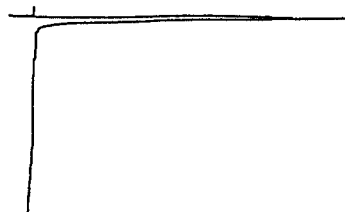
0 $\mu\text{g/ml}$

0.25 $\mu\text{g/ml}$

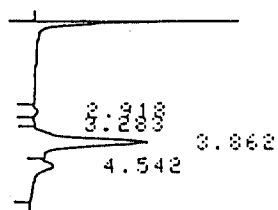
0.50 $\mu\text{g/ml}$

1.00 $\mu\text{g/ml}$

START

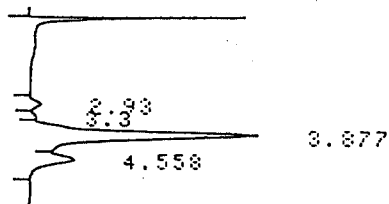


WARNING NO PEAK
START



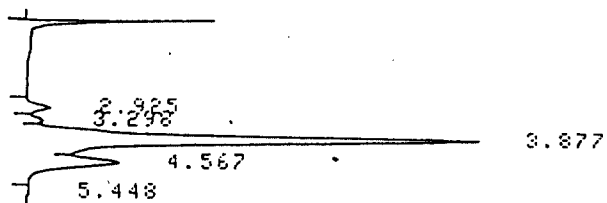
PKNO	TIME	AREA	HK	IDNO	CONC
1	2.918	8026			2.0726
2	3.283	5253			1.3564
3	3.862	299049	V		77.2208
4	4.542	74936	V		19.3502
TOTAL					100

START



PKNO	TIME	AREA	HK	IDNO	CONC
1	2.93	18629			2.4738
2	3.3	11731	V		1.5579
3	3.877	594555	V		78.9546
4	4.558	128119	V		17.0137
TOTAL					100

START



PKNO	TIME	AREA	HK	IDNO	CONC
1	2.925	40165			2.6298
2	3.298	26487	V		1.7342
3	3.877	1179476	V		77.2246
4	4.567	271999	V		17.8087
5	5.448	9205	V		0.6027
TOTAL					100

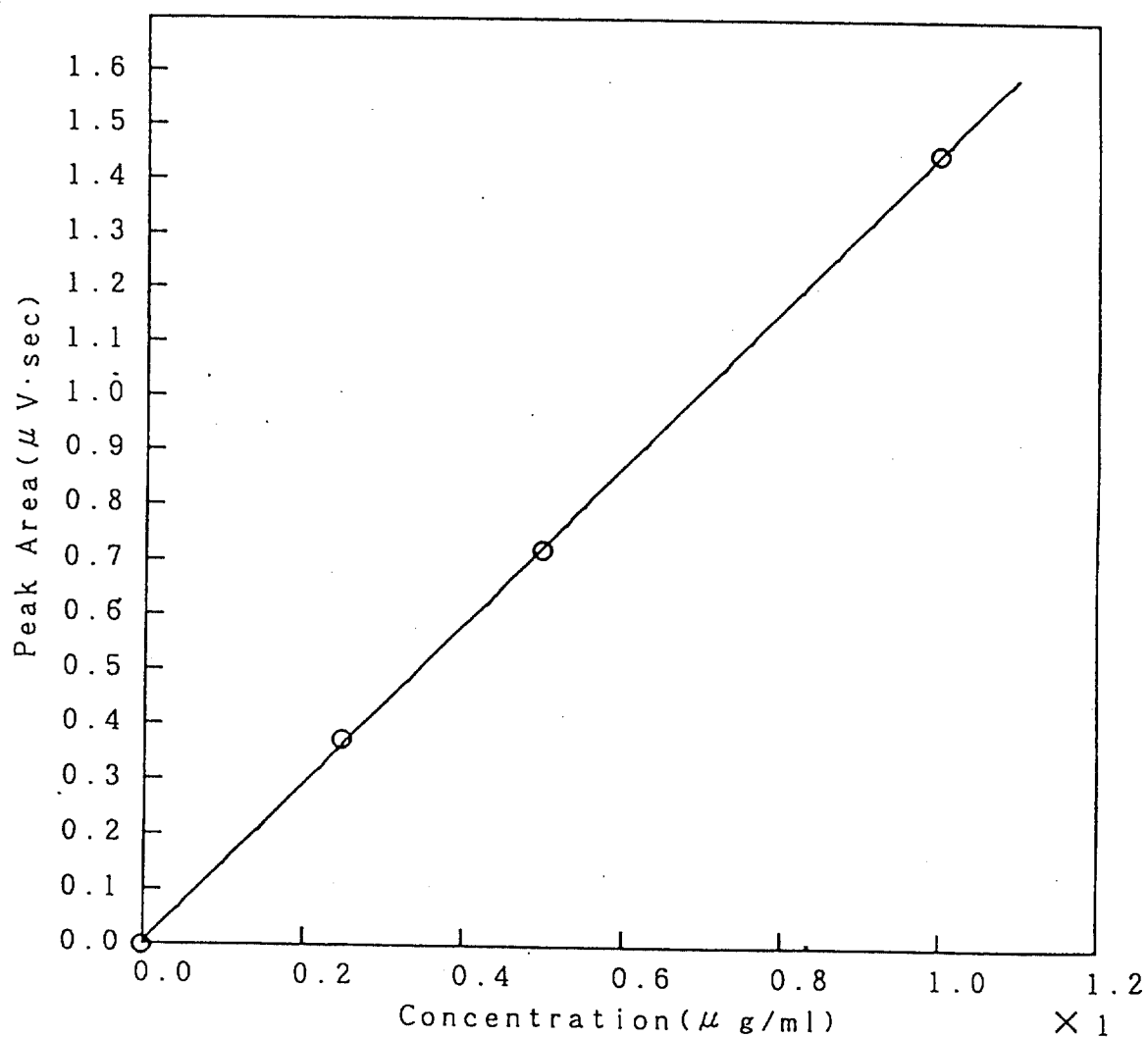
000576

Fig.5 Calibration curve of D-1

$$Y = 3.8338 \times 10^3 + 1.4473 \times 10^6 \times X^1$$
$$r = 0.99995$$

$\times 1000000$

Calibration Curve



000577

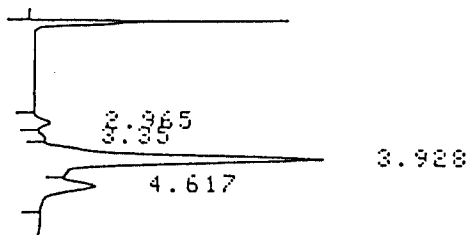
Fig. 6 Gas chromatograms (Blank test from water)

Std. 0.50 μ g/ml

1

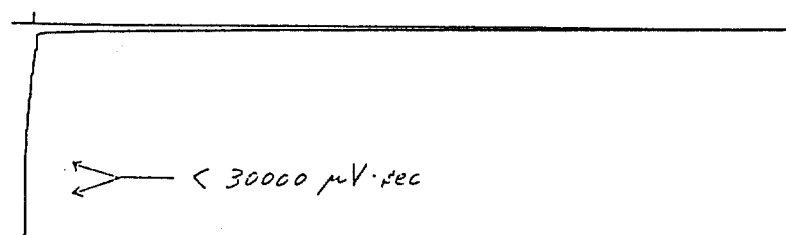
2

START



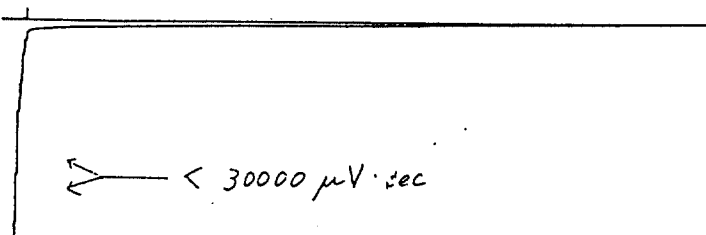
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.965	20297			2.5362
2	3.35	13721	V		1.7146
3	3.928	628854	V		78.5797
4	4.617	137403	V		17.1695
TOTAL		800275			100

START



WARNING NO PEAK

START



WARNING NO PEAK

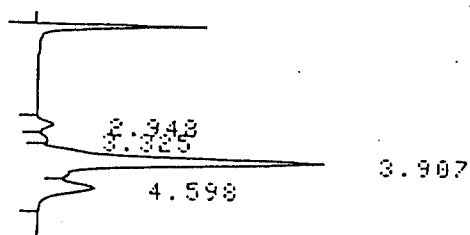
000578

Fig. 7-1 Gas chromatograms (Blank test from fish)

(before starting the study)

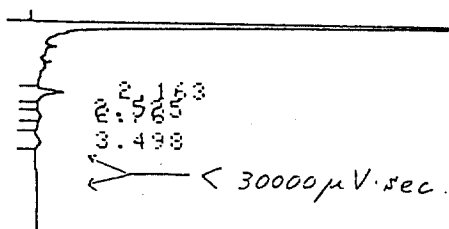
Std. 0.50 μ g/ml

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.948	21215			2.6632
2	3.325	14409	V		1.8088
3	3.907	625133	V		78.4757
4	4.598	135839	V		17.0524
TOTAL		796595			100

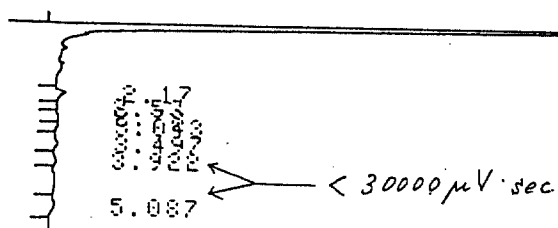
START



1

PKNO	TIME	AREA	MK	IDNO	CONC
1	2.163	26237			62.7341
2	2.78	5053	V		12.0813
3	3.498	10533			25.1846
TOTAL		41823			100

START



2

PKNO	TIME	AREA	MK	IDNO	CONC
1	2.17	11941			27.8025
2	2.78	8120	V		18.905
3	3.497	8755			20.3835
4	3.922	5657			13.1711
5	5.087	8477			19.7379
TOTAL		42949			100

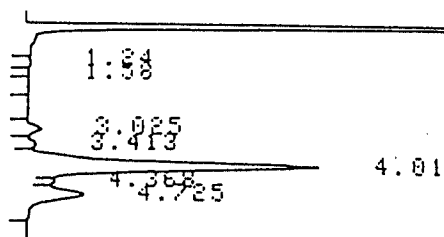
000579

Fig. 7-2 Gas chromatograms (Blank test from fish)

(at the termination of the study)

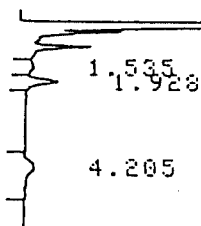
Std. 0.50 μ g/ml

START



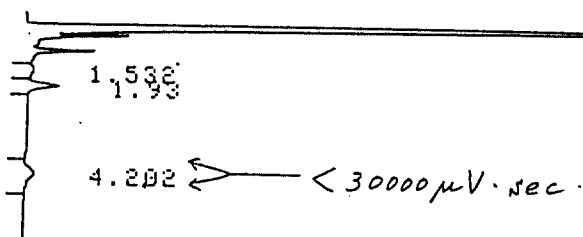
PKNO	TIME	AREA	MK	IDNO	CONC
1	3.025	19079			2.3534
2	3.413	12804	V		1.5793
3	4.01	639675	SV		78.9034
4	4.725	139148	V		17.1639
TOTAL		810706			100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	1.535	8144			10.3422
2	1.928	35586			45.1906
3	4.205	35016			44.4672
TOTAL		78746			100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	1.532	7659			11.3524
2	1.93	33256			49.2902
3	4.202	26554			39.3574
TOTAL		67470			100

Table 1 Recovery test from water

W		A	B	C	D	E	R	
amount of water		added amount	peak area		conc. in final volume	measured amount	recovery	Fig. No.
mL		μg	$\mu\text{V} \cdot \text{sec}$		$\mu\text{g/mL}$	μg	%	
			Sample	Std.				
1	1000	10	723856	762903	0.474	9.48	94.8	8
2	1000	10	720337	762903	0.472	9.44	94.4	8

Average : 94.6 %

Concentration of standard solution(Cstd.)
 Final volume (V)

: 0.56 mg/mL
 :20 mL

Calculation

$$D = \text{Cstd} \times B/C \quad ; \quad E = D \times V \quad ; \quad R = E/A \times 100$$

Table 2 Recovery test from fish

W		A	B	C	D	E	R	
Fish weight		added amount	peak area		conc. in final volume	measured amount	recovery	Fig. No.
g		μg	$\mu\text{V} \cdot \text{sec}$		$\mu\text{g/mL}$	μg	%	
			sample	Std.				
1	36.92	10	803266	813996	0.493	9.86	98.6	9
2	34.25	10	813149	813996	0.499	9.98	99.8	9

Concentration of standard solution(CStd.)
 Final volume (V)

Average : 99.2 %
 : 0.5 $\mu\text{g/mL}$
 :20.0mL

Calculation

$$D = \text{Cstd} \times B/C \quad ; \quad E = D \times V \quad ; \quad R = E/A \times 100$$

Table 3 Acute toxicity test (48 hrs)

D - 1 $\mu\text{g/mL}$	number of test fish	dead fish	mortality %
0 (control)	10	0	0
100	10	0	0

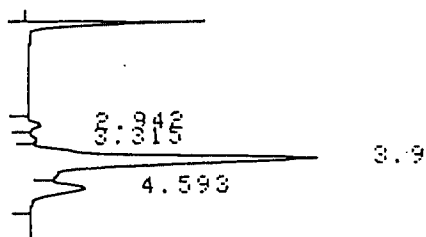
000581

48hr-LC₅₀ = >100 $\mu\text{g/mL}$

Gas chromatograms (Recovery test from water)

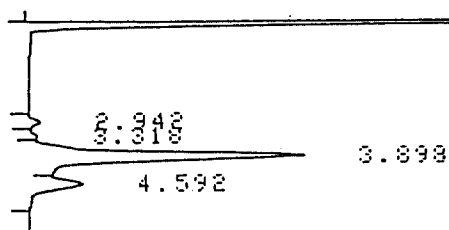
Std. 0.50 µg/ml

START



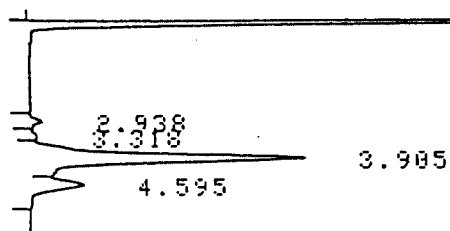
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.942	19610			2.4623
2	3.315	13901	V		1.7455
3	3.9	625788	V		78.5757
4	4.593	137115	V		17.2165
TOTAL					100

START



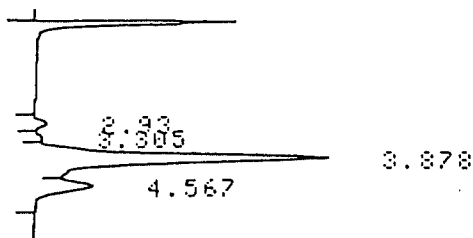
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.942	16717			2.2177
2	3.318	13213	V		1.7529
3	3.898	593258	V		78.7037
4	4.592	130598	V		17.3256
TOTAL					100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.938	16595			2.215
2	3.318	12282	V		1.6393
3	3.905	592950	V		79.1429
4	4.595	127387	V		17.0027
TOTAL					100

START

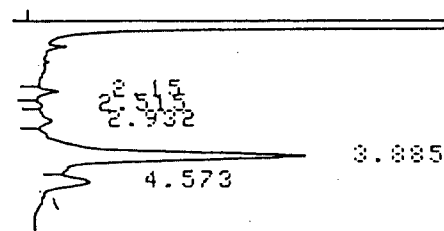


Std. 0.50 µg/ml

PKNO	TIME	AREA	MK	IDNO	CONC
1	2.93	20325			2.3961
2	3.305	13939	V		1.6432
3	3.878	667862	V		78.7332
4	4.567	146134	V		17.2275

TOTAL		848260			100

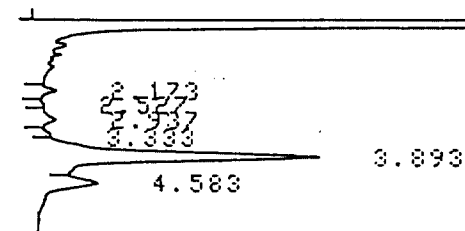
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.15	20166			2.3366
2	2.515	6354			0.7362
3	2.932	33246	V		3.8523
4	3.885	650164	V		75.3348
5	4.573	153102	V		17.74

TOTAL		863032			100

START

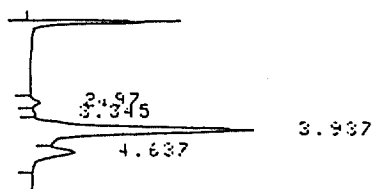


PKNO	TIME	AREA	MK	IDNO	CONC
1	2.173	15969			1.8185
2	2.937	32435	V		3.6937
3	3.333	16576	V		1.8876
4	3.893	657675	V		74.8951
5	4.583	155474	V		17.7051

TOTAL		878128			100

Fig.10 Gas chromatograms (Stability in water)

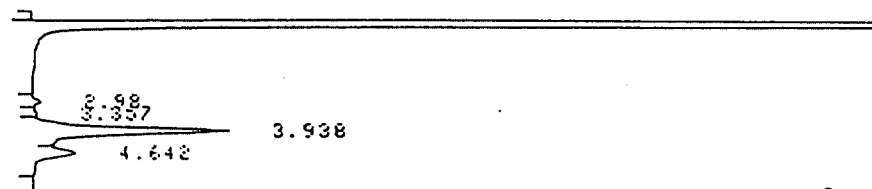
START



Std. 0.50 μ g/ml

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	2.97	18144			2.2954	
2	3.345	11585	V		1.4656	
3	3.937	624824	V		79.0451	
4	4.637	135911	V		17.1938	
TOTAL		790465			100	

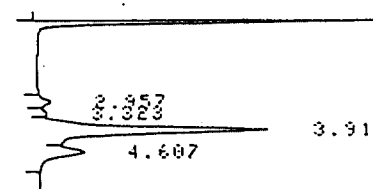
START



day 0

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	2.98	17187			2.405	
2	3.357	11017	V		1.5416	
3	3.938	551147	V		77.1226	
4	4.642	135287	V		18.9309	
TOTAL		714637			100	

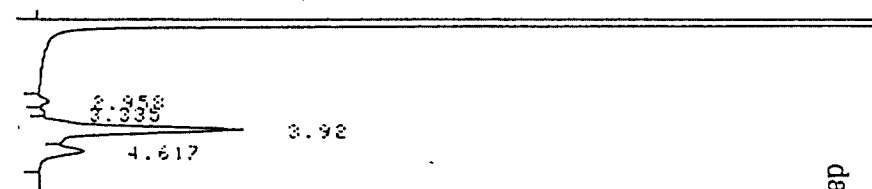
START



Std. 0.50 μ g/ml

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	2.957	20717			2.5946	
2	3.323	13763	V		1.7237	
3	3.91	627723	V		78.6225	
4	4.607	136211	V		17.0592	
TOTAL		798464			100	

START



day 2

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	2.958	17479			2.3471	
2	3.335	11633	V		1.5621	
3	3.92	576722	V		77.4419	
4	4.617	138882	V		18.6489	
TOTAL		744716			100	

000584

000585

Fig.11 Stability in water

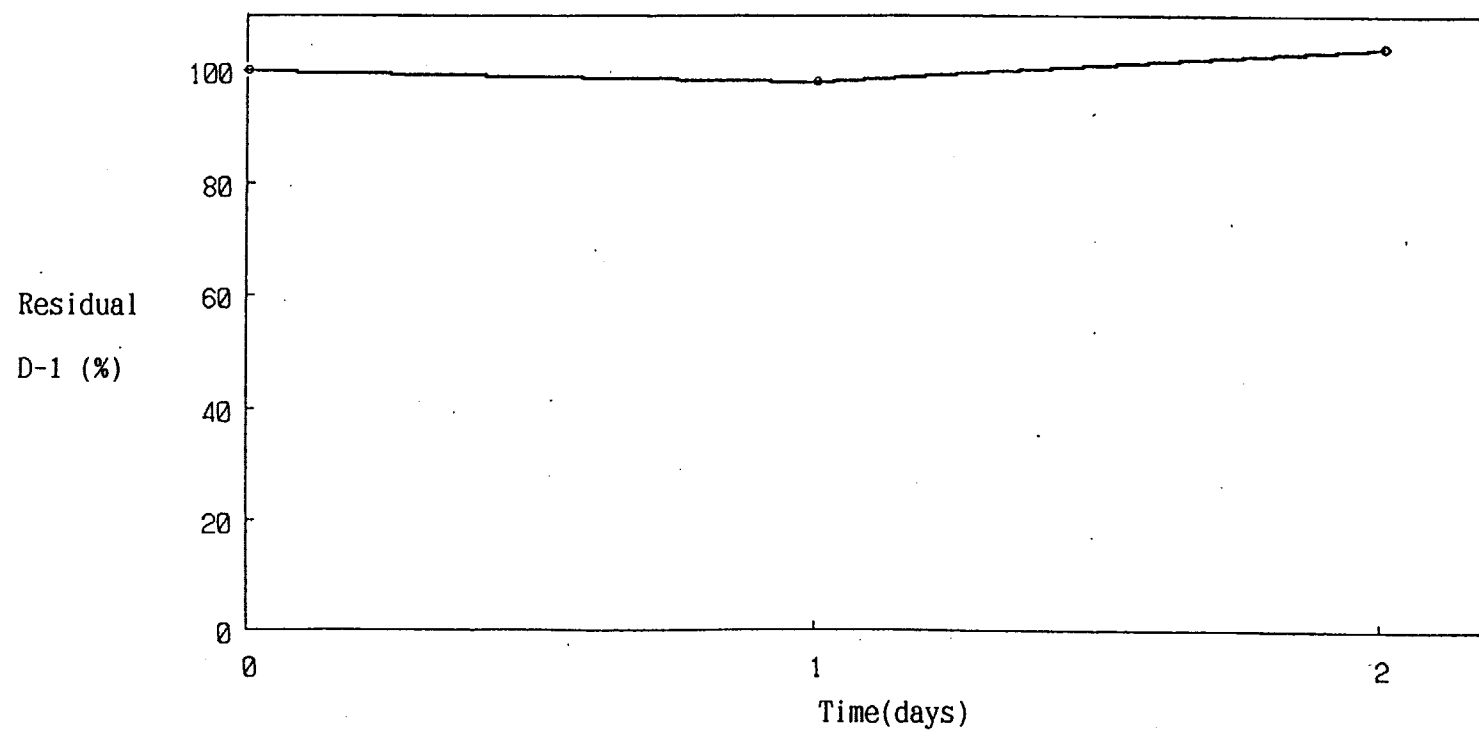


Fig. 12 Stability in feed solution in bioaccumulation test

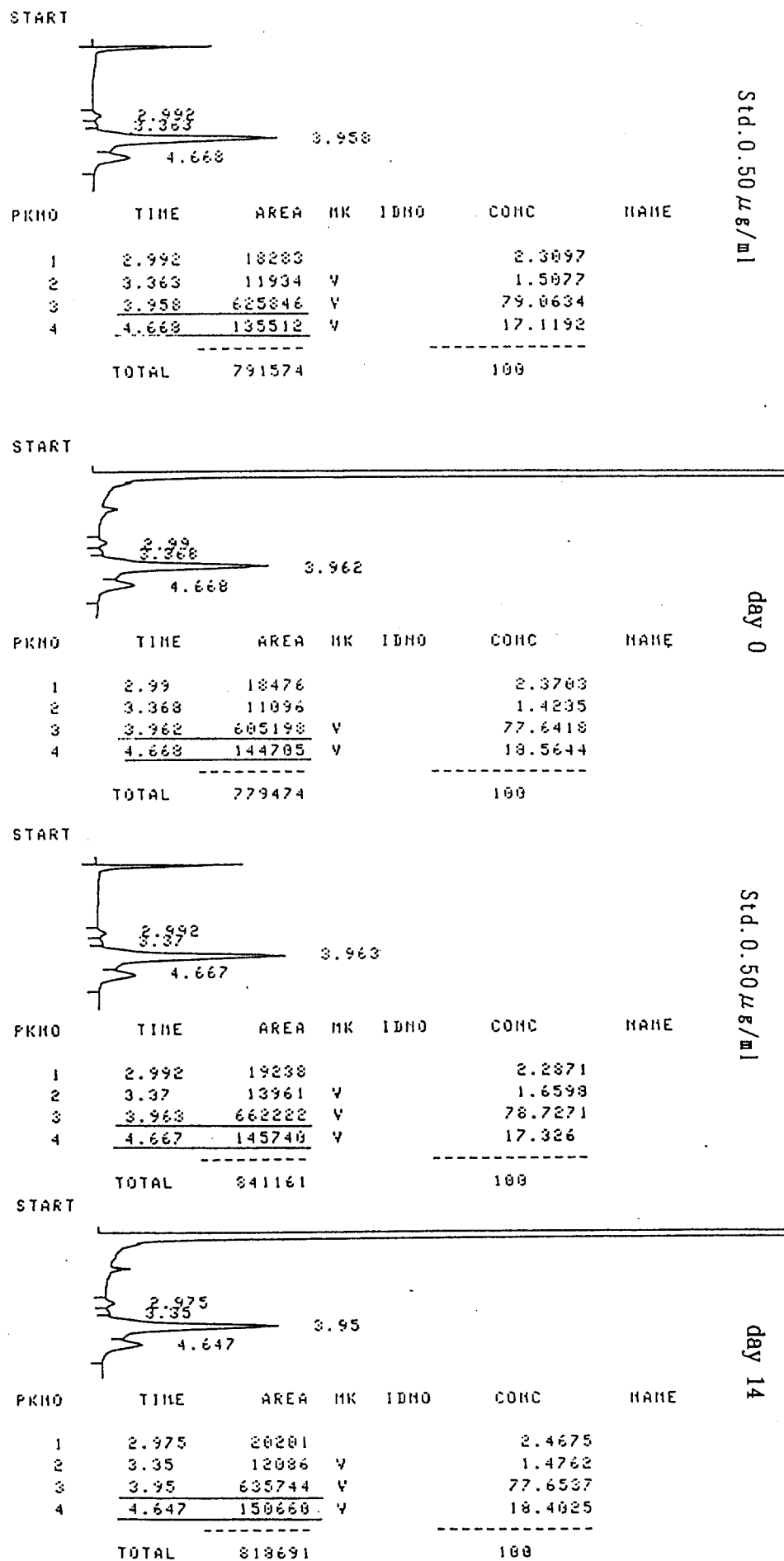
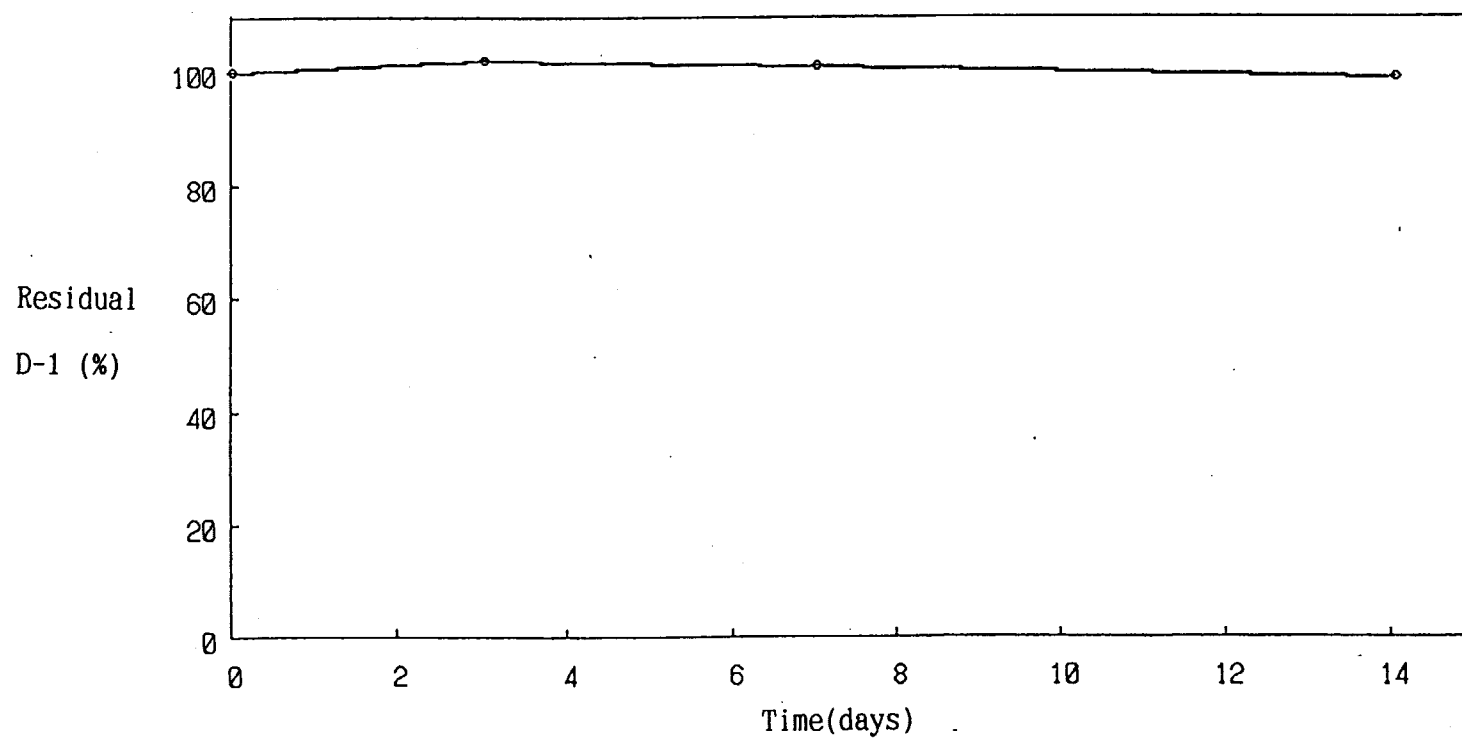


Fig.13 Stability in feed solution



000587

Table 4 Analysis of D-1 in test water (High exposure level)

			i	A	B	C	E	
sampling date	exposure period			peak area $\mu V \cdot sec$		conc. in water	mean conc.	Fig. No. *
	day	week		Sample	Std.	$\mu g/mL$	$\mu g/mL$	
5.26, '92	0	0	1	691668	761885	0.096	0.096	1-1
29	3		2	551911	768251	0.076	0.086	2
6. 2	7	1	3	665450	759199	0.093	0.088	3
5	10		4	684891	764544	0.095	0.090	4
9	14	2	5	655152	752597	0.092	0.090	5
12	17		6	742762	794882	0.099	0.092	6
16	21	3	7	600994	794596	0.080	0.090	7
19	24		8	705686	824875	0.090	0.090	8
23	28	4	9	716171	792816	0.095	0.091	9
26	31		10	687002	809639	0.090	0.091	10
30	35	5	11	776203	831148	0.099	0.091	11
7. 3	38		12	709193	787890	0.095	0.092	12
7	42	6	13	674771	771065	0.093	0.092	13
10	45		14	742351	808504	0.097	0.092	14
14	49	7	15	701150	791505	0.094	0.092	15
17	52		16	544487	775383	0.074	0.091	16
21	56	8	17	733512	779453	0.099	0.092	17

(* Appendix-2)

Concentration of standard solution
 Final volume
 Sample volume
 Recovery

Cstd : 0.5 $\mu g/mL$
 V : 20 mL
 W : 100 mL
 R : 94.6 %

Calculation

$$C = Cstd \times A / B \times F \quad ; \quad (F = V / W / (R / 100))$$

000588 $E = \sum C_i / i$ (i: number of analysis)

Mean concentration in water $\pm$ standard deviation : $0.092 \pm 0.0077 \mu g/mL$

Table 5 Analysis of D-1 in test water (Low exposure level)

			i	A	B	C	E	
sampling date	exposure period			peak area $\mu V \cdot sec$		conc. in water	mean conc.	Fig. No. *
	day	week		Sample	Std.	$\mu g/mL$	$\mu g/mL$	
5.26, '92	0	0	1	738542	761885	0.0102	0.0102	1-1
29	3		2	359497	768251	0.0049	0.0076	2
6. 2	7	1	3	515797	759199	0.0072	0.0047	3
5	10		4	599070	764544	0.0083	0.0077	4
9	14	2	5	570779	752597	0.0080	0.0077	5
12	17		6	572050	794882	0.0076	0.0077	6
16	21	3	7	659682	794596	0.0088	0.0079	7
19	24		8	572309	824875	0.0073	0.0078	8
23	28	4	9	676137	792816	0.0090	0.0079	9
26	31		10	689946	809639	0.0090	0.0080	10
30	35	5	11	701085	831148	0.0089	0.0081	11
7. 3	38		12	636904	787890	0.0085	0.0081	12
7	42	6	13	615794	771065	0.0084	0.0082	13
10	45		14	662294	808504	0.0087	0.0082	14
14	49	7	15	606422	791505	0.0081	0.0082	15
17	52		16	591058	775383	0.0081	0.0082	16
21	56	8	17	600028	779453	0.0081	0.0082	17

(* Appendix-2)

Concentration of standard solution

Cstd : 0.5 $\mu g/mL$

Final volume

V : 20 mL

Sample volume

W : 1000 mL

Recovery

R : 94.6 %

Calculation

$$C = Cstd \times A / B \times F \quad (F = V / W / (R / 100))$$

$$000589E = \sum C_i / i \quad (i: \text{number of analysis})$$

Mean concentration in water $\pm$ standard deviation : $0.0082 \pm 0.0011 ng/mL$

Fig.14 Concentrations of test substance in water High exposure level

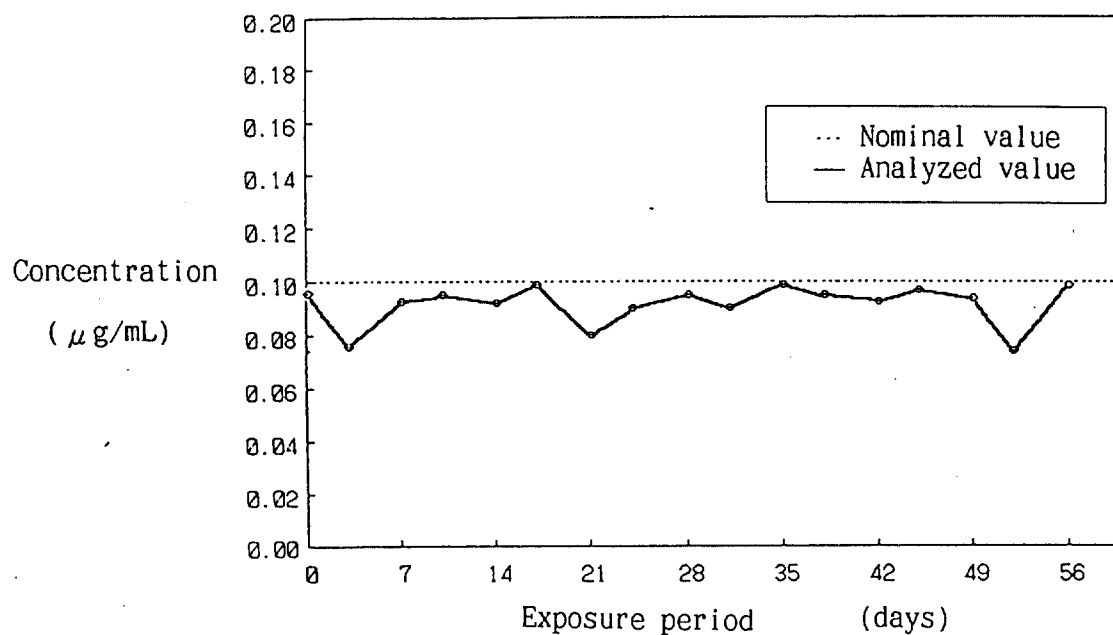
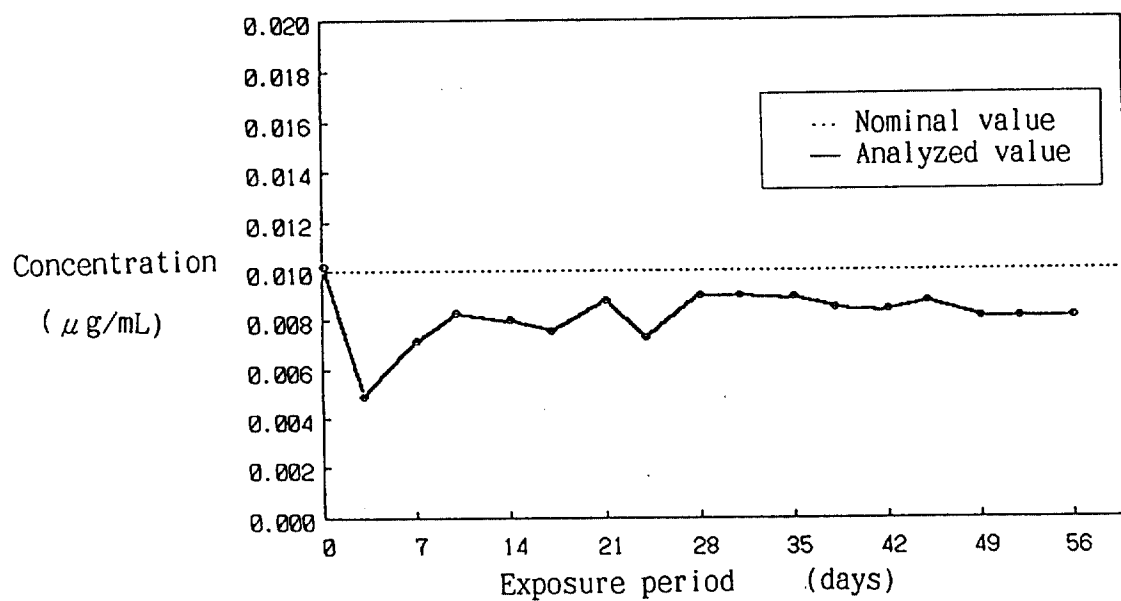


Fig.15 Concentrations of test substance in water Low exposure level



000590

Table 6 Analysis of D-1 in test fish (High exposure level)

		W	A	B	C	D	E	F	
exposure period		fish weight g	peak area $\mu V \cdot \text{sec}$		conc. in final volume	conc. in fish measured	mean conc. in water	BCF	Fig. No.
week			Sample	Std.	$\mu\text{g/mL}$	$\mu\text{g/g}$	$\mu\text{g/mL}$		
2	1	34.14	90324	839568	0.054	0.032	0.090	0.4	18-1
	2	34.80	94243	796908	0.059	0.034	0.090	0.4	18-1
4	1	37.18	168720	807176	0.105	0.057	0.091	0.6	18-2
	2	39.96	192918	790270	0.122	0.062	0.091	0.7	18-2
6	1	36.95	161768	839289	0.096	0.052	0.092	0.6	18-3
	2	37.47	113668	804605	0.071	0.038	0.092	0.4	18-3
8	1	35.54	132230	879847	0.075	0.043	0.092	0.5	18-4
	2	36.77	145406	885954	0.082	0.045	0.092	0.5	18-4

Concentration of standard solution(Std.)

Final volume

Recovery

Cstd : $0.5 \mu\text{g/mL}$

V : 20 mL

R : 99.2 %

Calculation

$$C = \text{Cstd} \times A / B \quad ; \quad D = C \times V / W \times 100 / R$$

$$F = D / E$$

Table 7 Analysis of D-1 in test fish (Low exposure level)

		W	A	B	C	D	E	F	
exposure period		fish weight g	peak area $\mu V \cdot sec$		conc. in final volume $\mu g/mL$	conc. in fish measured $\mu g/g$	mean conc. in water $\mu g/mL$	BCF	Fig. No.
week			Sample	Std.					
2	1	34.73	72837	839568	0.043	0.025	0.0077	3	18-5
	2	35.61	61435	796908	0.039	0.022	0.0077	3	18-5
4	1	34.23	93655	807176	0.058	0.034	0.0079	4	18-6
	2	35.70	63936	790270	0.040	0.023	0.0079	3	18-6
6	1	37.23	38016	804605	0.024	0.013	0.0082	2	18-7
	2	40.00	53795	798557	0.034	0.017	0.0082	2	18-7
8	1	35.27	45555	851984	0.027	0.015	0.0082	2	18-8
	2	35.73	46433	864383	0.027	0.015	0.0082	2	18-8

Concentration of standard solution(Std.)	Cstd	: 0.5 μ g/mL
Final volume	V	: 20 mL
Recovery	R	: 99.2 %

Calculation

$$C = C_{std} \times A / B \quad ; \quad D = C \times V / W \times 100 / R$$

$$F = D \diagup E$$

Fig.16 Bioconcentration factors (BCFs) of D-1 High exposure level

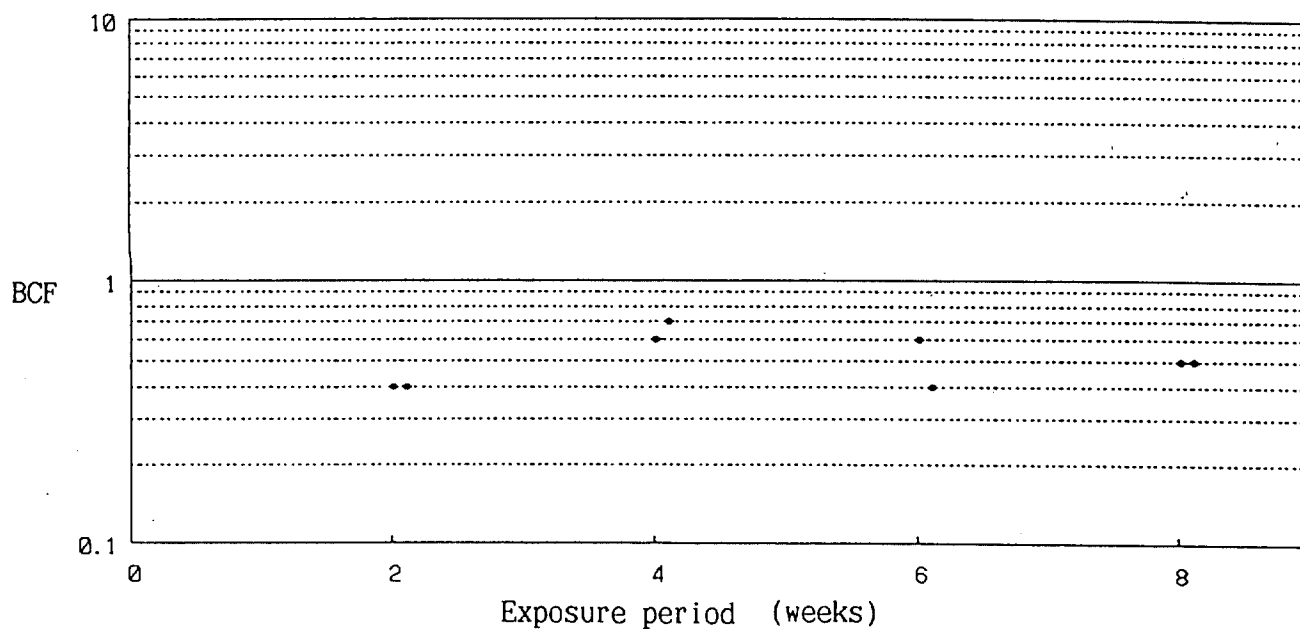


Fig.17 Bioconcentration factors (BCFs) of D-1 Low exposure level

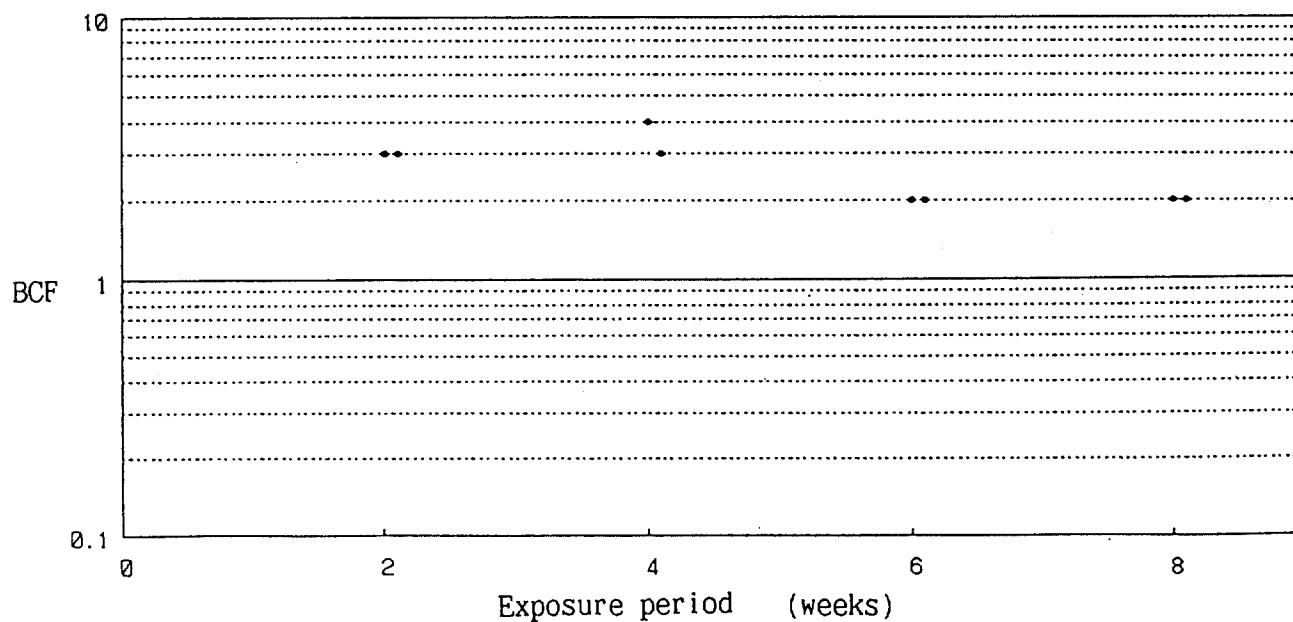
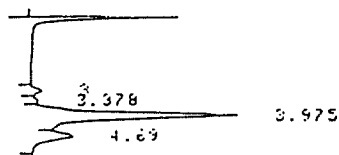


Fig. 18-1 Gas chromatograms (Analysis of test fish)

— High exposure level —

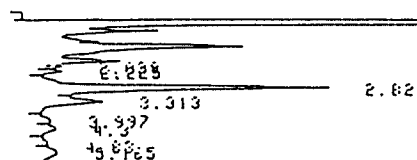
Std. 0.50 μ g/ml

START



PKNO	TIME	AREA	HK	IDHO	CONC	NAME
1	3	21899			2.4988	
2	3.378	14921	V		1.7026	
3	3.975	688849	V		78.6009	
4	4.69	156719	V		17.1977	
TOTAL					100	

START

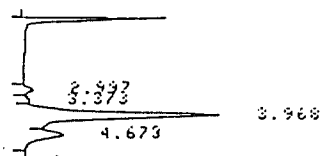


PKNO	TIME	AREA	HK	IDHO	CONC	NAME
1	2.225	16851			1.746	
2	2.82	617839			64.0153	
3	3.313	177252	V		18.3654	
4	3.997	27456			2.8448	
5	4.3	43991	V		4.558	
6	4.93	18877	V		1.9559	
7	5.165	62874	V		6.5145	
TOTAL					100	

Week 2
No. 1

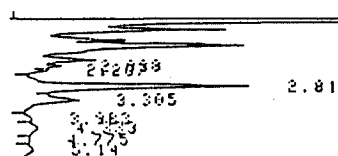
Std. 0.50 μ g/ml

START



PKNO	TIME	AREA	HK	IDHO	CONC	NAME
1	2.997	18595			2.2492	
2	3.373	11236	V		1.3591	
3	3.968	655877	V		79.3329	
4	4.673	141031	V		17.0587	
TOTAL					100	

START



PKNO	TIME	AREA	HK	IDHO	CONC	NAME
1	2.038	6617			0.8577	
2	2.207	9363			1.2136	
3	2.81	469854			60.9007	
4	3.305	156703	V		20.3113	
5	3.983	20688			2.6807	
6	4.333	55854	V		7.2396	
7	4.775	17707	V		2.2951	
8	5.14	34727	V		4.5012	
TOTAL					100	

Week 2
No. 2

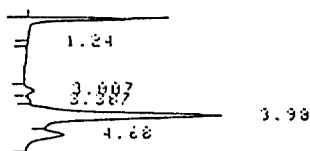
000594

Fig. 18-2 Gas chromatograms (Analysis of test fish)

— High exposure level —

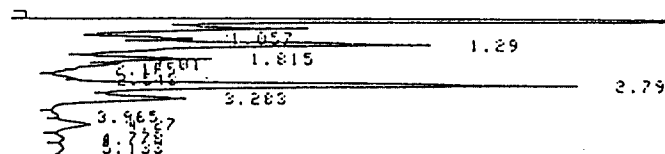
Std. 0.50 μ g/ml

START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	3.007	20342			2.4131	
2	3.387	13694	V		1.6279	
3	3.98	662318	V		78.7338	
4	4.68	144858	V		17.2201	
TOTAL		841211			100	

START

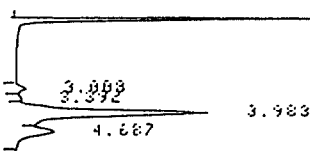


PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.057	30556			1.1685	
2	1.29	626677			23.9654	
3	1.815	194391			7.4339	
4	2.01	49740	V		1.9021	
5	2.175	5320			0.2034	
6	2.548	36284			1.3876	
7	2.79	1099665	V		42.0534	
8	3.283	359586	V		13.7513	
9	3.965	18810			0.7193	
10	4.27	114698	V		4.3863	
11	4.778	35212	V		1.3466	
12	5.133	43937	V		1.6822	
TOTAL		2614927			100	

week 4
No. 1

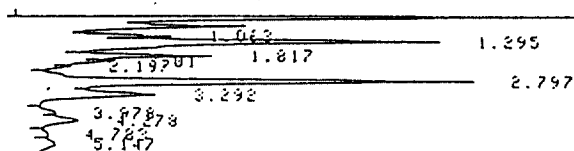
Std. 0.50 μ g/ml

START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	3.008	18556			2.257	
2	3.392	13323	V		1.6205	
3	3.983	647973	V		78.8145	
4	4.687	142297	V		17.303	
TOTAL		822150			100	

START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.063	33016			1.3371	
2	1.295	676331			27.3909	
3	1.817	197896			8.0147	
4	2.01	50849	V		2.0593	
5	2.197	12586			0.5097	
6	2.797	930669			37.6914	
7	3.292	306954	V		12.4314	
8	3.978	42276			1.7122	
9	4.278	119995	V		4.8597	
10	4.783	30647	V		1.2412	
11	5.147	67961	V		2.7524	
TOTAL		2469181			100	

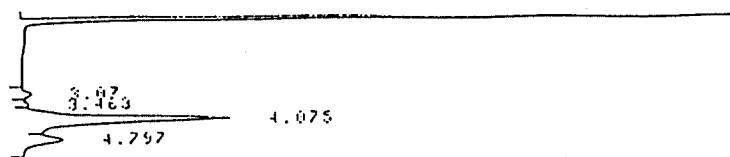
week 4
No. 2

000595

Fig. 18-3 Gas chromatograms (Analysis of test fish)

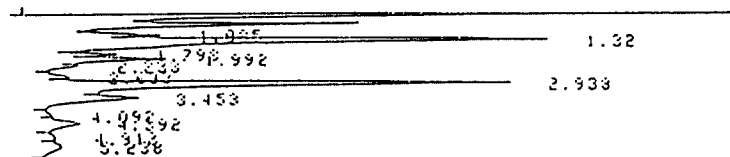
— High exposure level —

START



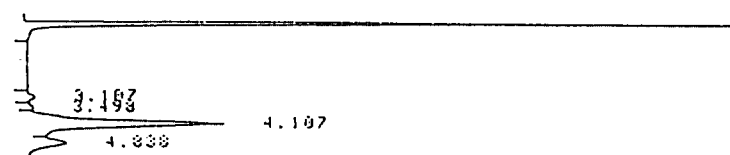
PKNO	TIME	AREA	HK	IDHO	CONC	NAME
1	3.07	20996			2.4017	
2	3.463	13919	V		1.5921	
3	4.075	689292	V		78.8595	
4	4.797	149897	V		17.1467	
TOTAL					100	

START



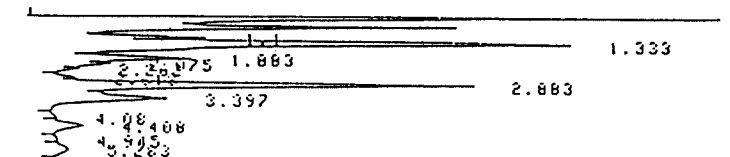
PKNO	TIME	AREA	HK	IDHO	CONC	NAME
1	1.085	51438			1.8378	
2	1.32	960403	V		34.3138	
3	1.798	58126			2.0767	
4	1.992	164295	V		5.87	
5	2.233	34433	V		1.2302	
6	2.433	34848			1.2451	
7	2.938	1027735	V		36.7195	
8	3.453	236231	V		8.4402	
9	4.092	15458			0.5523	
10	4.392	111682	V		3.9902	
11	4.912	34628	V		1.2372	
12	5.238	69606	V		2.4869	
TOTAL					100	

START



PKNO	TIME	AREA	HK	IDHO	CONC	NAME
1	3.107	17820			2.1328	
2	3.493	13081	V		1.5656	
3	4.107	659094	V		78.8857	
4	4.838	145511	V		17.4159	
TOTAL					100	

START



PKNO	TIME	AREA	HK	IDHO	CONC	NAME
1	1.1	103145			3.7683	
2	1.333	1029239	V		37.604	
3	1.883	171012			6.2478	
4	2.075	39547	V		1.4448	
5	2.262	9979			0.3646	
6	2.618	31831			1.1647	
7	2.883	862603	V		31.5143	
8	3.397	323153	V		11.3061	
9	4.08	10321			0.3771	
10	4.408	89255	V		3.2603	
11	4.945	14092	V		0.5148	
12	5.283	52906	V		1.9327	
TOTAL					100	

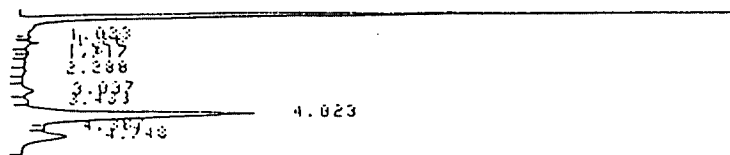
Std. 0.50 μ g/ml

week 6
No. 1

Std. 0.50 μ g/ml

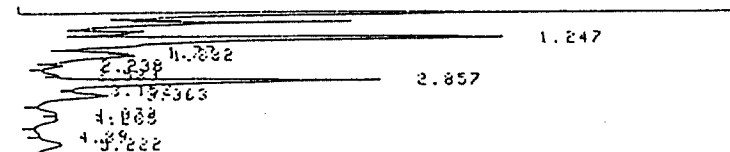
week 6
No. 2

START



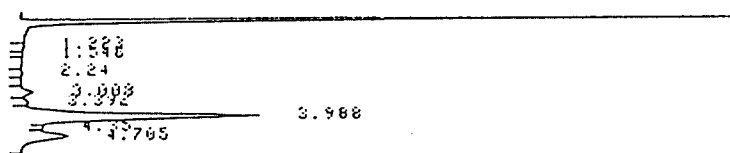
PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.26	10192			1.0889	
2	1.6	5476			0.5851	
3	3.037	25349			2.7083	
4	3.433	15119	V		1.6154	
5	4.023	724795	SV		77.4367	
6	4.748	155052	V		16.5656	
TOTAL		935983			100	

START



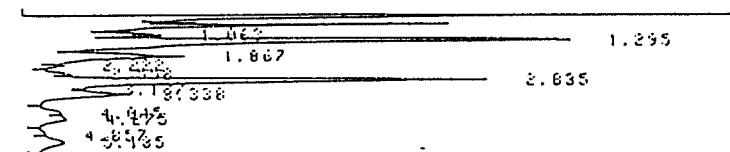
PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.247	876064			39.2078	
2	1.77	73667			3.2969	
3	1.882	127216	V		5.6935	
4	2.238	18456			0.826	
5	2.583	29488			1.3197	
6	2.857	708399	SV		31.704	
7	3.363	171384	V		7.6702	
8	4.023	55055			2.464	
9	4.285	62817	V		2.8113	
10	4.82	14353			0.6426	
11	5.222	97511	V		4.3641	
TOTAL		2234414			100	

START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	3.008	23440			2.5347	
2	3.392	15362	V		1.6612	
3	3.988	728895	SV		78.8203	
4	4.705	157059	V		16.9839	
TOTAL		924756			100	

START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.063	18429			0.7088	
2	1.295	962267			36.5932	
3	1.867	253568			9.6427	
4	2.222	12999			0.4943	
5	2.558	43157			1.6412	
6	2.835	911925	SV		34.6783	
7	3.338	193849	V		7.3717	
8	4.045	51793			1.9698	
9	4.275	78581	V		2.9883	
10	4.857	15027			0.5714	
11	5.185	88035	V		3.3478	
TOTAL		2629632			100	

Std. 0.50 μ g/mlweek 8
No. 1Fig. 18-4 Gas Chromatograms (Analysis of test fish)
— High exposure level —Std. 0.50 μ g/mlweek 8
No. 2

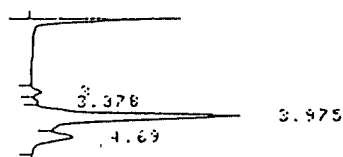
000597

Fig. 18-5 Gas chromatograms (Analysis of test fish)

— Low exposure level —

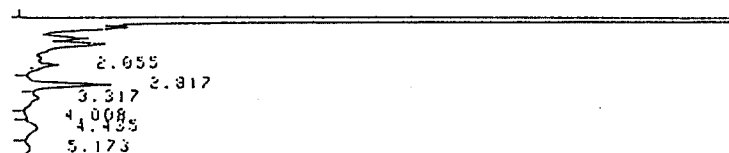
Std. 0.50 μ g/ml

START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	3	21899			2.4983	
2	3.378	14921	V		1.7026	
3	3.975	688849	V		78.6009	
4	4.69	150713	V		17.1977	
TOTAL		876388			100	

START

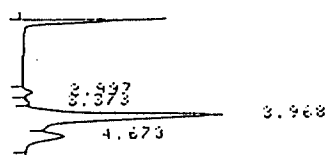


PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	2.055	9129			2.7017	
2	2.817	174936			51.7735	
3	3.317	53912	V		15.9557	
4	4.008	8412			2.4896	
5	4.435	64425	V		19.0671	
6	5.173	27073	V		8.0125	
TOTAL		337888			100	

week 2
No. 1

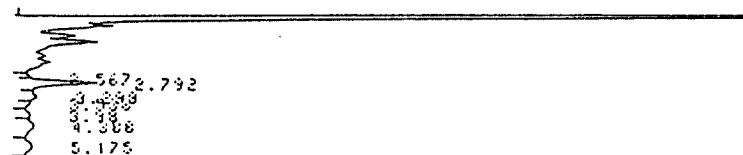
Std. 0.50 μ g/ml

START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	2.997	18595			2.2492	
2	3.373	11236	V		1.3591	
3	3.968	655877	V		79.3329	
4	4.673	141031	V		17.0587	
TOTAL		826740			100	

START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	2.567	7704			2.5412	
2	2.792	150318	V		49.5867	
3	3.298	37928	V		12.5117	
4	3.538	9787	V		3.2235	
5	3.98	15856			5.2305	
6	4.388	45579	V		15.0355	
7	5.175	35970	V		11.8659	
TOTAL		303141			100	

week 2
No. 2

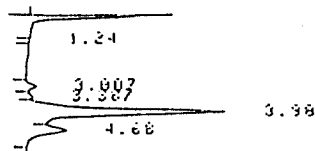
000598

Fig. 18-6 Gas chromatograms (Analysis of test fish)

— Low exposure level —

Std. 0.50 μ g/ml

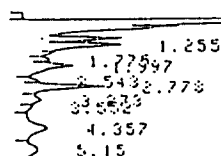
START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	3.007	20342			2.4181	
2	3.387	13694	V		1.6279	
3	3.98	662318	V		78.7338	
4	4.68	144358	V		17.2201	
TOTAL		841211			100	

week 4
No. 1

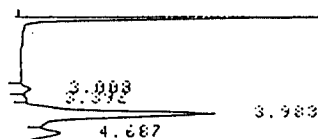
START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.255	95976			16.8791	
2	1.775	18292			3.2169	
3	1.997	84906	V		14.9322	
4	2.543	14179			2.4936	
5	2.778	154964	V		27.2532	
6	3.273	44587	V		7.3413	
7	3.552	9302	V		1.6359	
8	4.357	93655			16.471	
9	5.15	52749	V		9.2768	
TOTAL		568608			100	

Std. 0.50 μ g/ml

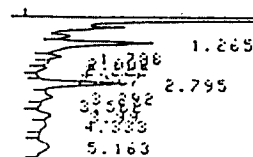
START



PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	3.008	18556			2.257	
2	3.392	13323	V		1.6205	
3	3.983	647973	V		78.8145	
4	4.687	142297	V		17.308	
TOTAL		822159			100	

week 4
No. 2

START



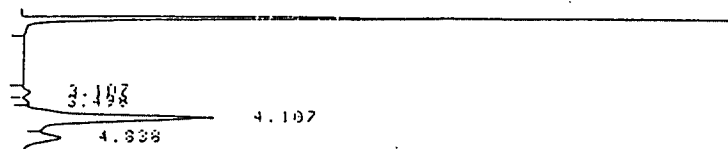
PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.265	192021			32.3603	
2	1.788	26276			4.4282	
3	2.002	10004	V		1.636	
4	2.222	9218			1.5535	
5	2.567	6386			1.0761	
6	2.795	179136	V		30.1889	
7	3.292	52169	V		8.7918	
8	3.562	8808	V		1.4843	
9	3.99	37967			6.3983	
10	4.323	25369	V		4.3764	
11	5.163	45430			7.6561	
TOTAL		593382			100	

000 599

Fig. 18-7 Gas chromatograms (Analysis of test fish)

— Low exposure level —

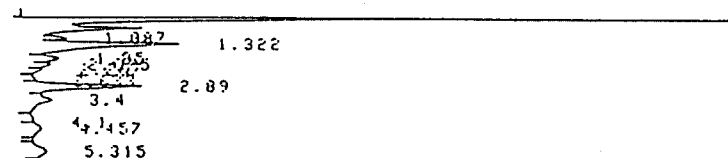
START



Std. 0.50 μ g/ml

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	3.107	17829			2.1328	
2	3.498	13081	V		1.5656	
3	4.107	652054	V		78.8857	
4	4.838	145511	V		17.4159	
TOTAL		835505			100	

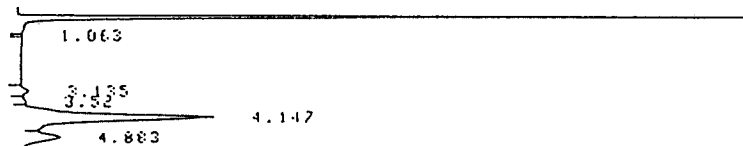
START



week 6
No. 1

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.087	17334			2.3713	
2	1.322	286125	V		39.1416	
3	1.85	33241			4.5473	
4	2.075	15858	V		2.1694	
5	2.633	6451			0.8825	
6	2.89	225211	V		30.8086	
7	3.4	68115	V		9.3181	
8	4.1	6259			0.952	
9	4.457	31052	V		4.2486	
10	5.315	40648			5.5606	
TOTAL		731060			100	

START



Std. 0.50 μ g/ml

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	3.135	16317			1.9734	
2	3.52	11983	V		1.4492	
3	4.147	654873	V		79.2803	
4	4.883	143684	V		17.3771	
TOTAL		826856			100	

START



week 6
No. 2

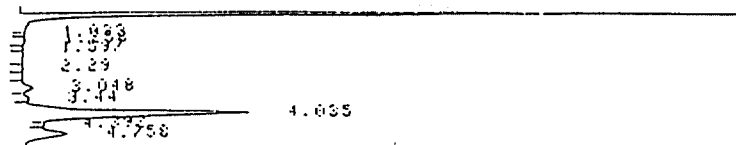
PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.078	48008			4.346	
2	1.323	296921			32.2542	
3	1.867	42823			4.6518	
4	2.092	55448	V		6.0233	
5	2.658	13616			1.4791	
6	2.917	288337	V		31.3272	
7	3.428	93474	V		10.154	
8	4.167	6861			0.7453	
9	4.51	46934	V		5.0983	
10	5.363	36094			3.9209	
TOTAL		920567			100	

000600

Fig. 18-8 Gas chromatograms (Analysis of test fish)

— Low exposure level —

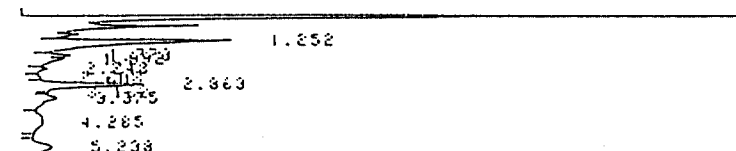
START



Std. 0.50 µg/ml

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	3.048	23924			2.6823	
2	3.44	15990	V		1.7928	
3	4.035	698118	SV		78.2733	
4	4.758	153866	V		17.2515	
TOTAL					100	

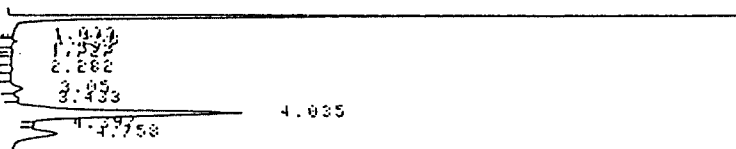
START



week 8
No. 1

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.252	357169			42.5117	
2	1.773	32220			3.835	
3	1.892	34497	V		4.1059	
4	2.248	10112			1.2036	
5	2.608	7523			0.8955	
6	2.863	225477	SV		26.8371	
7	3.375	62919	V		7.4889	
8	4.285	45555			5.4221	
9	5.238	64694			7.7002	
TOTAL					100	

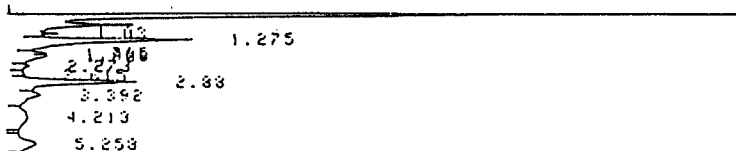
START



Std. 0.50 µg/ml

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.258	5224			0.5736	
2	3.05	24542			2.6946	
3	3.433	16652	V		1.8293	
4	4.035	708383	SV		77.8307	
5	4.758	155500	V		17.0728	
TOTAL					100	

START



week 8
No. 2

PKNO	TIME	AREA	HK	IDNO	CONC	NAME
1	1.275	292876			38.5866	
2	1.795	23210			3.0579	
3	1.908	37124	V		4.8911	
4	2.273	7754			1.0216	
5	2.88	238992	V		31.4874	
6	3.392	57528	V		7.5794	
7	4.213	46433			6.1175	
8	5.258	55093			7.2595	
TOTAL					100	

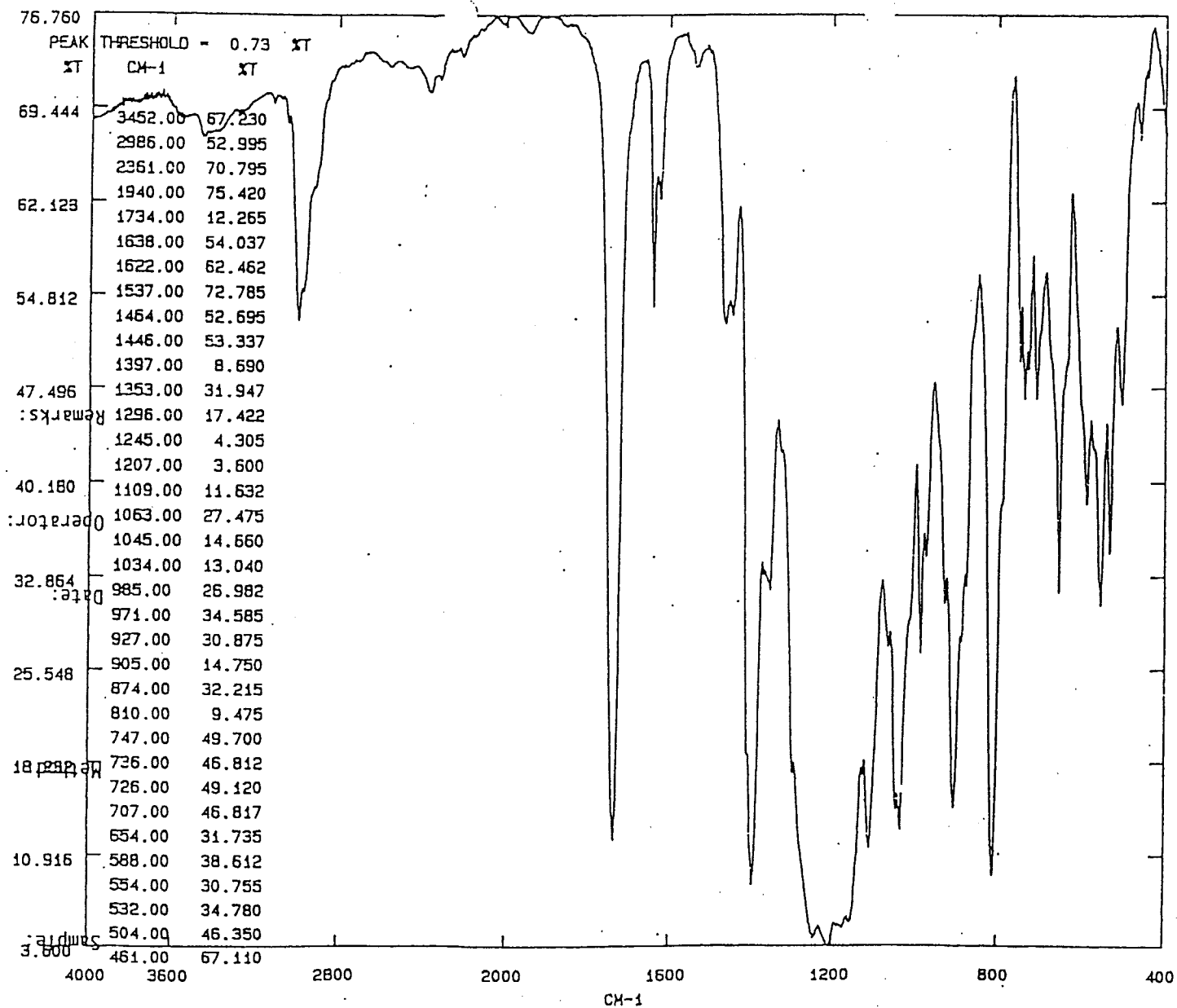
000601

A p p e n d i x - 1

Infrared spectrum of D-1 (Data provided by the sponsor)

(1 page in all)

000603



サンプル名

酢酸 "D-1"

lot 101

測定担当者

瀬川晴貴

測定年月日

H4.3.27

測定方法

70/113に

溶解させ

KBr板にて測定

A p p e n d i x - 2

Gas chromatograms (Analysis of test water)

(17 pages in all)

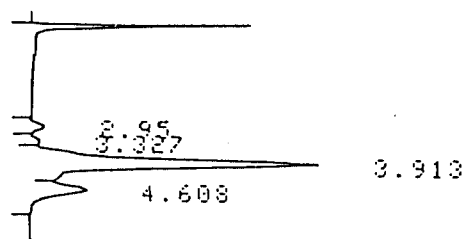
000604

Fig.1-1 Gas chromatogram (Analysis of test water)

Std. 0.50 μ g/ml

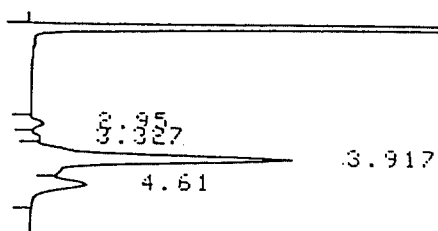
day 0

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.95	18728			2.3618
2	3.327	12342	V		1.5564
3	3.913	626699	V		79.0334
4	4.608	135186	V		17.0484
TOTAL		792955			100

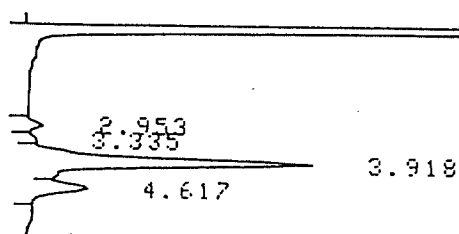
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.95	17757			2.458
2	3.327	12979	V		1.7967
3	3.917	560655	V		77.6097
4	4.61	131013	V		18.1357
TOTAL		722403			100

High exposure level

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.953	18199			2.3672
2	3.335	12061			1.5688
3	3.918	609365	V		79.2617
4	4.617	129177	V		16.8023
TOTAL		768802			100

Low exposure level

Fig. 1-2 Gas chromatogram (Analysis of test water)

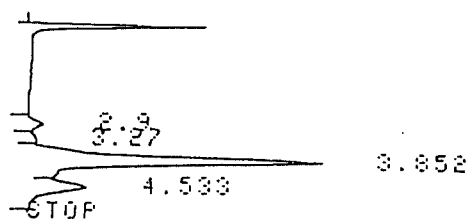
day 3

Std. 0.50 μ g/ml

High exposure level

Low exposure level

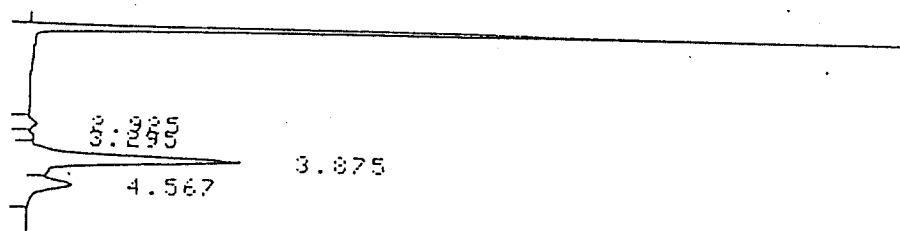
START



PKNO	TIME	AREA	HK	IDNO	CONC
1	2.9	18925			2.3642
2	3.27	13281	V		1.6591
3	3.852	631636	V		78.9095
4	4.533	136615	V		17.0671

TOTAL		800456			100

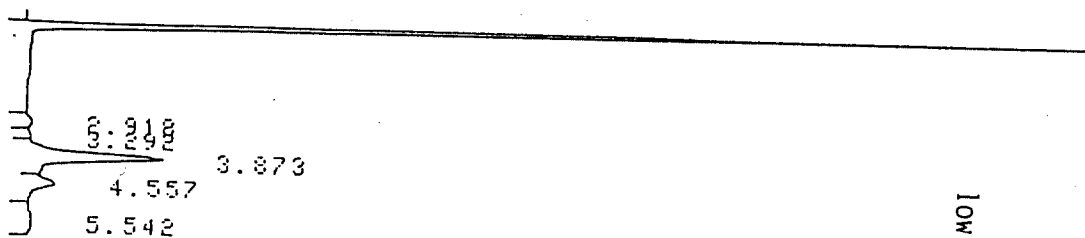
START



PKNO	TIME	AREA	HK	IDNO	CONC
1	2.925	12801			2.23
2	3.295	9318	V		1.6232
3	3.875	443612	V		77.2803
4	4.567	108299	V		18.8665

TOTAL		574030			100

START



PKNO	TIME	AREA	HK	IDNO	CONC
1	2.918	9094			2.32
2	3.292	5616			1.4326
3	3.873	291049	V		74.2463
4	4.557	68448	V		17.461
5	5.542	17797	V		4.5401

TOTAL		392004			100

000606

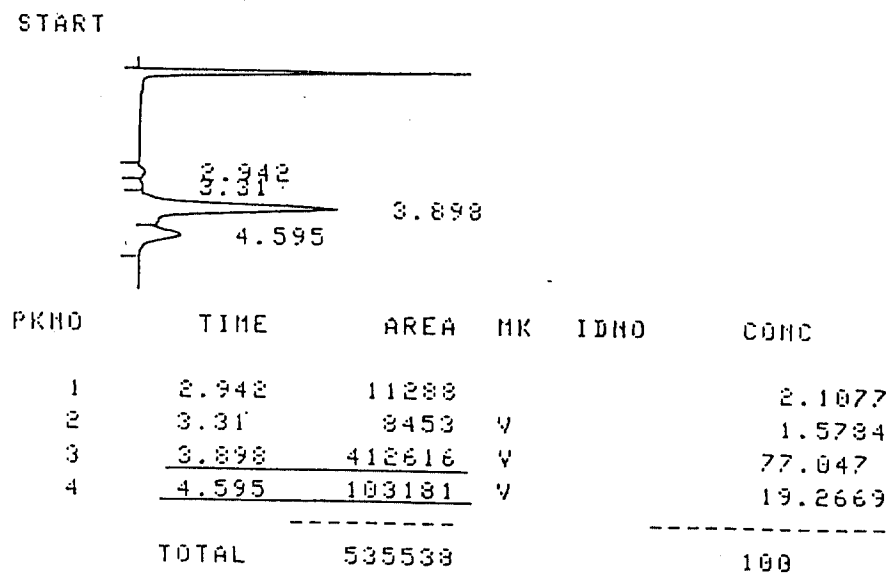
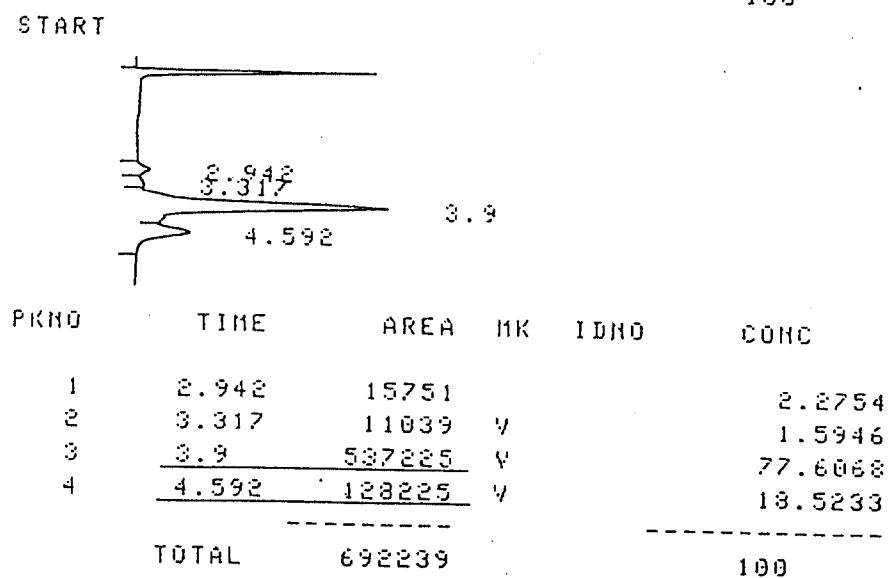
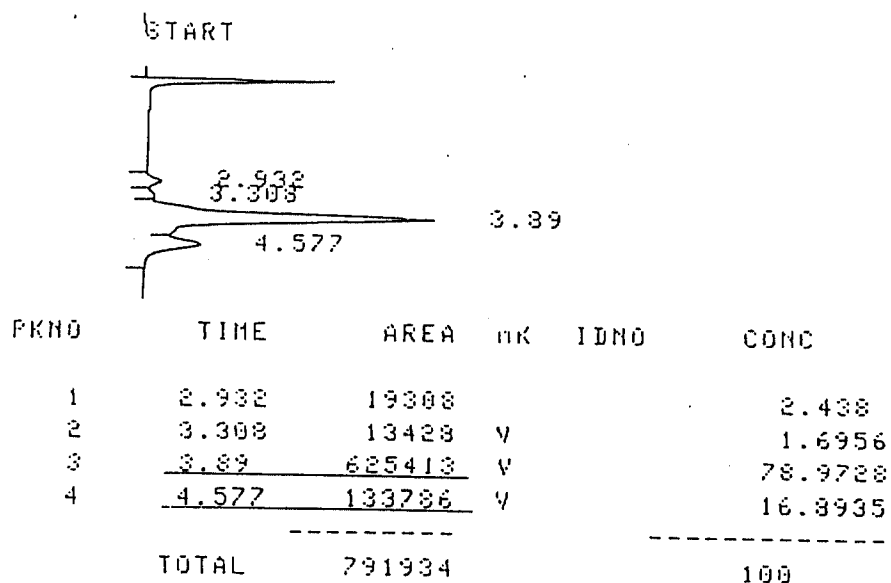
Fig. 1-3 Gas chromatogram (Analysis of test water)

day 7

Std. 0.50 μ g/ml

High exposure level

Low exposure level



000607

Fig. 1-4 Gas chromatogram (Analysis of test water)

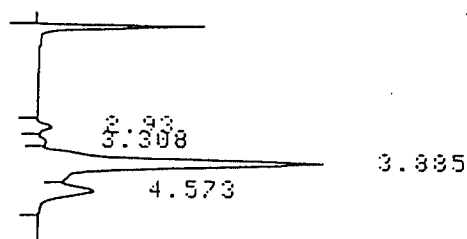
day 10

Std. 0.50 μ g/ml

High exposure level

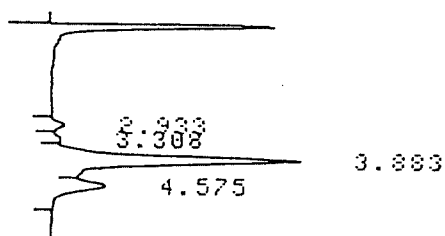
low exposure level

START



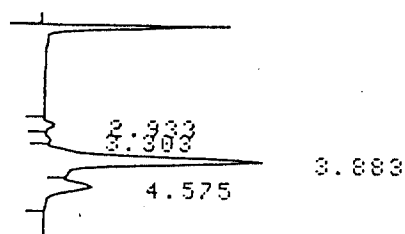
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.93	20002			2.5071
2	3.308	13275	V		1.6639
3	3.885	628548	V		78.7831
4	4.573	135996	V		17.0459
TOTAL					100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.933	18308			2.5583
2	3.308	12432	V		1.7371
3	3.883	551308	V		77.038
4	4.575	133583	V		18.6665
TOTAL					100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.933	15078			2.4128
2	3.303	10755	V		1.721
3	3.883	479951	V		76.8042
4	4.575	119119	V		19.062
TOTAL					100

000608

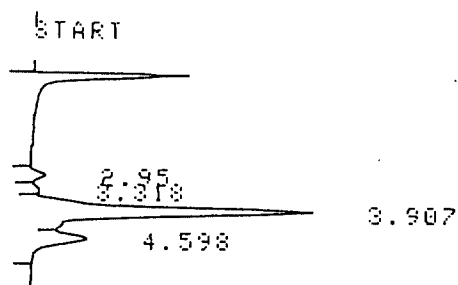
Fig. 1-5 Gas Chromatogram (Analysis of test water)

day 14

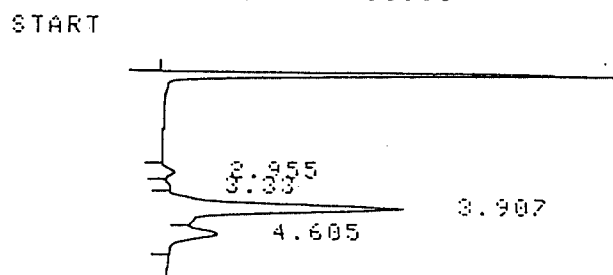
Std. 0.50 μ g/ml

High exposure level

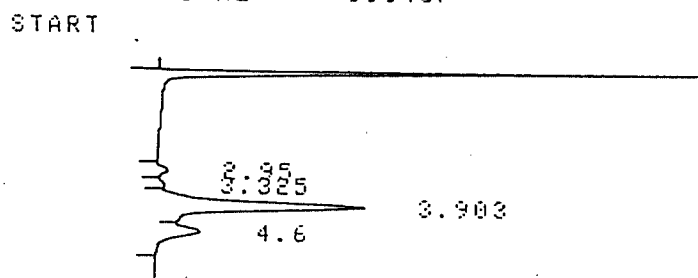
Low exposure level



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.95	19573			2.4931
2	3.318	12944	V		1.6487
3	3.907	618105	V		78.728
4	4.598	134492	V		17.1302
TOTAL					100



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.955	16633			2.4338
2	3.33	11652	V		1.7049
3	3.907	526234	V		76.9982
4	4.605	128918	V		18.8632
TOTAL					100

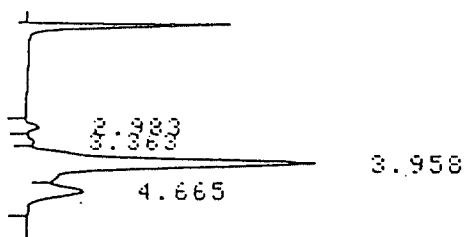


PKNO	TIME	AREA	MK	IDNO	CONC
1	2.95	14798			2.4802
2	3.325	11091	V		1.8538
3	3.903	459129	V		76.9487
4	4.6	111650	V		18.7123
TOTAL					100

000609

Fig.1-6 Gas chromatogram (Analysis of test water)

START

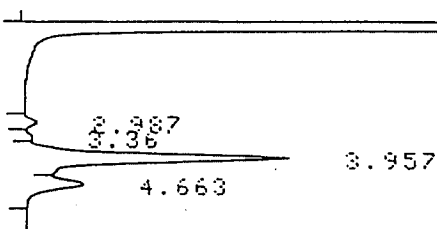


Std. 0.50 μ g/ml

day 17

PKNO	TIME	AREA	MK	IDNO	CONC
1	2.983	18752			2.2682
2	3.363	13121	V		1.587
3	3.958	651281	V		78.7756
4	4.665	143601	V		17.3692
TOTAL		826754			100

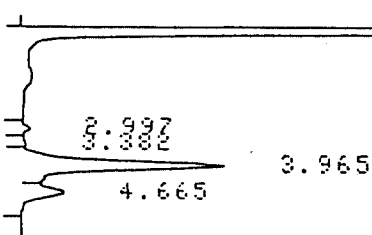
START



High exposure level

PKNO	TIME	AREA	MK	IDNO	CONC
1	2.987	17961			2.3229
2	3.36	12491	V		1.6154
3	3.957	596662	V		77.1664
4	4.663	146100	V		18.8952
TOTAL		773214			100

START



low exposure level

PKNO	TIME	AREA	MK	IDNO	CONC
1	2.997	12765			2.1484
2	3.382	9348	V		1.5733
3	3.965	459404	V		77.3195
4	4.665	112646	V		18.9588
TOTAL		594163			100

000610

Fig. 1-7 Gas chromatogram (Analysis of test water)

day 21

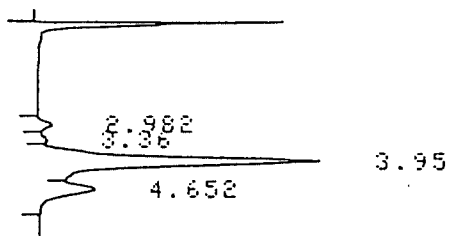
Std. 0.50 μ g/ml

High exposure level

low exposure level

START

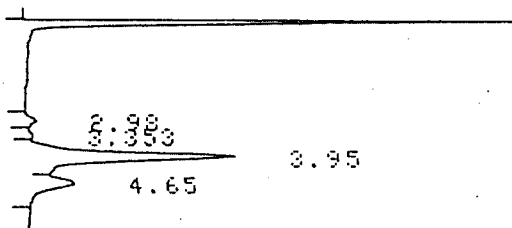
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.982	18998			2.2975
2	3.36	13311	V		1.6098
3	3.95	651927	V		78.8394
4	4.652	142669	V		17.2533

TOTAL		826905			100

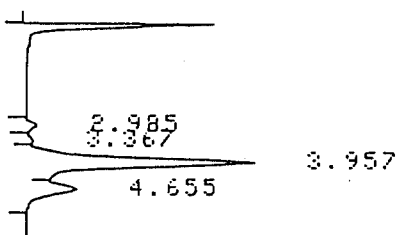
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.98	14537			2.3224
2	3.353	10426	V		1.6656
3	3.95	483481	V		77.2387
4	4.65	117513	V		18.7733

TOTAL		625957			100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.985	15518			2.2609
2	3.367	11180	V		1.6289
3	3.957	529472	V		77.1397
4	4.655	130210	V		18.9705

TOTAL		686380			100

000611

Fig. 1-8 Gas chromatogram (Analysis of test water)

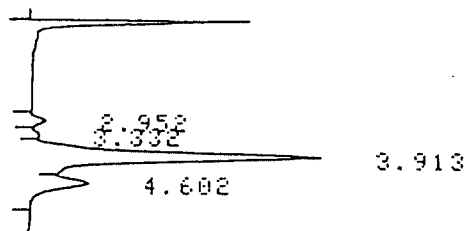
day 24

Std. 0.50 μ g/ml

High exposure level

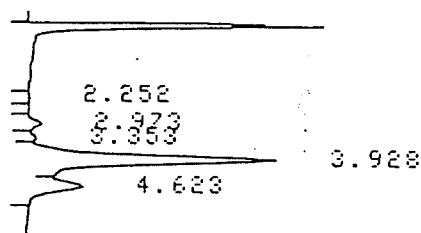
Low exposure level

START



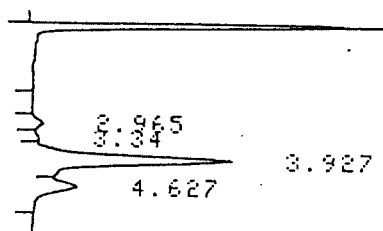
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.952	20893			2.4306
2	3.332	13828	V		1.6087
3	3.913	675116	V		78.5387
4	4.602	149759	V		17.422
TOTAL					100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.973	19175			2.6038
2	3.353	11557	V		1.5693
3	3.928	567817	V		77.1053
4	4.623	137869	V		18.7216
TOTAL					100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.965	13667			2.2947
2	3.34	9585	V		1.6094
3	3.927	457676	V		76.848
4	4.627	114633	V		19.2479
TOTAL					100

000612

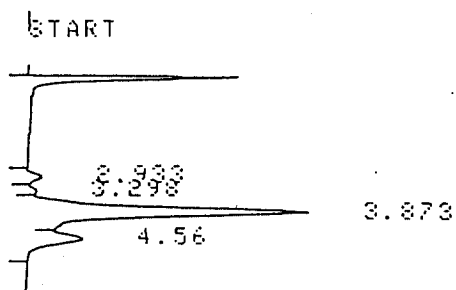
Fig.1-9 Gas chromatogram (Analysis of test water)

day 28

Std. 0.50 μ g/ml

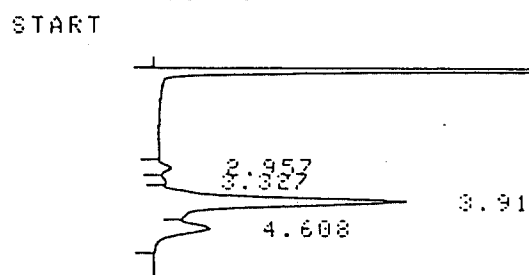
High exposure level

low exposure level



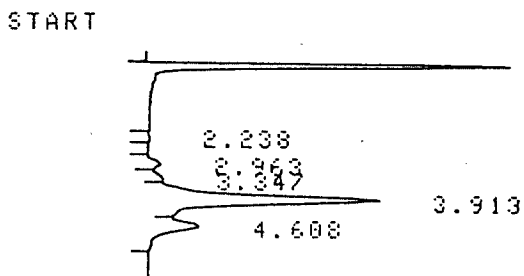
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.933	21055			2.5425
2	3.298	14268	V		1.7229
3	3.873	648705	V		78.3328
4	4.56	144111	V		17.4018

TOTAL		828140			100



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.957	18743			2.5062
2	3.327	12935	V		1.7297
3	3.91	575386	V		76.9388
4	4.608	140785	V		18.8253

TOTAL		747850			100



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.963	18682			2.5926
2	3.347	25742	V		3.5724
3	3.913	548095	V		76.0652
4	4.608	128042	V		17.7697

TOTAL		720560			100

000613

Fig. 1-10 Gas chromatogram (Analysis of test water)

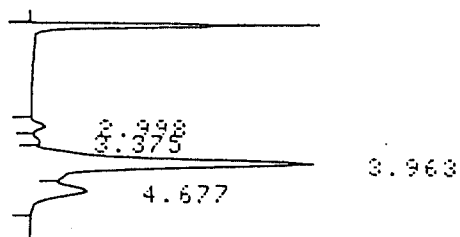
day 31

Std. 0.50 μ g/ml

High exposure level

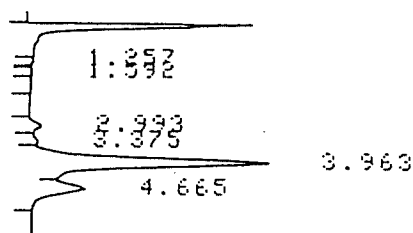
low exposure level

START



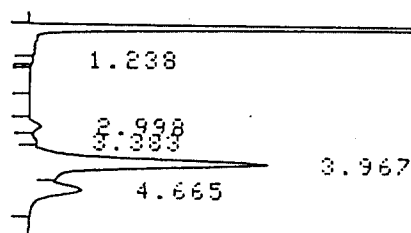
PKNO	TIME	AREA	MK	IDNO	CONC
1	2.998	19746			2.3422
2	3.375	13661	V		1.6204
3	3.963	662568	V		78.5922
4	4.677	147071	V		17.4452
TOTAL		843045			100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.993	16703			2.3366
2	3.375	11146	V		1.5593
3	3.963	552060	V		77.2272
4	4.665	134942	V		18.8769
TOTAL		714852			100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.998	17037			2.3687
2	3.383	12302	V		1.7103
3	3.967	552129	V		76.7607
4	4.665	137817	V		19.1603
TOTAL		719286			100

000614

Fig. 1-11 Gas chromatogram (Analysis of test water)

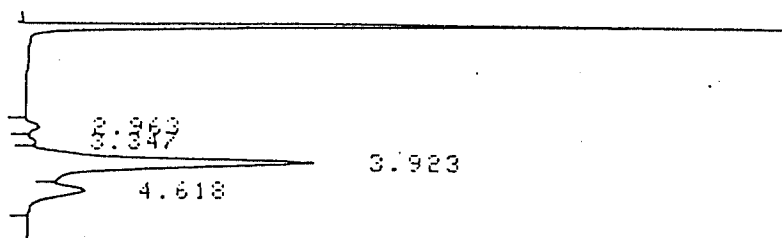
day 35

Std. 0.50 μ g/ml

High exposure level

low exposure level

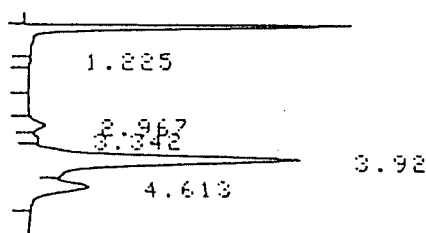
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.963	19804			2.2919
2	3.347	13140	V		1.5207
3	3.923	678328	V		78.5019
4	4.618	152820	V		17.6856

TOTAL		964091			100

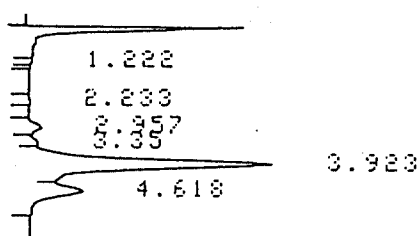
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.967	21191			2.6104
2	3.342	14378	V		1.7712
3	3.92	625275	V		77.026
4	4.613	150928	V		18.5924

TOTAL		811772			100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.957	16649			2.2839
2	3.35	11240	V		1.5419
3	3.923	569179	V		78.0794
4	4.618	131906	V		18.0948

TOTAL		728974			100

000615

Fig. 1-12 Gas chromatogram (Analysis of test water)

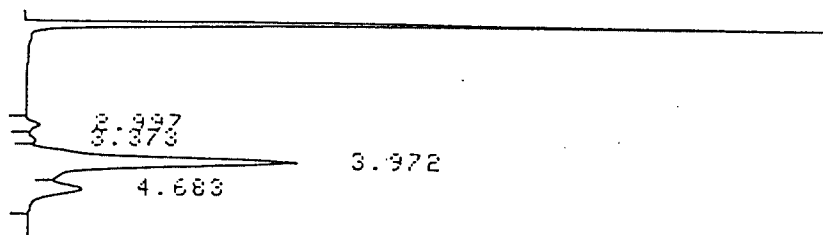
day 38

Std. 0.50 μ g/ml

High exposure level

low exposure level

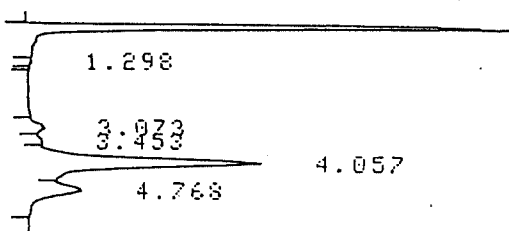
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.997	18649			2.2743
2	3.373	13448	V		1.6401
3	3.972	643757	V		78.5081
4	4.683	144133	V		17.5775

TOTAL		819988			100

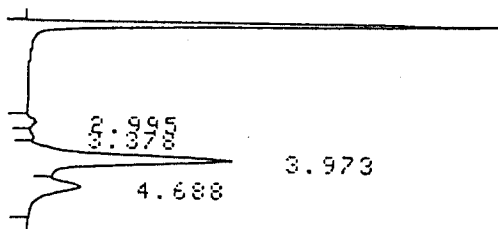
START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.073	20305			2.7173
2	3.453	17773	V		2.3784
3	4.057	569616	V		76.2262
4	4.768	139577	V		18.6782

TOTAL		747271			100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.995	13573			2.053
2	3.378	10671	V		1.614
3	3.973	479125	V		72.4686
4	4.688	157779	V		23.8644

TOTAL		661148			100

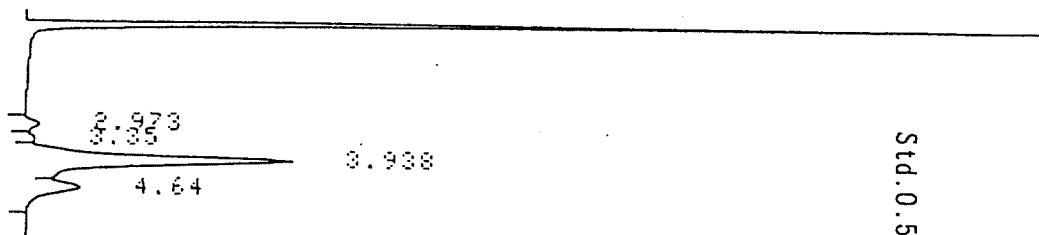
000616

Fig. 1-13 Gas chromatogram (Analysis of test water)

day 42

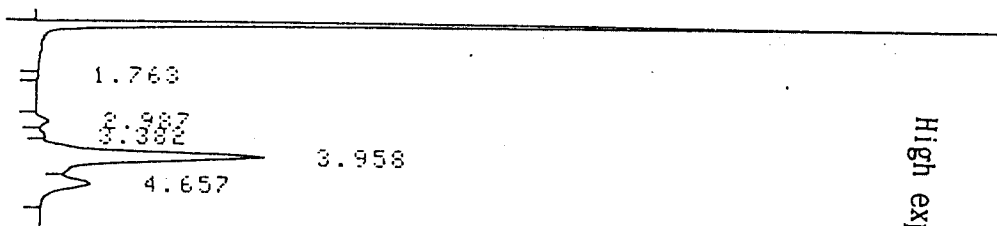
Std. 0.50 μ g/ml

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.973	19073			2.3741
2	3.35	13235	V		1.6475
3	3.938	633159	V		78.8126
4	4.64	137906	V		17.1658
TOTAL		803373			100

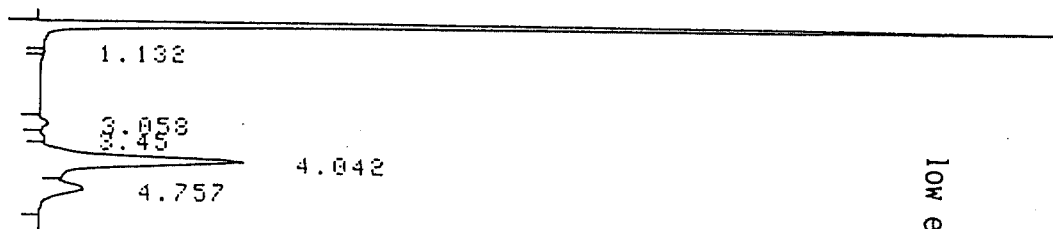
START



High exposure level

PKNO	TIME	AREA	MK	IDNO	CONC
1	2.987	16879			2.3965
2	3.382	12686	V		1.8012
3	3.958	539450	V		76.5898
4	4.657	135321	V		19.2125
TOTAL		704337			100

START



low exposure level

PKNO	TIME	AREA	MK	IDNO	CONC
1	3.058	14151			2.21
2	3.45	10364	V		1.6186
3	4.042	494294	V		77.1962
4	4.757	121500	V		18.9752
TOTAL		640308			100

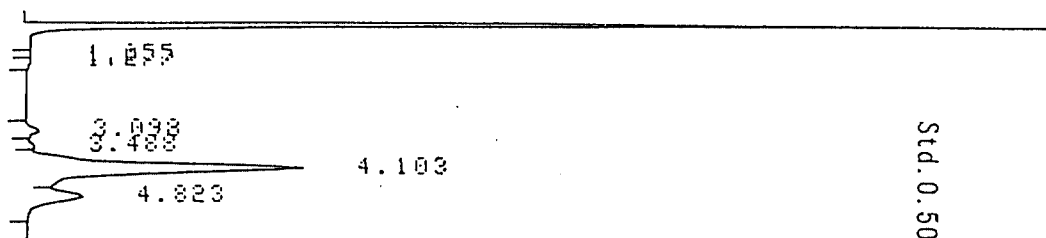
000617

Fig. 1-14 Gas chromatogram (Analysis of test water)

Std. 0.50 μ g/ml

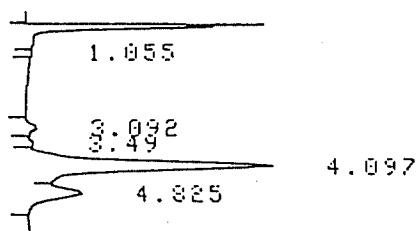
day 45

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.098	19202			2.2843
2	3.488	12900	V		1.5346
3	4.103	663595	V		78.9425
4	4.823	144909	V		17.2387
TOTAL		840606			100

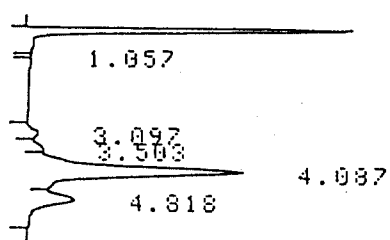
START



High exposure level

PKNO	TIME	AREA	MK	IDNO	CONC
1	3.092	18955			2.4487
2	3.49	12769	V		1.6496
3	4.097	594606	V		76.815
4	4.825	147745	V		19.0867
TOTAL		774075			100

START



low exposure level

PKNO	TIME	AREA	MK	IDNO	CONC
1	3.097	19555			2.7398
2	3.503	31894	V		4.4685
3	4.087	537210	V		75.2666
4	4.818	125084	V		17.525
TOTAL		713742			100

000618

Fig. 1-15 Gas chromatogram (Analysis of test water)

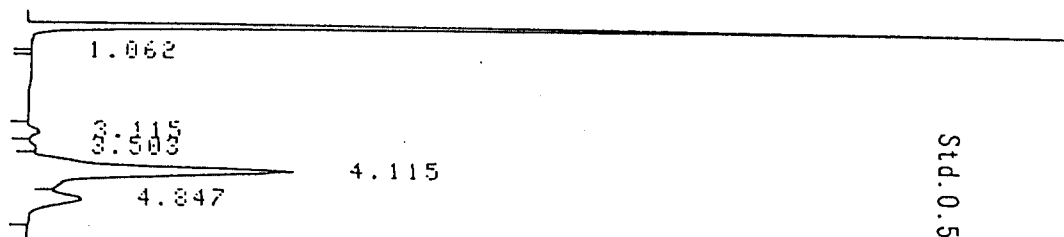
day 49

Std. 0.50 μ g/ml

High exposure level

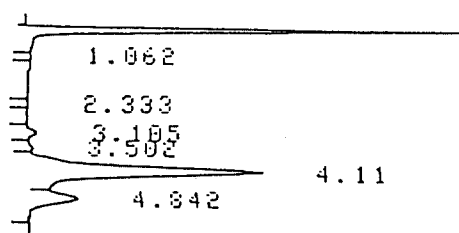
Low exposure level

START



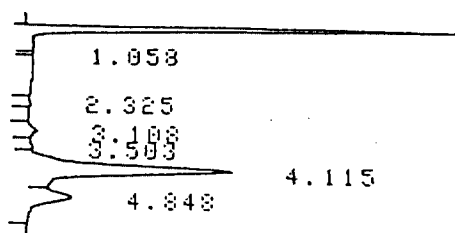
PKNO	TIME	AREA	MK	IDNO	CONC
1	3.115	18136			2.1992
2	3.503	15023	V		1.8218
3	4.115	645311	V		78.2513
4	4.847	146194	V		17.7277
TOTAL		824665			100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.105	15066			2.0726
2	3.502	10678	V		1.469
3	4.11	566263	V		77.9018
4	4.842	134887	V		18.5566
TOTAL		726893			100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.108	12808			2.0383
2	3.503	9152	V		1.4564
3	4.115	488226	V		77.6958
4	4.848	118196	V		13.8095
TOTAL		628382			100

000619

Fig. 1-16 Gas chromatogram (Analysis of test water)

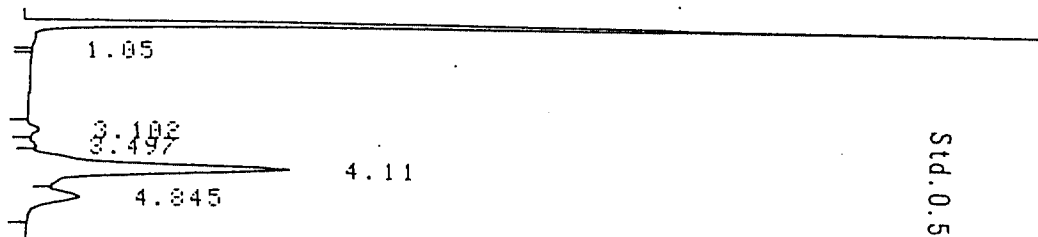
day 52

Std. 0.50 μ g/ml

High exposure level

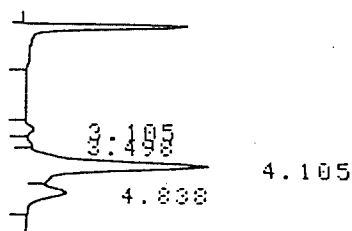
Low exposure level

START



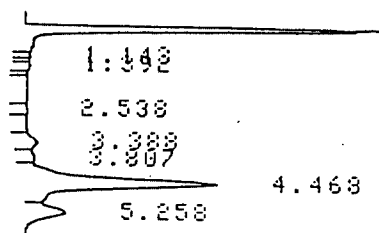
PKNO	TIME	AREA	MK	IDNO	CONC
1	3.102	16532			2.0554
2	3.497	12395	V		1.541
3	4.11	636203	V		79.0993
4	4.845	139180	V		17.3043
TOTAL					100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.105	11329			2.0103
2	3.498	7764	V		1.3776
3	4.105	441513	V		78.3407
4	4.838	102974	V		18.2714
TOTAL					100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.388	15406			2.4839
2	3.807	13754	V		2.2176
3	4.468	479148	V		77.2548
4	5.258	111910	V		18.0437
TOTAL					100

000620

Fig. 1-17 Gas chromatogram (Analysis of test water)

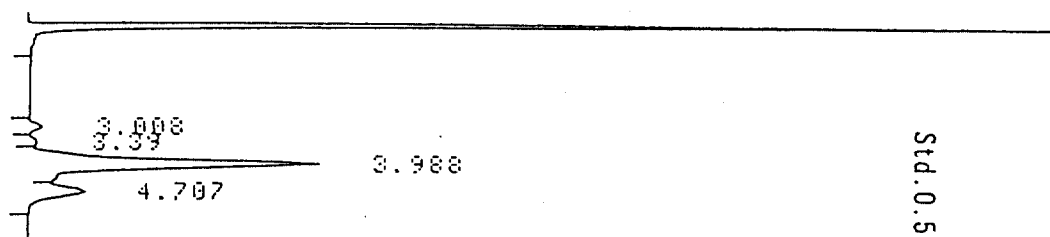
day 56

Std. 0.50 μ g/ml

High exposure level

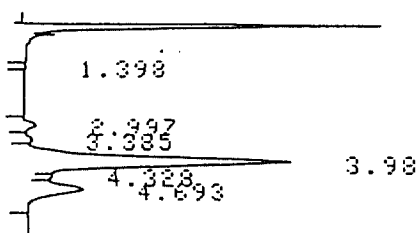
Low exposure level

START



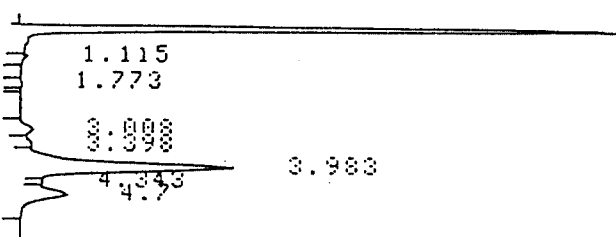
PKNO	TIME	AREA	MK	IDNO	CONC
1	3.008	17698			2.1876
2	3.39	11850	V		1.4648
3	3.988	642753	V		79.4501
4	4.707	136700	V		16.8974
TOTAL					100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	2.997	17988			2.3522
2	3.385	13222	V		1.729
3	3.98	557714	V		72.9303
4	4.328	32223	V		4.2137
5	4.693	143575	V		18.7747
TOTAL					100

START



PKNO	TIME	AREA	MK	IDNO	CONC
1	3.008	20549			3.2061
2	3.398	20365	V		3.1773
3	3.983	482711	SV		75.3127
4	4.7	117317	V		18.3039
TOTAL					100

000621