Ciba Specialty Chemicals
North America

8EHQ-1001-15021

52821

Ciba

October 26, 2001

Via Federal Express

US Environmental Protection Agency
OPPT Document Control Office TS 7407
Attention: Section 8(e)
Ariel-Rios Building
1200 Pennsylvania Avenue NW
Washington, DC 20460

8EHQ-01-15021

Contain NO CBI

Subject: TSCA 8(e) Notice - Tinuvin 343/Tinuvin 350

Dear Section 8(e) Coordinator:

This letter does not contain Confidential Business Information.

In accordance with EPA's March 16, 1978 Policy Statement on Section 8(e) reporting under the Toxic Substances Control Act (TSCA), the EPA's June, 1991 TSCA Section 8(e) Reporting Guide, Ciba Specialty Chemicals Corporation wishes to bring to the attention of the Environmental Protection Agency the results observed in a bioconcentration study with a commercial UV stabilizer, Tinuvin 343 (also sold under the trade name Tinuvin 350). The chemical nomenclature for Tinuvin 343 is 2-(3-(sec)-Butyl-5-(tert)-butyl-2-hydroxyphenyl)benzotriazole, CASRN 36437-37-3.

We are enclosing a copy of the study ***"Bioconcentration Test of Tinuvin 343 in Carp"*** conducted by Ciba Specialty Chemicals – Japan at Kurume Laboratory, Chemicals Evaluation and Research Institute, in Fukuoka.

A fish bioconcentration study was conducted with Carp (*Cyprinus carpio*) following OECD Guideline 305. The material was tested at 2 concentrations (1 ug/L and 0.1 ug/L) for 60 days with a 32 day depuration period. Tinuvin 343 bioconcentrated at 7700 and 13000 times the ambient water concentration (for the 1 ug/L and 0.1 ug/L exposure levels, respectively). The compound was rapidly excreted in the depuration phase with a half-life of ~14 days. No adverse effects were reported for the fish, moreover, a companion acute toxicity test conducted concurrently showed that the 96-h LC50 was greater than 250 mg/L, indicating the compound has low toxicity to fish. A previously conducted acute toxicity test with daphnia magna indicated the compound also had low toxicity to aquatic invertebrates with an EC50 1100 mg/L.

540 White Plains Road
Tarrytown, New York 10591

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88020000007

The use pattern for this product in the United States indicates the likelihood of significant environmental releases are minimal. The primary uses in the United States are as a UV absorber in photographic films and polycarbonate. In both applications, the Tinuvin 343 is encapsulated in either the film or polycarbonate, and is biologically unavailable.

Based upon the intent of current EPA guidelines, and Ciba's Responsible Care commitment, it is felt that these results should be evaluated by the Agency though it is not clear that they qualify as a significant adverse effect as defined under TSCA 8(e). We do not believe the chemical has a potential for widespread exposure nor is there evidence that exposure would be coupled with a non-trivial adverse effect. Please call the undersigned if you have any questions concerning this submittal.

Respectfully,

Ciba Specialty Chemicals Corporation

Thomas Barber
Manager, Product Compliance

Accepted number	S00-3705
Test number	43705

FINAL REPORT

Bioconcentration test of TINUVIN 343 in carp

August 21, 2001

Kurume Laboratory
Chemicals Evaluation and Research Institute, Japan

STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor	Ciba Specialty Chemicals K.K. Japan
Title	Bioconcentration test of TINUVIN 343in carp
Test number	43705

I, the undersigned, hereby declare that this report provides a correct English translation of the Final Report (Test No.43705, issued on August 21, 2001).

Date October 3, 2001

Study director N. Yakata

Naoaki Yakata

GLP STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor Ciba Specialty Chemicals K.K. Japan

Title Bioconcentration test of TINUVIN 343 in carp

Test number 43705

This test was conducted in compliance with Good Laboratory Practice Standards for industrial chemicals, "Basic standards to be observed by testing facilities in conducting tests stipulated in article 4 of the Order Prescribing Those Items of the Test Relating to the New Chemical Substances and Study on Harmful Effects of Designated Chemical Substances" (March 31, 1984, Revised March 1, 2000, Kanpogyo No.39, Planning and Coordination Bureau, Environment Agency, Yakuhatu No.229, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, and 59 Kikyoku No.85, Basic Industries Bureau, Ministry of International Trade and Industry, Japan) and "OECD Principles of Good Laboratory Practice" (November 26, 1997).

It has been confirmed that this final report reflects the raw data accurately and the test data are valid.

Date August 21, 2001

Study director Signed in original

Naoaki Yakata

QUALITY ASSURANCE STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor Ciba Specialty Chemicals K.K. Japan

Title Bioconcentration test of TINUVIN 343 in carp

Test number 43705

Study audits and inspections of this test were carried out and the results were reported to the management and the study director by Quality Assurance Unit of Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan as follows.

Item of audit or inspection	Date of audit or inspection	Date of report to study director	Date of report to management
Protocol	April 13, 2001	April 13, 2001	April 13, 2001
	April 18, 2001	April 18, 2001	April 18, 2001
	April 19, 2001	April 19, 2001	April 19, 2001
	June 27, 2001	June 27, 2001	June 27, 2001
	July 13, 2001	July 16, 2001	July 16, 2001
	July 23, 2001	July 23, 2001	July 23, 2001
Test Conduct	April 17, 2001	April 17, 2001	April 17, 2001
	May 8, 2001	May 8, 2001	May 8, 2001
	May 29, 2001	June 5, 2001	June 5, 2001
	June 4, 2001	June 5, 2001	June 5, 2001
	August 2, 2001	August 8, 2001	August 8, 2001
	August 7, 2001	August 8, 2001	August 8, 2001
	August 8, 2001	August 8, 2001	August 8, 2001
Raw Data and Final Report	August 21, 2001	August 21, 2001	August 21, 2001

It has been assured that the final report describes accurately the test method used, that details in the report are in compliance with the protocol and Standard Operating Procedures and that the final report reflects the raw data accurately.

Date August 21, 2001

Quality Assurance Unit, Head Signed in original

Kyoshiro Hori

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Test No. 43705

Title

Bioconcentration test of TINUVIN 343 in carp

Sponsor

Ciba Specialty Chemicals K.K. Japan
10-66 Miyuki-cho, Takarazuka, Hyougo 665-8666, Japan

Testing facility

Kurume Laboratory
Chemicals Evaluation and Research Institute, Japan
19-14 Chuomachi, Kurume, Fukuoka 830-0023, Japan

Objective

This test was performed to evaluate the bioconcentration potential of TINUVIN 343 in carp.

Test method

This test was conducted according to the "Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body" stipulated in the "Testing Methods for New Chemical Substances" (July 13, 1974, Revised October 8, 1998, Kanpogyo No.5, Planning and Coordination Bureau, Environment Agency, Yakuhatu No.615, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, and 49 Kikyoku No.392, Basic Industries Bureau, Ministry of International Trade and Industry, Japan). This test method is essentially the same as that in the OECD Guidelines for Testing of Chemicals, "Bioconcentration : Flow-through Fish Test (Guideline 305, June 14, 1996)".

Applied GLP

(1) Chemical GLP

This test complied with "Basic standards to be observed by testing facilities in conducting tests stipulated in article 4 of the Order Prescribing Those Items of the Test Relating to the New Chemical Substances and Study on Harmful Effects of Designated Chemical Substances (hereafter referred to as "GLP standards")" (March 31, 1984, Revised March 1, 2000, Kanpogyo No.39, Planning and Coordination Bureau, Environment Agency, Yakuhatu No.229, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, and 59 Kikyoku No.85, Basic Industries Bureau, Ministry of International Trade and Industry, Japan).

(2) OECD-GLP

This test complied with "OECD Principles of Good Laboratory Practice" (November 26, 1997).

Test schedule

Start of test	April 13, 20001
Experimental starting date	April 27, 2001
Experimental completion date	August 2, 2001
End of test	August 21, 2001

Storage of test substance, raw data, etc.

(1) Test substance

About 5 g of the item supplied by the sponsor is sealed in a store vessel and stored in a archive in this laboratory for ten years after receipt of notification that the test item belong to No.1, No.2 or No.3 in Clause 1, Article 4 of "Law Concerning Examination and Regulation of Manufacture, etc. of Chemical Substances". Treatment of the item supplied by the sponsor after the storage period is discussed with sponsor. If it is not stable for the storage period, it is stored while it is kept stable and it is disposed with approval of sponsor.

(2) Raw data and materials, etc.

Raw data used for the preparation of the final report, the protocol, documents about the test presented by the sponsor, the final report and necessary materials are stored in a storage room in this laboratory for the same term as the test substance. Treatment of raw data and materials, etc. after the storage period is discussed with sponsor.

Personnel

Study director	Naoaki Yakata
Personnel (Operation of bioconcentration test)	Akemi Inoue Yurika Mouri Kaori Taniguchi Hiromi Sugioka
Staff for fish care	Yasuro Kawashima
Person to conduct of fish acute toxicity test	Yasuro Kawashima Tadayoshi Tonai

Approval of final report

Study director	Date	August 21, 2001
	Signature	Signed in original
		Naoaki Yakata

SUMMARY

Title

Bioconcentration test of TINUVIN 343 in carp

Test conditions

Acute toxicity test

(1) Test fish	Orange-red killifish (Oryzias latipes)
(2) Duration of exposure	96 hrs.
(3) Exposure method	Semi static system (Renewal of test water, at every 8 - 16 hrs.)

Bioconcentration test

(1) Test fish	Carp (Cyprinus carpio)
(2) Nominal concentrations of test substance	
	High exposure level (Level 1) 1 μg/L
	Low exposure level (Level 2) 0.1 μg/L
(3) Duration of exposure	60 days
(4) Exposure method	Continuous flow system
(5) Analytical method	High-performance liquid chromatography

Results

(1) 96-hour LC50 value	> 250 mg/L
(2) Bioconcentration factors at a steady state	
Level 1	7700
Level 2	13000
(3) Depuration half-value ($t_{1/2}$)	
Level 1	15 day
Level 2	14 day
(4) Bioconcentration factors analysis in parts of test fish	
	See Table-5 (see page 28)

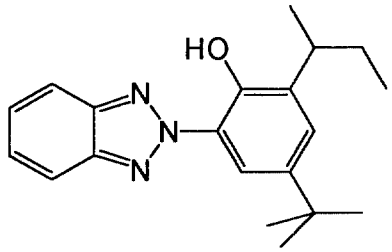
1. Test substance

In this report, TINUVIN 343 has the following chemical name, etc.

1.1 Chemical name*1 2-(2H-benzotriazol-2-yl)-4-(*tert*-butyl)-6-(*sec*-butyl)phenol

1.2 Chemical structure, etc.*1

Structural formula



Molecular formula	$C_{20}H_{25}N_3O$
Molecular weight	323.43

*1 Information supplied by the sponsor

2. Substance supplied by the sponsor

2.1 Supplier and lot number*1

(1) Supplier	Ciba Specialty Chemicals K.K. Japan
(2) Lot number	0101678S

2.2 Purity*1

Test substance	100 %

2.3 Confirmation of test substance

Two infrared (IR) spectra of the test substance provided by the sponsor and measured at this laboratory were confirmed to be identical (see Fig.21 and Reference 2).

2.4 Physicochemical properties*1

Appearance	Slightly yellow powder
Melting point	81 - 84 °C
Vapor pressure	4×10^{-5} Pa (20 °C)
Density	1.15 g/cm^3 (20 °C)
Solubility	Water < 1 mg/L

*1 Information supplied by the sponsor

2.5 Storage and stability

(1) Storage condition

Room temp.

(2) Stability

The test substance was stable under the storage condition as shown by the finding that IR spectra of the test substance before and after the experiment were identical (see Fig.21).

2.6 Stability under testing conditions

Prior to the bioconcentration test, a stability of the test substance under the testing conditions was confirmed by a preliminary test.

3. Performance of acute toxicity test

3.1 Test method

The test was performed in accordance with Japanese Industrial Standard (JIS K 0102-1998-71.), "Testing methods for industrial waste water, Acute toxicity test with fish".

3.2 Test fish

(1) Species Orange-red killifish (Oryzias latipes)
Reason for selection : This species is similar in sensitivity to carp and readily available as test fish.

(2) Supplier
Nakashima fish farm
(Address : 2029 Ooaza Nagasu Nagasu-cho Tamana-gun, Kumamoto 869-0123, Japan)

(3) Conditions for fish care before acclimatization
The fish were checked visually at the receiving and those demonstrating any abnormality were removed. The fish were reared for 14 days in a flow through system following an external disinfection.

(4) Conditions for acclimatization
After rearing, the fish were transferred to an acclimatizing aquarium and acclimatized there after the second external disinfection. The fish demonstrating any abnormality during this period were removed and the remainder of the fish were reared for 7 days in a flow through system at the temperature of 25 ± 2 ℃. The fish were checked for health conditions and reared another 47 days after the external disinfection.

(5) Weight average 0.24 g

(6) Length average 3.0 cm

(7) Certification
The 48-hour LC50 value of the reference substance for the fish of the same lot (TFO-010216) was 0.463 mg/L.

3.3 Dilution water for test

(1) Origin

Underground water from the premises of Kurume Laboratory.

(2) Water quality assessment

The dilution water for test was taken out on February 16, 2001, (the dilution water for analysis of total organic carbon was taken out on March 19, 2001) and it was analyzed and measured (once every six months in this laboratory). The results are shown in Reference 1.

It was confirmed that the dilution water met the ministerial ordinance of the Ministry of Health and Welfare (December 21, 1992), water quality criteria for fisheries (Shadanhozin Nihon Suisansigen Hogokyokai, March 1983), OECD Guidelines for Testing of Chemicals, "Fish, Early-life Stage Toxicity Test" (Guideline 210, July 17, 1992) and environmental quality standards for water pollutants No. 14 (Revised February 22, 1999, Environment Agency) or OECD Guidelines for Testing of Chemicals, "Bioconcentration: Flow-through Fish Test (Guideline 305, June 14, 1996)".

3.4 Test conditions

(1) Test tank — Round glass vessel

(2) Volume of test water — 4 L / level

(3) Temperature of test water

At initial exposure	24.2 °C
Before renewal of test water	24.0 °C

(4) Concentration of dissolved oxygen in test water

At initial exposure	8.0 mg/L
Before renewal of test water	6.5 mg/L

(5) pH of test water

At initial exposure	7.7
Before renewal of test water	7.6

(6) Number of fish — 10 / level

(7) Duration of exposure — 96 hrs.

(8) Exposure method — Semi static system
(Renewal of test water, at every 8 - 16 hrs.)

3.5 Preparation of stock solution

(1) Dispersant

HCO-40 (Hydrogenated castor oil)

(2) Preparation

The substance supplied by the sponsor and HCO-40 (20 times amount of the test substance) were dissolved with tetrahydrofuran. After tetrahydrofuran was evaporated from the solution, ion-exchanged water was added to the mixture to prepare 1000 mg/L stock solution.

3.6 Performance of test

(1) Place 214 LC50 room

(2) Date April 16, 2001 – April 20, 2001

3.7 Estimation of 96-hour LC50 value

The 96-hour LC50 value was estimated by the Doudoroff method.

3.8 Result of test

96-hour LC50 value > 250 mg/L*2 (see Fig.3)

*2 The concentration of the dispersant at this time was 5000 mg/L. Taking into account of the toxicity of the dispersant, the test at higher concentration was not performed, because the 96-hour LC50 value of the dispersant was 50000 mg/L.

4. Performance of bioconcentration test

4.1 Test fish

(1) Species Carp (Cyprinus carpio)
Reason for selection : The previous data conducted with this species can be compared and the size of this species is adequate for handling.

(2) Supplier
Fukuokaken yabegawa fishermen's cooperative association
(Address : 748 Yamauchi, Yame-shi, Fukuoka 834-0012, Japan)

Date received February 9, 2001

(3) Conditions for fish care before acclimatization
The fish were checked visually in the receiving and those demonstrating any abnormality were removed. The fish were reared for 14 days in a flow through system following an external disinfection.

(4) Conditions for acclimatization
After rearing, the fish were medicated to eliminate parasites and transferred to an acclimatizing aquarium. After the second external disinfection, they were acclimatized. The fish demonstrating any abnormality during this period were removed and the remainder of the fish were reared for 34 days in a flow through system at the temperature of 25 ± 2 °C. The fish were then transferred to test tanks and reared at the same temperature in the flow through system for another 23 days, following the external disinfection.

(5) Length 6.7 – 11.4 cm

(6) Lot No. TFC-010209- II

(7) Age Yearling fish

(8) Feeding

Feed	Feed for fry of carp
Composition	Proteins content ≧ 43.0 %
	Lipid content ≧ 3.0 %
Manufacturer	Nippon Formula Feed Mfg. Co., Ltd.

Feeding amount and interval
Amount corresponding to 2 % of total body weight was fed twice a day in halves.
The fish were starved for 24 hours before sampling.

4.2 Dilution water for test

The same as described in 3.3.

4.3 Conditions of test and circumstances

(1) Supply of test water

Flow through system assembled at this laboratory was used.

(2) Test tank

100-L glass tank

(3) Flow rate of test water

Duration of exposure

At 2 mL/min. for stock solution and 1600 mL/min. for dilution water; 2307 liters/day of test water were supplied.

Duration of depuration

At 1600 mL/min. for dilution water; 2304 liters/day of test water were supplied.

(4) Stock solution bottle 25-L glass bottle
(Frequency of renewal 1 - 2 times/week)

(5) Temperature of test water

Level 1	24.7 – 25.6 °C
Level 2	24.8 – 25.8 °C
Control	25.1 – 25.7 °C

(6) Concentrations of dissolved oxygen in test water

Level 1	7.9 – 8.1 mg/L (see Fig.17)
Level 2	7.7 – 8.1 mg/L (see Fig.18)
Control	8.1 mg/L (see Fig.19)

(7) pH of test water

Level 1	7.7 – 7.9
Level 2	7.7 – 7.9
Control	7.7 – 7.9

(8) Time of irradiation with light

Artificial light of white fluorescent lamp (14 hrs./day)

(9) Number of fish (at the beginning of exposure)

Level 1 and 2	47
Control	10

(10) Duration of exposure 60 days
Reason for decision : The time to reach a steady-state was not estimated to be within 28 days from preliminary test results.

(11) Duration of depuration 36 days

(12) Place 213 Aquatron room

4.4 Preparation of stock solutions

(1) Dispersant
The same as described in 3.5 (1).

(2) Preparation
- Level 1
0.8 mg/L stock solution was prepared in the same way as described in 3.5 (2).

- Level 2
0.08 mg/L stock solution was prepared in the same way as described in 3.5 (2).

- Control
HCO-40 was dissolved with ion-exchanged water to prepare 16 mg/L stock solution.

4.5 Test concentrations

Based on preliminary test results for the 96-hour LC50 value and analytical detection limits, test concentrations of the test substance were decided as follows. The control was set as a blank test.

Level 1	1 μg/L
Level 2	0.1 μg/L

4.6 Observation, measurement, etc.

(1) Observation of test fish

Condition of test fish was observed visually twice a day.

(2) Flow rate of test water

Flow rate of stock solution and dilution water were measured with graduated cylinder and recorded once a day.

(3) Temperature of test water

Temperature of test water was measured with alcohol thermometer and recorded once a day.

(4) Concentration of dissolved oxygen in test water

Concentration of dissolved oxygen in test water was measured with dissolved oxygen probe and recorded twice a week.

(5) pH of test water

pH of test water was measured with pH meter once a week or more.

(6) Cleaning of test tank

In experimental period, excreta of carp, dirt on test tank, etc. were removed about once a day.

4.7 Analysis of test water and fish

Analysis of the test substance in test water and test fish was performed with high-performance liquid chromatography (HPLC) analysis.

4.7.1 Frequency of analysis

(1) Test water analysis

The test water of each level was analyzed once before first analysis of test fish and at the same time as analysis of test fish.

(2) Test fish analysis

Analysis of test fish was performed five times at each in duration of exposure. As BCFs were over 1000, depuration test was performed. Analysis of test fish was performed at least 4 times in duration of depuration. Analysis of control fish was performed before the experimental starting and after the experimental completion.

Four fish were taken out at each sampling time and divided into two groups, then both were analyzed individually*3.

*3 Because one fish was too small to take out the stored sample for the measurement of lipid content, two fish a group were employed.

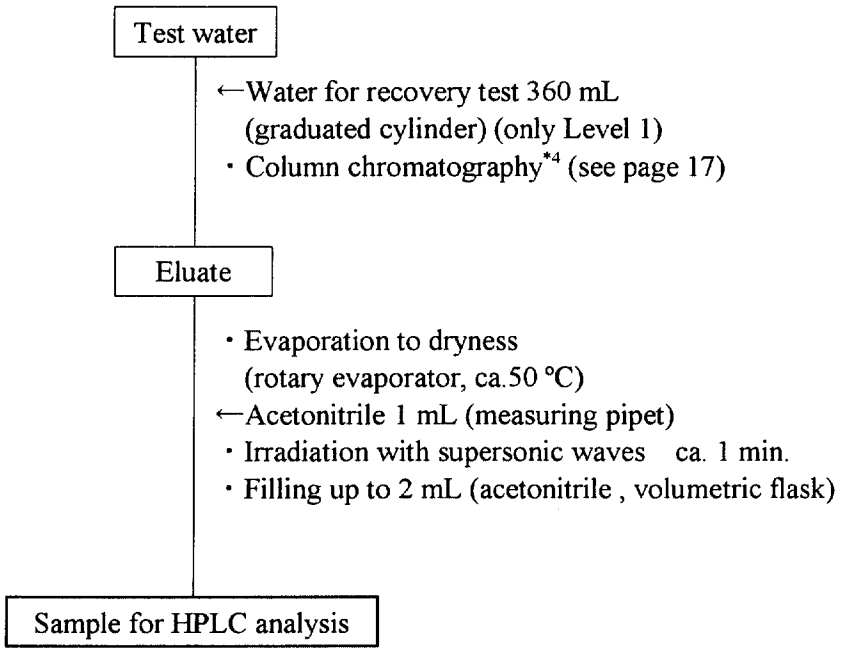
4.7.2 Pretreatment for analysis

(1) Test water

An aliquot of the test water,

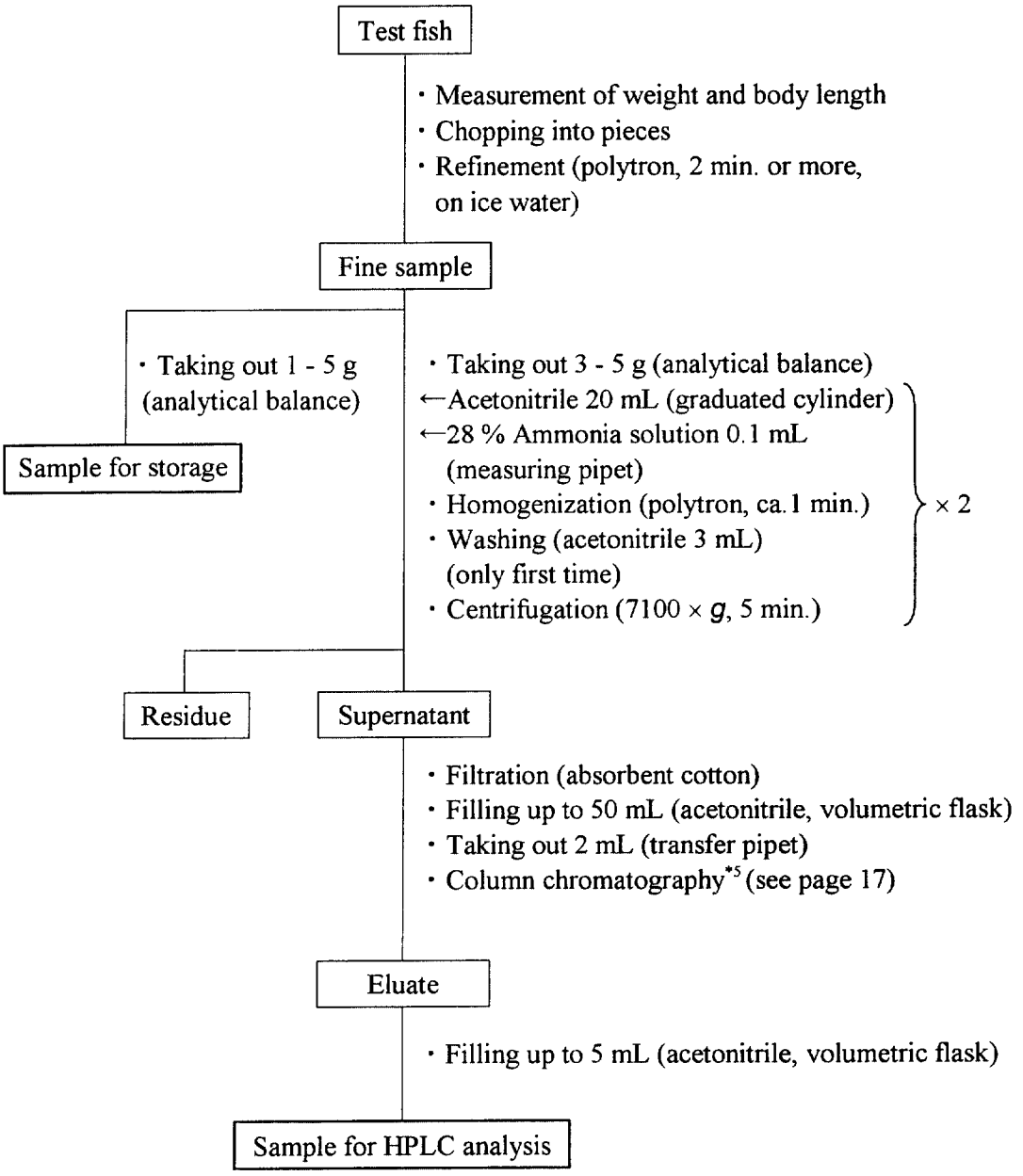
Level 1	40 mL
Level 2	400 mL

was taken from each test tank, and pretreated for high-performance liquid chromatography (HPLC) analysis as follows :



(2) Test fish

Test fish were taken from each test tank and pretreated for HPLC analysis as follows :



*4 Conditions of column chromatography

MEGA BOND ELUT C8

Conditionings	Tetrahydrofuran	ca.10 mL
	Ion-exchanged water	ca.10 mL
Loading	Whole volume of the solution was loaded.	
Elution	Eluate 1 Tetrahydrofuran	10 mL

Test substance was eluted with the eluate 1.

*5 Conditions of column chromatography

Sep-Pak C_{18}

Conditionings	Acetonitrile	ca.10 mL
Loading	Whole volume of the solution was loaded.	
Elution	Eluate 1 Acetonitrile	2.5 mL

Test substance was eluted with the load and the eluate 1.

4.7.3 Quantitative analysis for test substance

The samples for HPLC analysis in pretreatment were analyzed by high-performance liquid chromatography under the following analytical conditions. When the concentration of the test substance in the sample for HPLC analysis exceeded the range of the calibration curve in test fish analysis, the sample was analyzed after it was diluted to a concentration within the range of the calibration curve. The concentration of the test substance in each sample solution was determined on the basis of a comparison of the peak area on the chromatogram of the sample solution with that of a standard solution (see Tables-7, 8, Fig.6 and Tables-10, 11, 12, 13, 14, 15, 16 , Figs.8, 9, 10, 11, 14, 15, 16).

(1) Analytical conditions

Instrument	High-performance liquid chromatograph
Pump	Shimadzu Corporation type LC-10AT
Detector	Shimadzu Corporation type SPD-10AV
Auto sampling injector	M&S Instruments Trading Inc. type 231XL
Column	L-column ODS
	15 cm × 4.6 mmI.D. stainless steel
Temperature	35 °C
Eluent	Acetonitrile / tetrahydrofuran (98/2 V/V)
Flow rate	1.0 mL/min
Measurement wave length	338 nm (see Fig.20)
Sample size	100 μL
Detector output	2 V/AU

(2) Preparation of standard solution

The standard solution to determine the concentration of the test substance in the sample solutions was prepared as follows.

100 mg of the substance supplied by the sponsor was dissolved in acetonitrile to prepare 1000 mg/L solution of the test substance. 20.0 μg/L standard solution was then prepared from this solution by dilution with acetonitrile.

(3) Calibration curve

10.0, 20.0 and 40.0 μg/L standard solutions were prepared by the same method as described in (2). These solutions were analyzed according to the analytical conditions described in (1). A calibration curve was drawn on the basis of the relation between the peak area on the chromatograms and the respective concentrations.

In consideration of the noise level, the lowest detectable peak area of the test substance was regarded as 545 μV·sec for analysis of test water and test fish, which corresponded to 1.0 μg/L of the test substance concentration respectively (see Fig.4).

4.7.4 Recovery and blank test

(1) Method

Water and fine sample of fish (10 g) spiked a specified amount of the test substance for the recovery test were prepared in the same way as described in 4.7.2. The blank test was also performed in the same manner without the test substance. All the recovery and blank tests were performed in duplicate.

(2) Results of recovery test

In the blank test, the chromatogram of HPLC had no peaks interfering with determination of the test substance concentration. The duplicate recovery rates and the average of them in the pretreatment are shown below (see Tables-6, 9 and Figs.5, 7). The average recovery rate was used as correction factors for the determination of the test substance concentrations in the analytical samples.

For analysis of test water (40 ng test substance added)
90.4 %, 87.7 % average 89.1 %

For analysis of test fish (5000 ng test substance added)
71.3 %, 76.3 % average 73.8 %

4.7.5 Lipid content in test fish

Lipid contents in the sample for storage of the control test fish (see 4.7.2(2)) were determined after chloroform-methanol extraction with gravimetric analysis.

4.7.6 Calculation of the test substance concentration in sample and minimum limit of determination

(1) Calculation of the test substance concentration in test water

The equation in Tables-7, 8 was used to obtain the concentrations, and they were rounded off to 3 figures.

(2) Determination limit of the test substance in test water

The determination limit*6 of the test substance in test water was calculated on the basis of that obtained from the calibration curve in 4.7.3 (3) as follows.

Level 1	0.056 μg/L
Level 2	0.0056 μg/L

(3) Calculation of the test substance concentration in test fish

The equations in Tables-10, 11, 12, 13, 14, 15, 16 were used to obtain the concentrations, and they were rounded off to 3 figures.

(4) Determination limit of the test substance in test fish

Assuming the fine sample of fish to be 5 g, the determination limit*6 of the test substance in test fish was calculated to be 34 ng/g on the basis of that obtained from the calibration curve in 4.7.3 (3).

*6 Minimum determination limit of the test substance (μg/L or ng/g)

$$= \frac{A}{\frac{B}{100} \times \frac{C \times E}{D}}$$

where

A : Minimum determination limit of the test substance on the calibration curve (μg/L)

B : Recovery rate (%)

C : Sampling volume of test water (mL) or fine sample of fish (g)

D : Final volume of sample solution (mL)

E : Ratio of the portion, used for analysis to whole volume

Results were rounded off to 2 figures.

4.7.7 Calculation of mean concentration of the test substance in test water (duration of exposure)

$$\overline{Cwt} = \{ Cw(1) + \cdots\cdots \cdot + Cw(n) \} / n$$

where

$\overline{Cwt}$	The mean concentration of the test substance in test water (μg/L)
n	Number of analysis for test water (mesurement times)
Cw(1)	Concentration of the test substance in 1st analysis of test water (μg/L)
Cw(n)	Concentration of the test substance in *n*-th analysis of test water (μg/L)

4.7.8 Calculation of bioconcentration factor (BCF)

Bioconcentration factor (BCF) was calculated as follows.

(1) Calculation of mean concentration of the test substance in test water for calculating BCF

$$Cw = \{ Cw(n-1) + Cw(n) \} / 2 \quad \text{(only 1st analysis of test fish)}$$
$$\overline{Cw} = \{ Cw(n-2) + Cw(n-1) + Cw(n) \} / 3 \quad \text{(from 2nd analysis of test fish)}$$

where

$\overline{Cw}$	The mean concentration of the test substance in test water for calculating BCF (μg/L)
Cw(n)	Concentration of the test substance in *n*-th analysis of test water (μg/L)

(2) Calculation of bioconcentration factor

$$BCF = \frac{Cf}{\overline{Cw}}$$

where

BCF	Bioconcentration factor
Cf	Concentration of the test substance in test fish (ng/g)
$\overline{Cw}$	The mean concentration of the test substance in test water for calculating BCF (μg/L)

(3) The mean bioconcentration factor in m-th analysis

$$BCFm = (BCFa + BCFb) / n$$

BCFm : The mean bioconcentration factor in m-th analysis (number of individual or group 2 (a,b))
BCFa,b : Each bioconcentration factor in m-th analysis of test fish
n : Number of individual or group in m-th analysis of test fish

4.7.9 Confirmation of the steady-state was reached

It was evaluated that a steady-state had been reached when three successive analyses of BCFs made on samples taken at intervals of at least 48 hours were within ± 20 % of each other. When BCFs were less than 100, it was evaluated that a steady-state had been reached after 60 days even if BCFs were over ± 20 % of each other.

Criterion of the steady-state was reached : V(m-2), V(m-1), V(m) $\leqq$ 20 (%)

$$V(m-2) = \frac{\left| BCF(m-2) - \overline{BCF} \right|}{\overline{BCF}} \times 100$$

$$V(m-1) = \frac{\left| BCF(m-1) - \overline{BCF} \right|}{\overline{BCF}} \times 100$$

$$V(m) = \frac{\left| BCF(m) - \overline{BCF} \right|}{\overline{BCF}} \times 100$$

V(m-2), V(m-1), V(m) : Variation of bioconcentration factor (%)
BCF(m-2), BCF(m-1), BCF(m) : The mean bioconcentration factor in m-2, m-1, m-th analysis of test fish
$\overline{BCF}$: { BCF(m-2) + BCF(m-1) + BCF(m) } / 3

4.7.10 Calculation of bioconcentration factor at a steady-state (BCFss)

Bioconcentration factor at a steady-state (BCFss) was calculated as follows.

(1) Calculation of the mean concentration of the test substance in test water for calculating BCFss

$$\overline{Cws} = \{ Cw(n-2) + Cw(n-1) + Cw(n) \} / 3$$

where

$\overline{Cws}$ The mean concentration of the test substance in test water for calculating BCFss, which is calculated from three successive analyses of test water before last analysis of test fish as a general rule (μg/L)

Cw(n) Concentration of the test substance in *n*-th analysis of test water (μg/L)

(2) Calculation of the mean concentration of the test substance in test fish at a steady-state

$$\overline{Cfs} = \{ Cf(m-2) + Cf(m-1) + Cf(m) \} / 3$$

where

$\overline{Cfs}$ The mean concentration of the test substance in test fish at a steady-state (ng/g)

Cf(m) The mean concentration of the test substance, from which FB is subtracted, in *m*-th analysis of test fish (ng/g)

FB The arithmetical mean concentration of the test substance or blank in the control test fish analyzed before and after the experiment (ng/g)

(3) Calculation of BCFss

$$BCFss = \overline{Cfs} / \overline{Cws}$$

where

BCFss Bioconcentration factor at a steady-state (ng/g)

$\overline{Cfs}$ The mean concentration of the test substance in test fish at a steady-state (μg/L)

$\overline{Cws}$ The mean concentration of the test substance in test water for calculating BCFss

4.7.11 Calculable BCF

On the basis of the minimum determination limit of the test substance in 4.7.6 (4), BCF can be obtained when BCF exceeds the following. The mean concentration of the test substance in test water obtained from all the analyzed sample was used to calculate the following calculable BCF.

Level 1	39
Level 2	470

4.7.12 Calculation of lipid content

Lipid contents were calculated according to the following equation.

$$\text{Lipid content (\%)} = (T - T_0) / S \times 100$$

where

T_0 Weight of vessel (g)

T Weight of sample for gravimetric analysis (containing vessel) (g)

S Weight of fine sample taken out for analysis of lipid content (g)

4.8 Treatment of numerical values

Values were rounded off in accordance with JIS Z 8401:1999 rule B. The each value used for calculation was used without rounding off on the way of the calculation.

The concentration values of the test substance in test water and fish were rounded off to 3 figures. BCFss values were rounded off to 2 figures.

5. Factors possibly affecting accuracy

No adverse effects on the reliability of this test were noted.

6. Results

6.1 Concentration of test substance in test water

The measured concentrations of the test substance in test water are shown in Table-1. Each concentration of the test substance was maintained at 61-92 % of each the nominated concentration. The variation of the concentrations of the test substance was within ± 20 % of the mean of the measured concentrations.

Table-1 Measured concentrations of the test substance in test water

(Unit : μg/L)

Level	After 4 days	After 11 days	After 18 days	After 32 days	After 46 days	After 60 days	Average (Standard deviation)	Table	Fig.
1	0.902	0.919	0.884	0.840	0.862	0.812	0.870 (0.0399)	7	6
2	0.0610	0.0808	0.0658	0.0611	0.0795	0.0813	0.0716 (0.01000)	8	

6.2 Bioconcentration factors

BCFs are shown in Table-2.

BCFs in Table-2 plotted against duration of exposure are shown in Fig.1 and 2.

These BCFs of the test substance ranged from 3300 to 10000 at Level 1 and from 6300 to 14000 at Level 2.

Table-2 BCFs ( ) : average value

Level	After 11 days	After 18 days	After 32 days	After 46 days	After 60 days	Table	Fig.
1	4800 3300 (4100)	4100 7100 (5600)	5000 8400 (6700)	5100 10000 (7600)	9500 6800 (8100)	10	8
2	8900 7700 (8300)	6300 8800 (7500)	14000 14000 (14000)	14000 14000 (14000)	13000 12000 (12000)	11	9

6.3 Calculation of BCFs at a steady-state (BCFss)

Because the variation of BCFs (average value) after 32, 46 and 60 days from the initiation of exposure were within ± 20 % of the average for these days' BCFs (see 6.2), it was evaluated that a steady-state was reached after 60 days. BCFss were calculated on the basis of these result.

(1) Concentrations of test substance in test water at a steady-state

The mean concentrations of the test substance in test water at a steady-state are shown in Table-3. The mean concentrations were 84 % at Level 1 and 74 % at Level 2 of their respective nominated concentrations.

Table-3 Concentrations of the test substance in test water at a steady-state

(Unit : μg/L)

Level	After 32 days	After 46 days	After 60 days	Average	Table	Fig.
1	0.840	0.862	0.812	0.838	7 , 10	6
2	0.0611	0.0795	0.0813	0.0740	8 , 11	

(2) BCFs at a steady-state (BCFss)

BCFss were calculated as follows.

Level 1	7700
Level 2	13000

6.4 Depuration test

After the test fish were exposed for 60 days, this depuration test was started by transferring them to another test tank (without test substance and dispersant).

The residual rates (%) of the test substance in test fish after 2, 15, 22 and 36 days from the initiation of depuration were calculated by comparison with the average concentration of the test substance in test fish at a steady-state (see Tables-12, 13, Figs. 10, 11).

The residual rates (%) plotted against duration of depuration are shown in Figs.12, 13.

On the basis of this result, the depuration half-value ($t_{1/2}$) is 15 days at Level 1 and 14 days at Level 2.

Table-4 Residual rate of test substance (Unit : %)

Level	After 2 days	After 15 days	After 22 days	After 36 days	Table	Fig.
1	110 110	45 47	30 12	27 22	12	10
2	110 140	56 41	67 34	32 14	13	11

6.5 Analysis in parts of test fish

The fish which were exposed for 60 days were separated into parts; integument, head, viscera and remaining matter were weighed separately. The integument consisted of the skin except head, scales, fin, alimentary canal or gills. The viscera consisted of internal organs except alimentary canal. The test substance in all the parts was determined in the same manner as 4.7. However, refinement and taking out of fine sample in the pretreatment for test fish (see 4.7.2(2)) were not performed.

Concentrations and BCFs of the test substance in each part were shown in Table-5.

Table-5 Concentrations and BCFs of the test substance in each part

Level	Parts	Concentration in each part (ng/g)	BCF	Table	Fig.
1	Integument	13300 7490	16000 8900	15	15
	Head	14100 9140	17000 11000		
	Viscera	24400 14100	29000 17000		
	Remainder Parts	9590 4610	11000 5500		
2	Integument	965 1270	13000 17000	16	16
	Head	1360 1840	18000 25000		
	Viscera	2310 3820	31000 52000		
	Remainder Parts	665 868	9000 12000		

6.6 Lipid content in test fish

The measured lipid contents in the test fish are shown as follows.

Before the experimental start	1.47 %
After the experimental completion	2.31 %

Lipid contents of after 32, 46 and 60 days are shown as follows.

(Unit : %)

Level	After 32 days	After 46 days	After 60 days	Average
1	2.31 2.56 (2.43)	3.11 2.12 (2.61)	2.54 2.09 (2.31)	2.46
2	3.08 1.63 (2.35)	2.77 2.31 (2.54)	1.77 1.80 (1.78)	2.23

( ) : average value

6.7 Results of test fish observation

No abnormality in behavior or appearance was noted.

7. Discussion

The change between lipid contents before the experimental starting and those after the experimental completion was over 25 %. Because the control of lipid contents in young fish of carp for test fish is very difficult and an individual difference of lipid contents is also large, it is considered that the lipid contents changed in this test.

8. Remarks

Instruments, apparatuses, special apparatuses and reagents, etc. for the test

(1) Instruments for fish care

Micro quantitative pump for supplying stock solution :
Tokyo Rika Kikai Co., Ltd. type GMW

Instrument for measuring concentration of dissolved oxygen :
Iijima Seimitsu Industries Co., Ltd.
type F-102

pH meter — Toa Electronics Ltd. type HM-14P

(2) Instruments, apparatuses, special apparatuses and reagents

Instruments and apparatuses

High-performance liquid chromatograph :
see page 18

Electronic analytical balance : Sartorius type LP4200S
Shimadzu Corporation type AEX-200B
Sartorius type BP301S
Metler Toledo type PB602

Infrared spectrophotometer : Shimadzu Corporation type FTIR-8200PC

Ultraviolet and visible spectrophotometer :
Shimadzu Corporation type UV-2200A

Rotary evaporator : Tokyo Rika Kikai Co., Ltd. type N
Yamato Scientific Co., Ltd type RE50
Tokyo Rika Kikai Co., Ltd. type N-NJ
Yamato Scientific Co., Ltd type RE52

Homogenizer (polytron) : Kinematica type PT3000
Kinematica type PT3100

Centrifuge : Kubota Syouji Co., Ltd type 6900
Hitachi Koki Co., Ltd. type CR21G

Special apparatuses

MEGA BOND ELUT C8 : Varian sample preparation products

Sep-Pak C_{18} : Nihon Waters K. K.

Reagents

Acetonitrile (HPLC grade) :	Wako Pure Chemical Industries, Ltd.
Tetrahydrofuran (HPLC grade) :	Kanto Chemical Co., Inc.
HCO-40 :	Nikko Chemicals Co., Ltd.
28 % Ammonia solution (guaranteed reagent):	Wako Pure Chemical Industries, Ltd.

(3) Instruments, apparatuses and reagents for gravimetric analysis of lipid content in test fish

Instruments and apparatuses

Electronic analytical balance :	Sartorius type BP301S
Rotary evaporator :	Tokyo Rika Kikai Co., Ltd. type N-1
	Tokyo Rika Kikai Co., Ltd. type N
Homogenizer (polytron) :	Kinematica type PT3000
	Kinematica type PT3100
Homogenizer (autocellmaster) :	Iuchiseieido Co., Ltd type CM-200
Vacuum pump :	Sinku Kiko Co., Ltd. type DA-20D
	Sinku Kiko Co., Ltd. type DAH-20C
Vacuum desiccator :	Iuchiseieido Co., Ltd. type VL

Reagents

Purified water :	Takasugi Seiyaku Co., Ltd.
Methanol (extra pure) :	Wako Pure Chemical Industries, Ltd.
Chloroform (guaranteed reagent) :	Kishida Chemical Co., Ltd.
Anhydrous sodium sulfate (extra pure) :	Katayama Chemical Industries Co., Ltd.

Table-6 Calculation table for recovery and blank test
(Analysis of test water)

Test No. 43705

Sample description	A	B	C	D	E	F
Standard 20.0μg/L	10955					
Recovery a	9903	1	2	-	36.2	90.4 %
Recovery b	9610	1	2	-	35.1	87.7 %
						Average
						89.1 %
Standard 20.0μg/L	10953					
Blank a	n.d.	1	2	-	-	
Blank b	n.d.	1	2	-	-	

(a, b : individual sample)

A : Peak area (μV·sec)

A(std) : Standard solution A(t) : Sample

B : Ratio of portion used for analysis

C : Final volume (mL)

D : Amount of blank in test water (ng)

E : Amount of test substance recovered (ng)

$E = P \times (A(t) / A(std)) / B \times C - D$

F : Recovery rate (%)

$F = E / Q \times 100$

P : Concentration of test substance in standard solution 20.0μg/L

Q : Amount of test substance added (40ng)

See Fig. 5

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Table-7 Calculation table for analysis of test water

(Level 1)

Test No. 43705

Sample description	A	I
Standard 20.0μg/L	10851	
Test water after 4 days	8713	0.902
Standard 20.0μg/L	10420	
Test water after 11 days	8531	0.919
Standard 20.0μg/L	10851	
Test water after 18 days	8545	0.884
Standard 20.0μg/L	10616	
Test water after 32 days	7942	0.840
Standard 20.0μg/L	10980	
Test water after 46 days	8431	0.862
Standard 20.0μg/L	10874	
Test water after 60 days	7863	0.812

Average concentration of test substance in test water 0.870 (S.D. 0.0399)

A : Peak area (μV·sec)

A(std) : Standard solution A(t) : Sample

B : Ratio of portion used for analysis 1

C : Final volume 2mL

F : Recovery rate 89.1%

H : Volume of test water taken out 40mL

I : Concentration of test substance in test water (μg/L)

$I = P \times (A(t) / A(std)) / B \times C / F \times 100 / H$

J : Average concentration of test substance in test water (μg/L)

$J = (I(1) + ... + I(n)) / n$

n : Number of test water analyses (n = 6)

I (1) : First analysis of test water I (n) : Last analysis of test water

$$S.D. = \sqrt{\frac{n \times \sum_{i=1}^{n} I(i)^2 - \left(\sum_{i=1}^{n} I(i) \right)^2}{n \times (n-1)}}$$

P : Concentration of test substance in standard solution 20.0μg/L

See Fig. 6

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Table-8 Calculation table for analysis of test water
(Level 2)

Test No. 43705

Sample description	A	I
Standard 20.0μg/L	10851	
Test water after 4 days	5892	0.0610
Standard 20.0μg/L	10420	
Test water after 11 days	7499	0.0808
Standard 20.0μg/L	10851	
Test water after 18 days	6357	0.0658
Standard 20.0μg/L	10616	
Test water after 32 days	5776	0.0611
Standard 20.0μg/L	10980	
Test water after 46 days	7775	0.0795
Standard 20.0μg/L	10874	
Test water after 60 days	7878	0.0813

Average concentration of test substance in test water 0.0716 (S.D. 0.01000)

A : Peak area (μV•sec)

A(std) : Standard solution A(t) : Sample

B : Ratio of portion used for analysis 1

C : Final volume 2mL

F : Recovery rate 89.1%

H : Volume of test water taken out 400mL

I : Concentration of test substance in test water (μg/L)

$I = P \times (A(t) / A(std)) / B \times C / F \times 100 / H$

J : Average concentration of test substance in test water (μg/L)

$J = (I(1) + ... + I(n)) / n$

n : Number of test water analyses (n = 6)

I (1) : First analysis of test water I (n) : Last analysis of test water

$$S.D. = \sqrt{\frac{n \times \sum_{i=1}^{n} I(i)^2 - \left(\sum_{i=1}^{n} I(i) \right)^2}{n \times (n-1)}}$$

P : Concentration of test substance in standard solution 20.0μg/L

See Fig. 6

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Table-9 Calculation table for recovery and blank test
(Analysis of test fish)

Test No. 43705

Sample description	A	C	D	E	F	G
Standard 20.0μg/L	10437					
Recovery a	7446	2/50	5	-	3570	71.3 %
Recovery b	7965	2/50	5	-	3820	76.3 %
						Average
						73.8 %
Standard 20.0μg/L	10688					
Blank a	n.d.	2/50	5	-	-	
Blank b	n.d.	2/50	5	-	-	

(a, b : individual sample)

A : Peak area (μV·sec)

A(std) : Standard solution A(t) : Sample

B : Ratio of portion used for analysis (fish homogenate) 1/2

C : Ratio of portion used for analysis (extracted solution)

D : Final volume (mL)

E : Amount of blank in test fish (ng)

F : Amount of test substance recovered (ng)

$F = P \times (A(t) / A(std)) / B / C \times D - E$

G : Recovery rate (%)

$G = F / Q \times 100$

P : Concentration of test substance in standard solution 20.0μg/L

Q : Amount of test substance added (5000ng)

See Fig. 7

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Table-10 Calculation table for analysis of test fish
(Level 1)

Test No. 43705

Sample description	A	D	G	K	H	J	M	O
Standard 20.0μg/L	10521							
Test fish after 11 days a	6820	10	5.00	4390	0.910	4800	4100	
Test fish after 11 days b	4701	10	5.00	3030	0.910	3300		
Standard 20.0μg/L	11141							
Test fish after 18 days a	6036	10	5.00	3670	0.902	4100	5600	
Test fish after 18 days b	10585	10	5.00	6430	0.902	7100		
Standard 20.0μg/L	10822							
Test fish after 32 days a	7015	10	5.00	4390	0.881	5000	6700	5500
Test fish after 32 days b	11814	10	5.00	7390	0.881	8400		
Standard 20.0μg/L	11106							
Test fish after 46 days a	7198	10	5.00	4390	0.862	5100	7600	6600
Test fish after 46 days b	14262	10	5.00	8700	0.862	10000		
Standard 20.0μg/L	10916							
Test fish after 60 days a	12879	10	5.00	7990	0.838	9500	8100	7500
Test fish after 60 days b	9128	10	5.00	5660	0.838	6800		
(a, b : individual sample)								
BCFss:	**7700**							

A : Peak area (μV·sec)
A(std) : Standard solution A(t) : Sample
B : Ratio of portion used for analysis 2/50
C : Final volume 5mL
D : Dilution factor
E : Average concentration of blank in analysis of control 0ng/g
F : Recovery rate 73.8%
G : Weight of fine sample (g)
K : Concentration of test substance in test fish (ng/g)
$K = \{ P \times (A(t) / A(std)) / B \times D \times C / G - E \} / F \times 100$
H : Average concentration of test substance in test water (μg/L)
$H = \{ I(n-2) + I(n-1) + I(n) \} / m$; n : Number of test water analyses ; m = 2 when n = 2, m = 3 when n ≧3
I : Concentration of test substance in test water (μg/L)
J : BCF
$J = K / H$
M : Average value of BCF(a) and BCF(b)
$M = \{ BCF(a) + BCF(b) \} / 2$
O : Average value of BCF
$O = \{ M(n-2) + M(n-1) + M(n) \} / 3$
P : Concentration of test substance in standard solution 20.0μg/L
$BCFss = [\{ K(n-2)a + K(n-2)b + K(n-1)a + K(n-1)b + K(n)a + K(n)b \} / 6] / [\{ I(n-2) + I(n-1) + I(n) \} / 3]$
See Fig. 8

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Table-11 Calculation table for analysis of test fish
(Level 2)

Test No. 43705

Sample description	A	D	G	K	H	J	M	O
Standard 20.0μg/L	10759							
Test fish after 11 days a	10052	1	5.00	633	0.0709	8900	8300	
Test fish after 11 days b	8695	1	5.00	547	0.0709	7700		
Standard 20.0μg/L	10630							
Test fish after 18 days a	6803	1	5.00	433	0.0692	6300	7500	
Test fish after 18 days b	9561	1	5.00	609	0.0692	8800		
Standard 20.0μg/L	10584							
Test fish after 32 days a	14613	1	5.00	935	0.0692	14000	14000	9900
Test fish after 32 days b	15383	1	5.00	984	0.0692	14000		
Standard 20.0μg/L	10556							
Test fish after 46 days a	14480	1	5.00	929	0.0688	14000	14000	12000
Test fish after 46 days b	15247	1	5.00	978	0.0688	14000		
Standard 20.0μg/L	10792							
Test fish after 60 days a	15105	1	5.00	948	0.0740	13000	12000	13000
Test fish after 60 days b	13748	1	5.00	863	0.0740	12000		
(a, b : individual sample)								
BCFss:	**13000**							

A : Peak area (μV·sec)
A(std) : Standard solution A(t) : Sample
B : Ratio of portion used for analysis 2/50
C : Final volume 5mL
D : Dilution factor
E : Average concentration of blank in analysis of control 0ng/g
F : Recovery rate 73.8%
G : Weight of fine sample (g)
K : Concentration of test substance in test fish (ng/g)
$K = \{ P \times (A(t) / A(std)) / B \times D \times C / G - E \} / F \times 100$
H : Average concentration of test substance in test water (μg/L)
$H = \{ I(n-2) + I(n-1) + I(n) \} / m$; n : Number of test water analyses ; m = 2 when n = 2, m = 3 when n ≧3
I : Concentration of test substance in test water (μg/L)
J : BCF
$J = K / H$
M : Average value of BCF(a) and BCF(b)
$M = \{ BCF(a) + BCF(b) \} / 2$
O : Average value of BCF
$O = \{ M(n-2) + M(n-1) + M(n) \} / 3$
P : Concentration of test substance in standard solution 20.0μg/L
$BCFss = [\{ K(n-2)a + K(n-2)b + K(n-1)a + K(n-1)b + K(n)a + K(n)b \} / 6] / [\{ I(n-2) + I(n-1) + I(n) \} / 3]$
See Fig. 9

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Table-12 Calculation table for analysis of test fish

Depuration test (Level 1)

Test No. 43705

Sample description	A	D	G	K	M
Standard 20.0μg/L	10733				
Test fish after 2 days a	10745	10	5.00	6780	110
Test fish after 2 days b	11663	10	5.00	7360	110
Standard 20.0μg/L	10970				
Test fish after 15 days a	9272	5	5.00	2860	45
Test fish after 15 days b	9842	5	5.00	3040	47
Standard 20.0μg/L	10881				
Test fish after 22 days a	6125	5	5.00	1910	30
Test fish after 22 days b	11955	1	5.00	744	12
Standard 20.0μg/L	10683				
Test fish after 36 days a	13800	2	5.00	1750	27
Test fish after 36 days b	10905	2	5.00	1380	22

(a, b : individual sample)

A : Peak area (μV·sec)

A(std) : Standard solution A(t) : Sample

B : Ratio of portion used for analysis 2/50

C : Final volume 5mL

D : Dilution factor

E : Average concentration of blank in analysis of control 0ng/g

F : Recovery rate 73.8%

G : Weight of fine sample (g)

K : Concentration of test substance in test fish (ng/g)

$K = \{ P \times A(t) / A(std) / B \times D \times C / G - E \} / F \times 100$

L : Average concentration of test substance in test fish at steady-state 6420ng/g

M : Residual rate (%)

$M = K / L \times 100$

P : Concentration of test substance in standard solution 20.0μg/L

See Fig. 10

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Table-13 Calculation table for analysis of test fish

Depuration test (Level 2)

Test No. 43705

Sample description	A	D	G	K	M
Standard 20.0μg/L	10764				
Test fish after 2 days a	16475	1	5.00	1040	110
Test fish after 2 days b	21101	1	5.00	1330	140
Standard 20.0μg/L	10810				
Test fish after 15 days a	8397	1	5.00	526	56
Test fish after 15 days b	6175	1	5.00	387	41
Standard 20.0μg/L	10462				
Test fish after 22 days a	9792	1	5.00	634	67
Test fish after 22 days b	4918	1	5.00	318	34
Standard 20.0μg/L	10950				
Test fish after 36 days a	4887	1	5.00	302	32
Test fish after 36 days b	2078	1	5.00	129	14

(a, b : individual sample)

A : Peak area (μV·sec)

A(std) : Standard solution A(t) : Sample

B : Ratio of portion used for analysis 2/50

C : Final volume 5mL

D : Dilution factor

E : Average concentration of blank in analysis of control 0ng/g

F : Recovery rate 73.8%

G : Weight of fine sample (g)

K : Concentration of test substance in test fish (ng/g)

$K = \{ P \times A(t) / A(std) / B \times D \times C / G - E \} / F \times 100$

L : Average concentration of test substance in test fish at steady-state 940ng/g

M : Residual rate (%)

$M = K / L \times 100$

P : Concentration of test substance in standard solution 20.0μg/L

See Fig. 11

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Table-14 Calculation table for analysis of test fish
(Control)

Test No. 43705

Sample description	A	E	G	I
Standard 20.0μg/L	10922			
Before the experimental start a	n.d.	-	3.00	-
Before the experimental start b	n.d.	-	3.00	-
Standard 20.0μg/L	10984			
After the experimental completion a	n.d.	-	5.00	-
After the experimental completion b	n.d.	-	5.00	-

(a, b : individual sample)

A : Peak area (μV·sec)

A(std) : Standard solution A(t) : Sample

B : Ratio of portion used for analysis 2/50

C : Final volume 5mL

E : Amount of blank in analysis of control (ng)

$E = P \times (A(t) / A(std)) / B \times C$

G : Weight of fine sample (g)

I : Concentration of blank in test fish (ng/g)

$I = E / G$

P : Concentration of test substance in standard solution 20.0μg/L

See Fig. 14

August 6, 2001 Name A. INOUE

Table-15 Calculation table for analysis in parts of test fish
(Level 1)

Test No. 43705

Sample description	A	D	G	K	H	J
Standard 20.0μg/L	10852					
Teguments* a	14572	4	1.37	13300	0.838	16000
Teguments* b	6961	4	1.16	7490	0.838	8900
*Including intestine and gill						
Standard 20.0μg/L	10789					
Head a	12145	10	2.70	14100	0.838	17000
Head b	7454	10	2.56	9140	0.838	11000
Standard 20.0μg/L	10506					
Viscera a	8493	4	0.449	24400	0.838	29000
Viscera b	3874	4	0.355	14100	0.838	17000
Standard 20.0μg/L	10917					
Remainder parts a	12516	10	4.05	9590	0.838	11000
Remainder parts b	5145	10	3.46	4610	0.838	5500

(a, b : individual sample)

A : Peak area (μV·sec)
A(std) : Standard solution A(t) : Sample
B : Ratio of portion used for analysis 2/50
C : Final volume 5mL
D : Dilution factor
E : Average concentration of blank in analysis of control 0ng/g
F : Recovery rate 73.8%
G : Weight of part (g)
K : Concentration of test substance in test fish (ng/g)
$K = \{ P \times A(t) / A(std) / B \times D \times C / G - E \} / F \times 100$
H : Average concentration of test substance in test water at steady-state (μg/L)
J : BCF
$J = K / H$
P : Concentration of test substance in standard solution 20.0μg/L
See Fig. 15

August 17, 2001 Name A. INOUE

Table-16 Calculation table for analysis in parts of test fish
(Level 2)

Test No. 43705

Sample description	A	D	G	K	H	J
Standard 20.0μg/L	10795					
Teguments* a	3755	1	1.22	965	0.0740	13000
Teguments* b	4348	1	1.07	1270	0.0740	17000
*Including intestine and gill						
Standard 20.0μg/L	10561					
Head a	10697	1	2.52	1360	0.0740	18000
Head b	12431	1	2.17	1840	0.0740	25000
Standard 20.0μg/L	10802					
Viscera a	2844	1	0.386	2310	0.0740	31000
Viscera b	4004	1	0.329	3820	0.0740	52000
Standard 20.0μg/L	10489					
Remainder parts a	8158	1	3.96	665	0.0740	9000
Remainder parts b	10085	1	3.75	868	0.0740	12000
(a, b : individual sample)						

A : Peak area (μV·sec)
A(std) : Standard solution A(t) : Sample
B : Ratio of portion used for analysis 2/50
C : Final volume 5mL
D : Dilution factor
E : Average concentration of blank in analysis of control 0ng/g
F : Recovery rate 73.8%
G : Weight of part (g)
K : Concentration of test substance in test fish (ng/g)
$K = \{ P \times A(t) / A(std) / B \times D \times C / G - E \} / F \times 100$
H : Average concentration of test substance in test water at steady-state (μg/L)
J : BCF
$J = K / H$
P : Concentration of test substance in standard solution 20.0μg/L
See Fig. 16

August 17, 2001 Name A. INOUE

Reference 1 Analytical results of dilution water

Sampling date February 16, 2001

March 19, 2001

Item	Unit	Measured value	Standard value	Detection limit
Total hardness (Ca, Mg)	mg/L	106	< 300 *1	
Suspended solid	mg/L	< 1	< 20 *2	1
pH	—	7.8	6.5 ~ 8.5 *3	
Total organic carbon	mg/L	0.9	< 2 *2	0.1
Chemical oxygen demand	mg/L	0.3	< 5 *3	0.1
Residual chlorine	mg/L	< 0.01	< 0.02 *3	0.01
Ammonia nitrogen	mg/L	< 0.01	< 1 *3	0.01
Total cyan	mg/L	< 0.01	n.d. *3	0.01
Alkalinity	mg/L	92.7	—	
Electric conductivity	μs/cm	379	—	
Organic phosphorus	mg/L	< 0.1	n.d. *3	0.1
Alkylmercury	mg/L	< 0.0005	n.d. *3	0.0005
Mercury	mg/L	< 0.0005	< 0.0005 *3	0.0005
Cadmium	mg/L	< 0.001	< 0.01 *3	0.001
Cr^{6+}	mg/L	< 0.02	< 0.05 *3	0.02
Lead	mg/L	< 0.005	< 0.1 *3	0.005
Arsenic	mg/L	0.002	< 0.05 *3	0.002
Iron	mg/L	< 0.01	< 1.0 *3	0.01
Copper	mg/L	< 0.005	< 0.005 *3	0.005
Cobalt	mg/L	< 0.001	< 0.001 *5	0.001
Manganese	mg/L	< 0.01	< 0.05 *1	0.01
Zinc	mg/L	< 0.01	< 1.0 *1	0.01
Aluminium	mg/L	< 0.001	< 0.2 *1	0.001
Nickel	mg/L	< 0.001	< 0.01 *1	0.001
Silver	mg/L	< 0.0001	< 0.0001 *5	0.0001
Organochlorine pesticides				
1,2-Dichloropropane	mg/L	< 0.0001	< 0.06 *4	0.0001
Chlorothalonil	mg/L	< 0.0002	< 0.04 *4	0.0002
Propyzamide	mg/L	< 0.0002	< 0.008 *4	0.0002
Chlornitrofen	mg/L	< 0.0001	< 0.0001 *1	0.0001
Simazine	mg/L	< 0.0003	< 0.003 *4	0.0003
Thiobencarb	mg/L	< 0.001	< 0.02 *4	0.001
Organophosphorous pesticides				
Diazinon	mg/L	< 0.0002	< 0.005 *4	0.0002
Isoxathion	mg/L	< 0.0002	< 0.008 *4	0.0002
Fenitrothion	mg/L	< 0.0002	< 0.003 *4	0.0002
EPN	mg/L	< 0.0002	< 0.006 *4	0.0002
Dichlorvos	mg/L	< 0.0002	< 0.01 *4	0.0002
Iprobenfos	mg/L	< 0.0002	< 0.008 *4	0.0002
PCB	mg/L	< 0.0005	n.d. *4	0.0005

*1 Ministerial ordinance of the Ministry of Health and Welfare No.69 (Revised December 21, 1992)

*2 OECD Guidelines for Testing of Chemicals, Fish, Early-life Stage Toxicity Test (Guideline 210, July 17, 1992)

*3 Water quality criteria for fisheries (Shadanhozin Nihon Suisansigen Hogokyokai, March 1983)

*4 Environmental Quality Standards for Water Pollutants No.14 (Revised February 22, 1999, Environment Agency)

*5 OECD Guidelines for Testing of Chemicals, Bioconcentration : Flow-through Fish Test (Guideline 305, June 14, 1996)

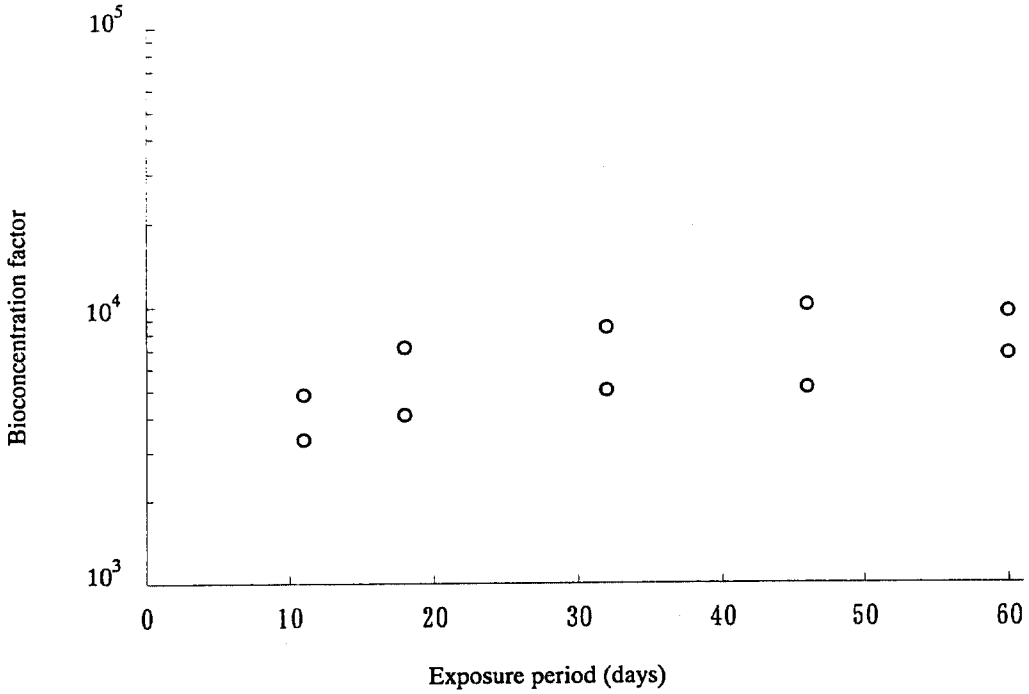


Fig. 1 Correlation between exposure period and bioconcentration factor (Level 1).

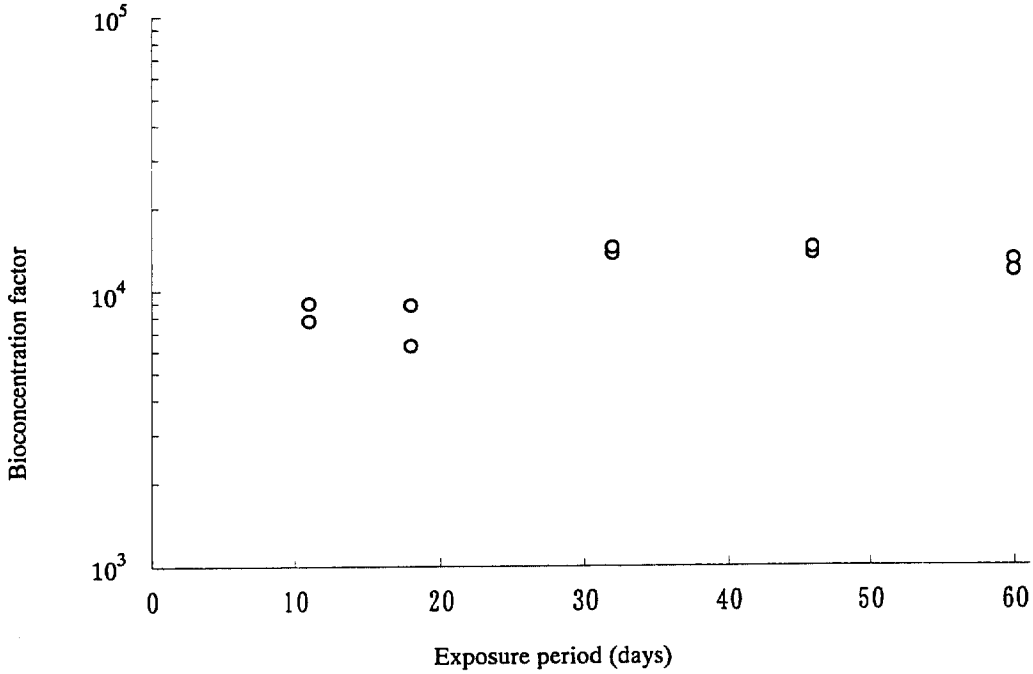
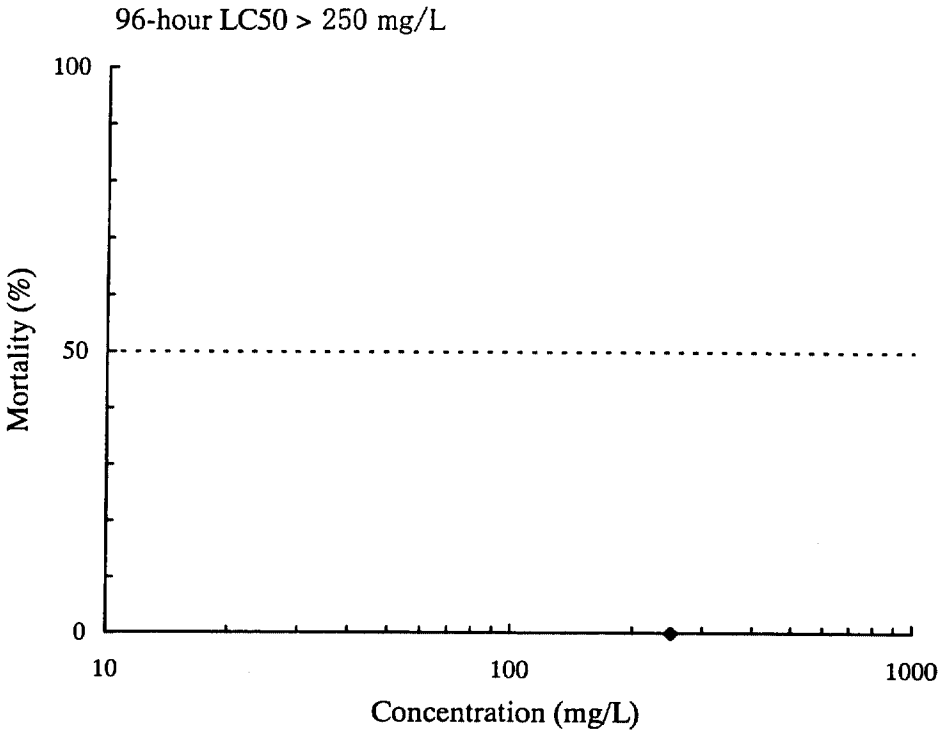


Fig. 2 Correlation between exposure period and bioconcentration factor (Level 2).

August 13, 2001 Name A. INOUE



Concentration (mg/L)	Cumulative Mortality (%)			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
250	0	0	0	0

Fig. 3 Concentration - Mortality Curve.

Date : April 24, 2001 Name Tadayoshi Tonai

Test No. 43705

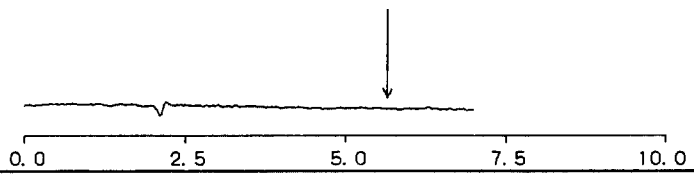
Solvent

Operating date : Apr. 6, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
Total		0		0.00

Peak position

n.d.

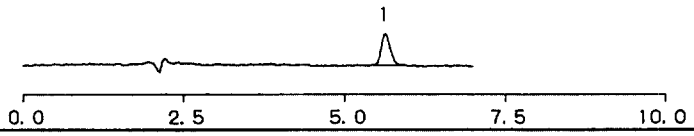


Operator : Akemi Inoue

Standard solution 10.0 μg/L

Operating date : Apr. 6, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.63	5237	538	100.00
Total		5237		100.00

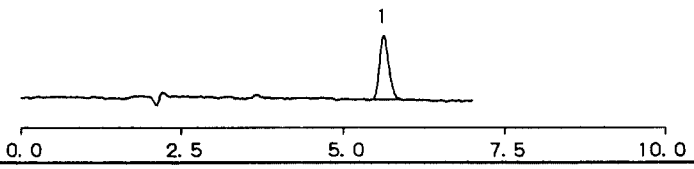


Operator : Akemi Inoue

Standard solution 20.0 μg/L

Operating date : Apr. 6, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.62	10893	1094	100.00
Total		10893		100.00

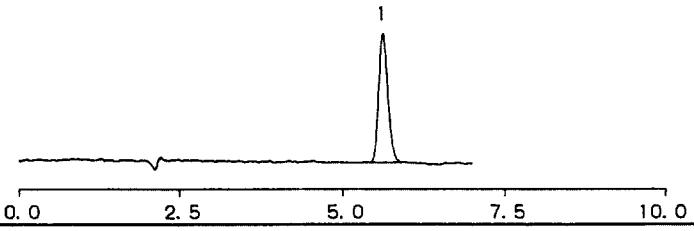


Operator : Akemi Inoue

Standard solution 40.0 μg/L

Operating date : Apr. 6, 2001

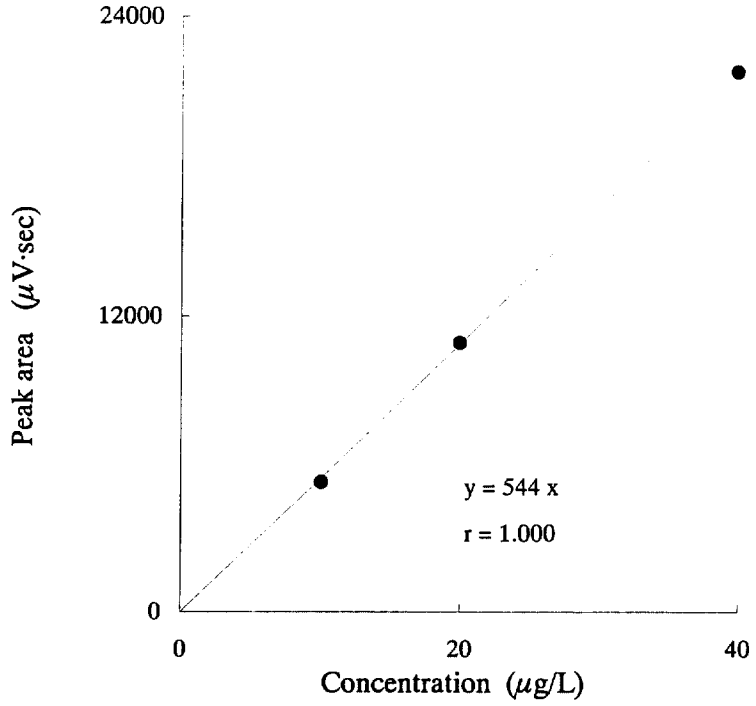
PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.62	21787	2192	100.00
Total		21787		100.00



Operator : Akemi Inoue

Fig. 4 - 1 Chromatogram of HPLC analysis for calibration curve.

Date : Apr. 6, 2001 Name : A. INOUE



Concentration (μg/L)	Peak area (μV·sec)
10.0	5237
20.0	10893
40.0	21787

Conditions of HPLC analysis

Instrument	:	Shimadzu LC-10AT, SPD-10AV
Sample	:	TINUVIN 343
Solvent	:	Acetonitrile
Auto samp. Injector	:	M&S 231XL
Injection vol.	:	100μL
Column	:	L-column ODS
Size	:	15cm × 4.6mm I.D.
Column temp.	:	35℃
Eluent	:	Acetonitrile / tetrahydrofuran (98/2 V/V)
Flow rate	:	1.0mL/min.
Detector	:	UV
Wave length	:	338nm
Output	:	2V/AU

Fig. 4 - 2 Calibration curve and conditions of HPLC analysis for TINUVIN 343 .

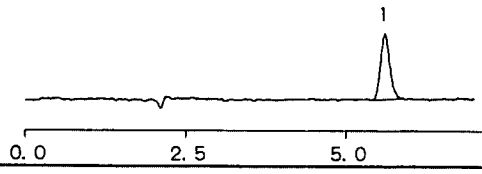
August 20, 2001 Name A. INOUE

Test No. 43705

Standard solution 20.0 ug/L

Operating date : Apr. 9, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.60	10955	1096	100.00
Total		10955		100.00

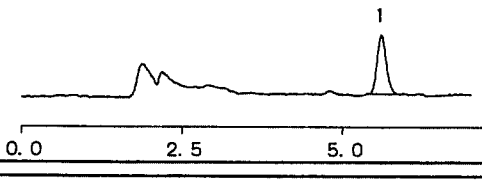


Operator : Akemi Inoue

Recovery test from test water a

Operating date : Apr. 9, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.59	9903	1010	100.00
Total		9903		100.00

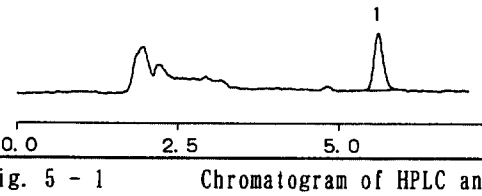


Operator : Akemi Inoue

Recovery test from test water b

Operating date : Apr. 9, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.60	9610	969	100.00
Total		9610		100.00



Operator : Akemi Inoue

Fig. 5 - 1 Chromatogram of HPLC analysis for recovery and blank test (analysis of test water).

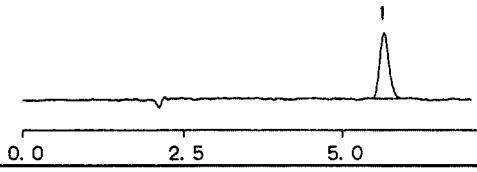
Date : Apr. 9, 2001 Name : A. INOUE

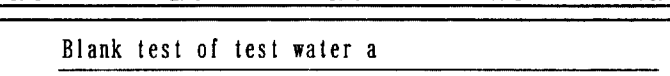
Test No. 43705

Standard solution 20.0 μg/L

Operating date : Apr. 9, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.64	10953	1099	100.00
Total		10953		100.00





Operator : Akemi Inoue

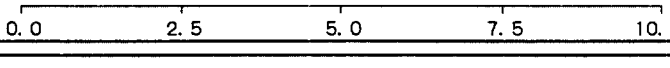
Blank test of test water a

Operating date : Apr. 9, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
Total		0		0.00

Peak position

n.d.



Operator : Akemi Inoue

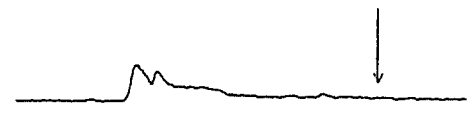
Blank test of test water b

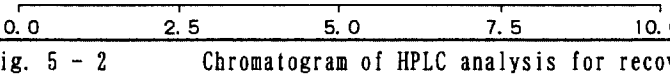
Operating date : Apr. 9, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
Total		0		0.00

Peak position

n.d.





Operator : Akemi Inoue

Fig. 5 - 2 Chromatogram of HPLC analysis for recovery and blank test (analysis of test water).

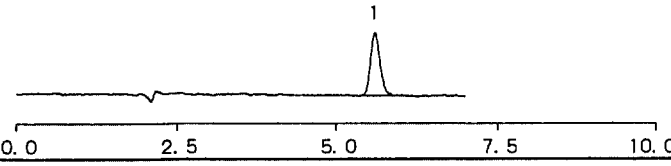
Date : Apr. 9, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : May. 1, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.59	10851	1054	100.00
Total		10851		100.00

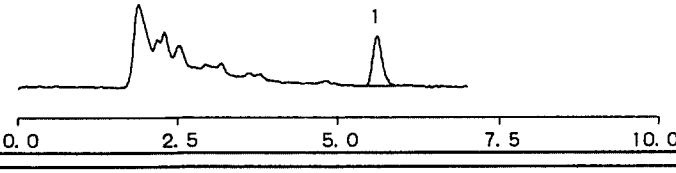


Operator : Akemi Inoue

Test water after 4 days (Level 1)

Operating date : May. 1, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.60	8713	848	100.00
Total		8713		100.00

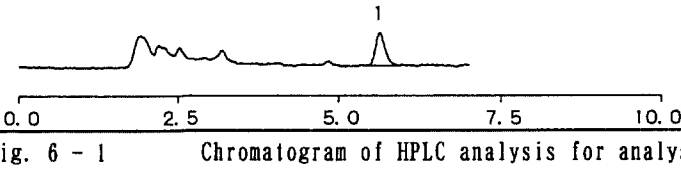


Operator : Akemi Inoue

Test water after 4 days (Level 2)

Operating date : May. 1, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.62	5892	562	100.00
Total		5892		100.00



Operator : Akemi Inoue

Fig. 6 - 1 Chromatogram of HPLC analysis for analysis of test water.

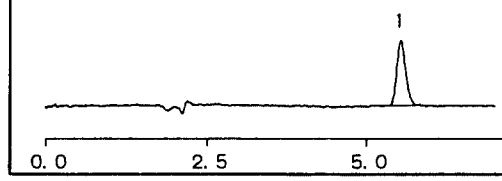
Date : May. 1, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : May. 8, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.53	10420	1082	100.00
Total		10420		100.00

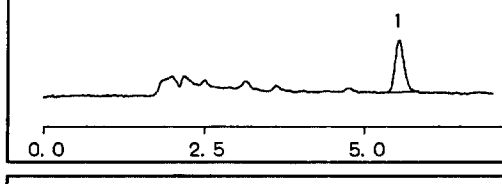


Operator : Akemi Inoue

Test water after 11 days (Level 1)

Operating date : May. 8, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.54	8531	883	100.00
Total		8531		100.00

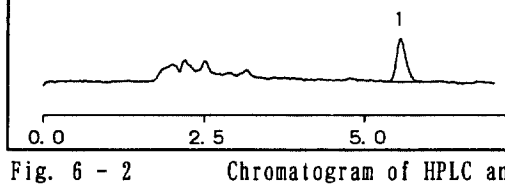


Operator : Akemi Inoue

Test water after 11 days (Level 2)

Operating date : May. 8, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.55	7499	740	100.00
Total		7499		100.00



Operator : Akemi Inoue

Fig. 6 - 2 Chromatogram of HPLC analysis for analysis of test water.

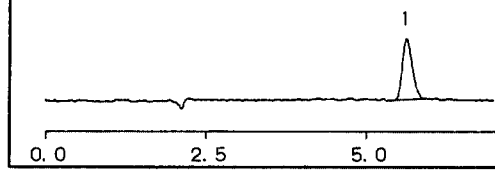
Date : May. 8, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : May. 15, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.63	10851	1019	100.00
Total		10851		100.00

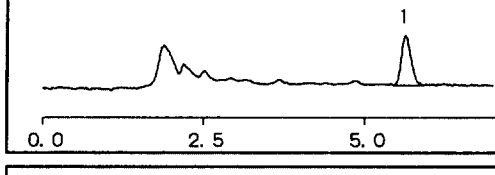


Operator : Akemi Inoue

Test water after 18 days (Level 1)

Operating date : May. 15, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.63	8545	841	100.00
Total		8545		100.00

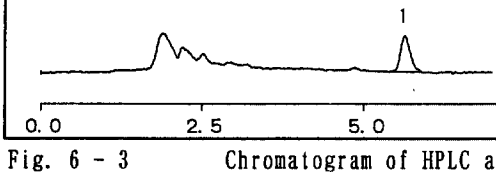


Operator : Akemi Inoue

Test water after 18 days (Level 2)

Operating date : May. 15, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.64	6357	621	100.00
Total		6357		100.00



Operator : Akemi Inoue

Fig. 6 - 3 Chromatogram of HPLC analysis for analysis of test water.

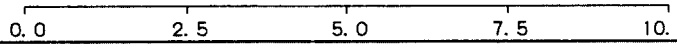
Date : May. 15, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : May. 29, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.66	10616	985	100.00
Total		10616		100.00

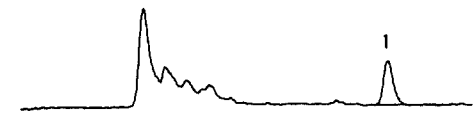


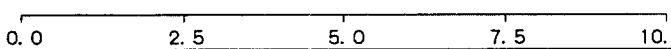
Operator : Akemi Inoue

Test water after 32 days (Level 1)

Operating date : May. 29, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.68	7942	748	100.00
Total		7942		100.00



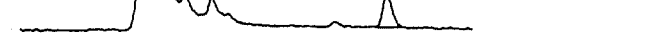


Operator : Akemi Inoue

Test water after 32 days (Level 2)

Operating date : May. 29, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.67	5776	564	100.00
Total		5776		100.00



Operator : Akemi Inoue

Fig. 6 - 4 Chromatogram of HPLC analysis for analysis of test water.

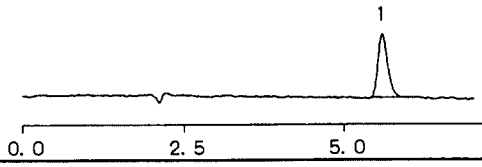
Date : May. 29, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jun. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	10980	1062	100.00
Total		10980		100.00

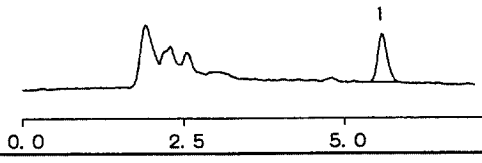


Operator : Akemi Inoue

Test water after 46 days (Level 1)

Operating date : Jun. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.56	8431	828	100.00
Total		8431		100.00

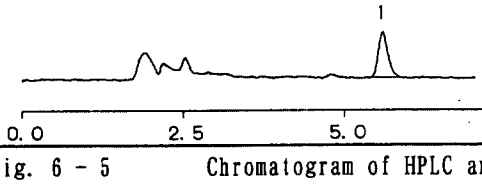


Operator : Akemi Inoue

Test water after 46 days (Level 2)

Operating date : Jun. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	7775	771	100.00
Total		7775		100.00



Operator : Akemi Inoue

Fig. 6 - 5 Chromatogram of HPLC analysis for analysis of test water.

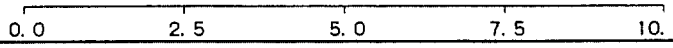
Date : Jun. 12, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jun. 26, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5. 59	10874	953	100. 00
Total		10874		100. 00

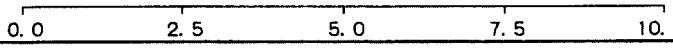


Operator : Akemi Inoue

Test water after 60 days (Level 1)

Operating date : Jun. 26, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5. 60	7863	695	100. 00
Total		7863		100. 00

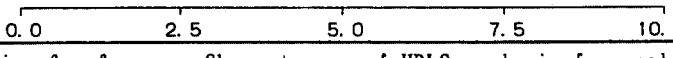


Operator : Akemi Inoue

Test water after 60 days (Level 2)

Operating date : Jun. 26, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5. 58	7878	721	100. 00
Total		7878		100. 00

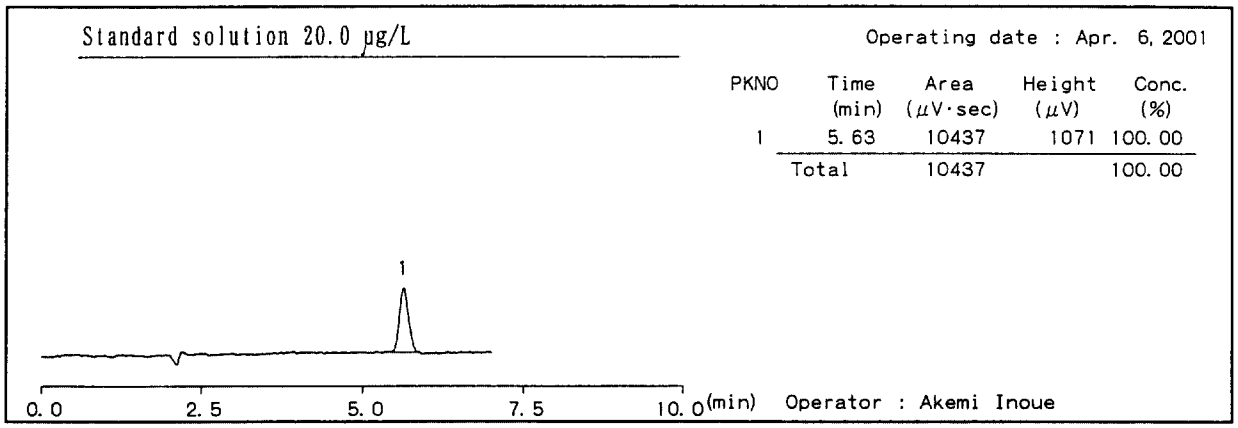


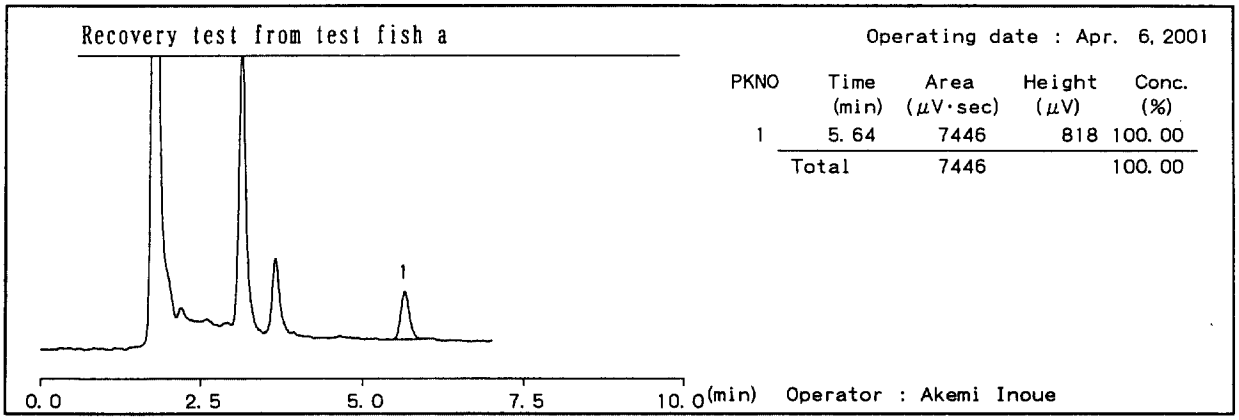
Operator : Akemi Inoue

Fig. 6 - 6 Chromatogram of HPLC analysis for analysis of test water.

Date : Jun. 26, 2001 Name : A. INOUE

Test No. 43705





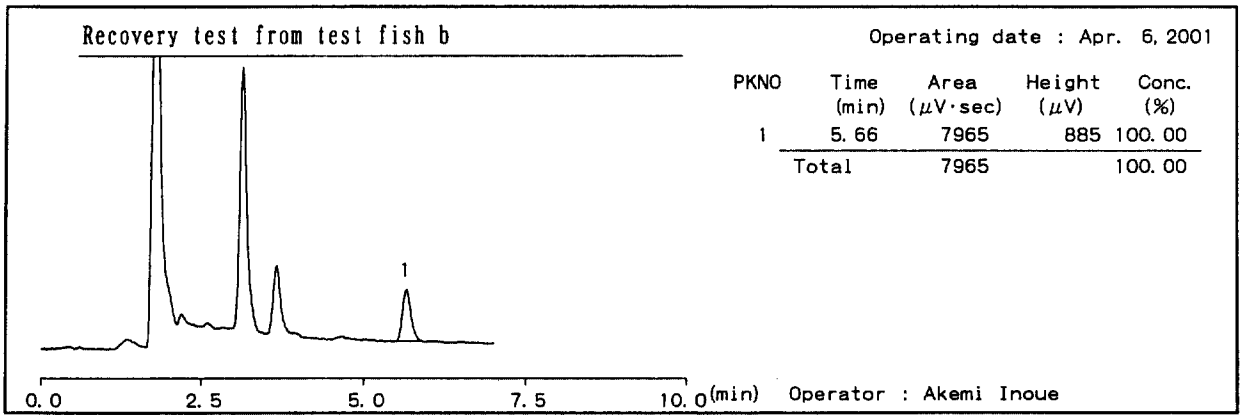


Fig. 7 - 1 Chromatogram of HPLC analysis for recovery and blank test (analysis of test fish).

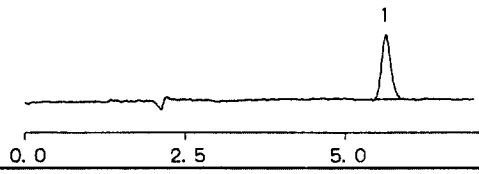
Date : Apr. 6, 2001 Name : A. INOUE

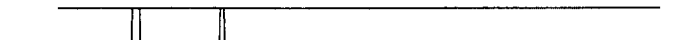
Test No. 43705

Standard solution 20.0 μg/L

Operating date : Apr. 6, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.62	10688	1077	100.00
Total		10688		100.00



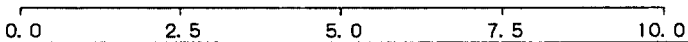


Operator : Akemi Inoue

Blank test of test fish a

Operating date : Apr. 6, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
Total		0		0.00

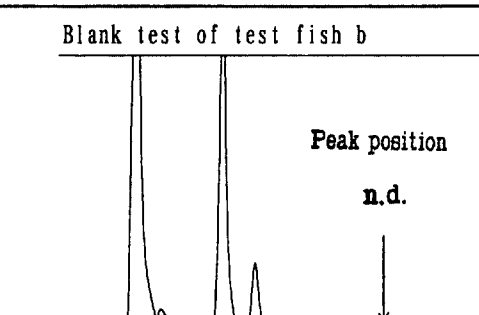


Operator : Akemi Inoue

Blank test of test fish b

Operating date : Apr. 6, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
Total		0		0.00



Operator : Akemi Inoue

Fig. 7 - 2 Chromatogram of HPLC analysis for recovery and blank test (analysis of test fish).

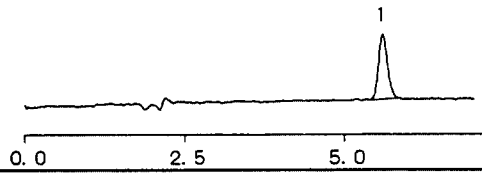
Date : Apr. 6, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : May. 8, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.59	10521	1078	100.00
Total		10521		100.00

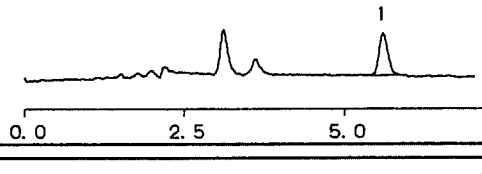


Operator : Akemi Inoue

Test fish after 11 days (Level 1-a) D·10

Operating date : May. 8, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.59	6820	711	100.00
Total		6820		100.00

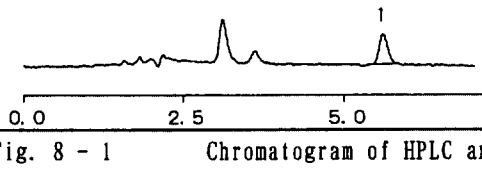


Operator : Akemi Inoue

Test fish after 11 days (Level 1-b) D·10

Operating date : May. 8, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.59	4701	510	100.00
Total		4701		100.00



Operator : Akemi Inoue

Fig. 8 - 1 Chromatogram of HPLC analysis for test fish (Level 1).

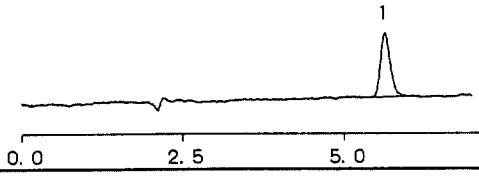
Date : May. 8, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : May. 15, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.63	11141	1073	100.00
Total		11141		100.00

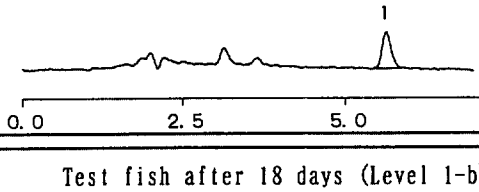


Operator : Akemi Inoue

Test fish after 18 days (Level 1-a) D·10

Operating date : May. 15, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.63	6036	618	100.00
Total		6036		100.00

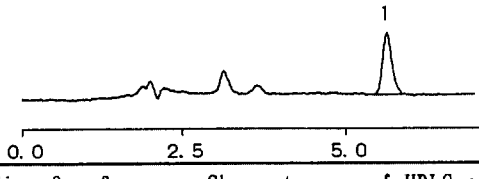


Operator : Akemi Inoue

Test fish after 18 days (Level 1-b) D·10

Operating date : May. 15, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.63	10585	1058	100.00
Total		10585		100.00

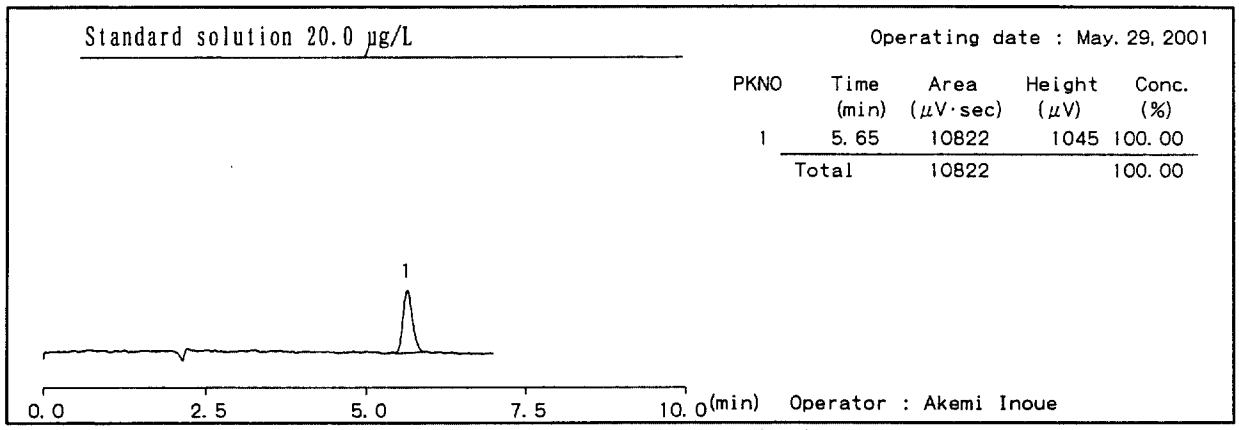


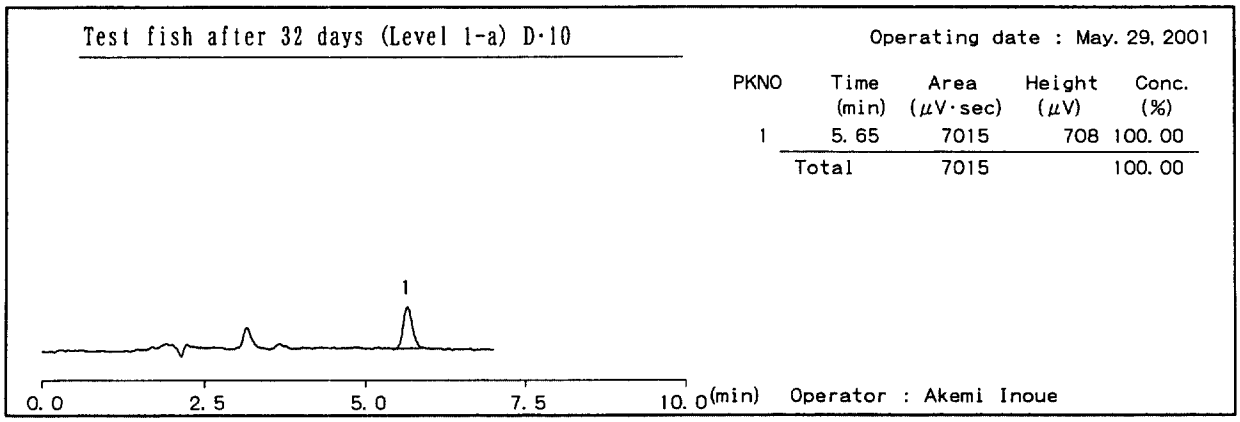
Operator : Akemi Inoue

Fig. 8 - 2 Chromatogram of HPLC analysis for test fish (Level 1).

Date : May. 15, 2001 Name : A. INOUE

Test No. 43705





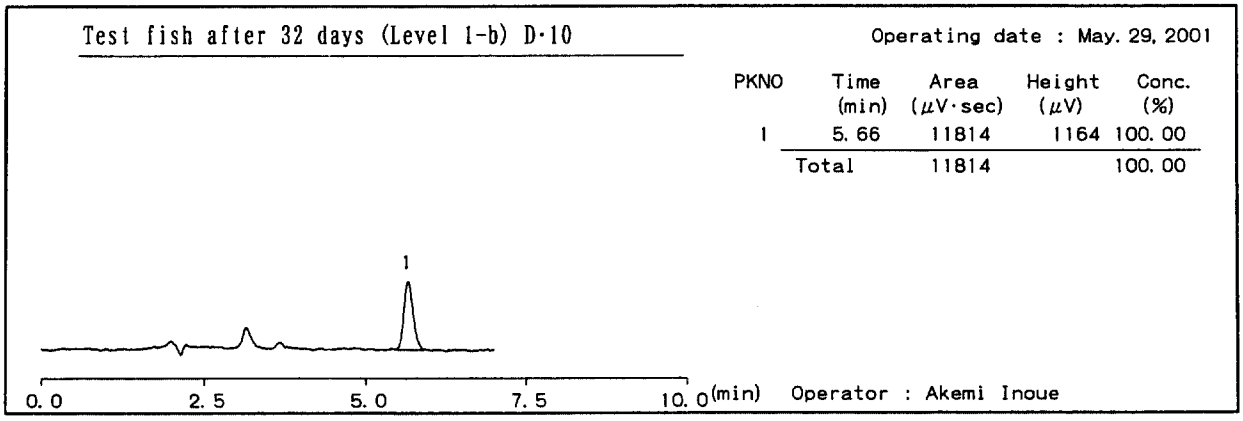


Fig. 8 - 3 Chromatogram of HPLC analysis for test fish (Level 1).

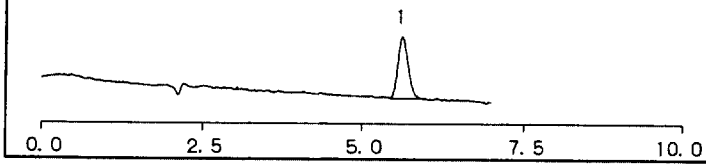
Date : May. 29, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jun. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.62	11106	1023	100.00
Total		11106		100.00

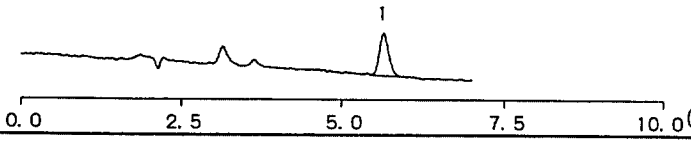


Operator : Akemi Inoue

Test fish after 46 days (Level 1-a) D·10

Operating date : Jun. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.64	7198	725	100.00
Total		7198		100.00

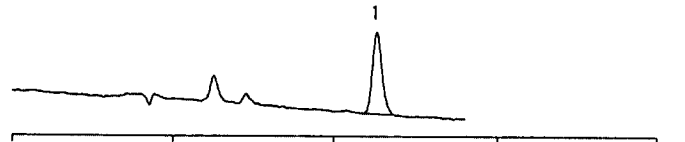


Operator : Akemi Inoue

Test fish after 46 days (Level 1-b) D·10

Operating date : Jun. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.65	14262	1398	100.00
Total		14262		100.00



Operator : Akemi Inoue

Fig. 8 - 4 Chromatogram of HPLC analysis for test fish (Level 1).

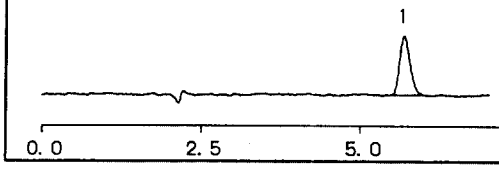
Date : Jun. 12, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jun. 26, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.67	10916	984	100.00
Total		10916		100.00

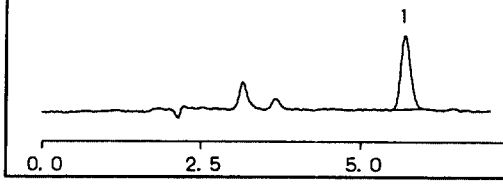


Operator : Akemi Inoue

Test fish after 60 days (Level 1-a) D·10

Operating date : Jun. 26, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.68	12879	1258	100.00
Total		12879		100.00

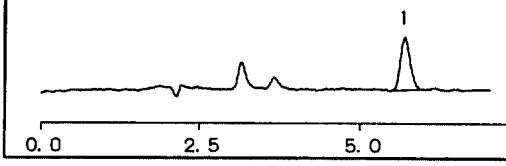


Operator : Akemi Inoue

Test fish after 60 days (Level 1-b) D·10

Operating date : Jun. 26, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.69	9128	902	100.00
Total		9128		100.00

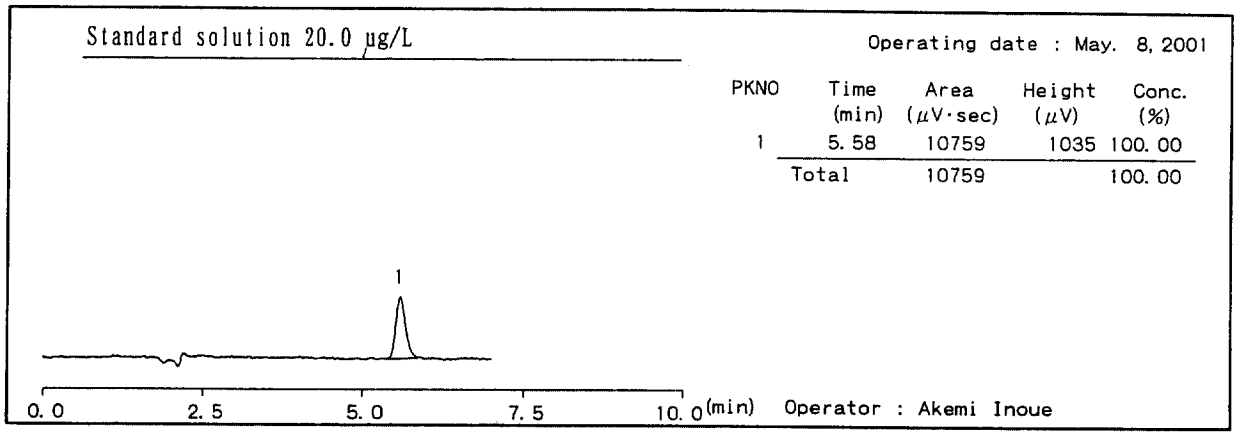


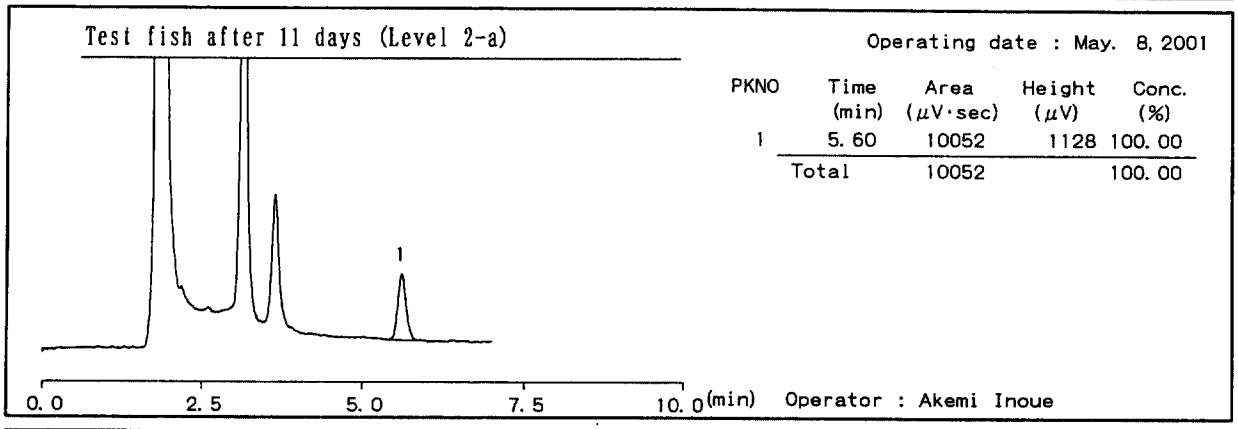
Operator : Akemi Inoue

Fig. 8 - 5 Chromatogram of HPLC analysis for test fish (Level 1).

Date : Jun. 26, 2001 Name : A. INOUE

Test No. 43705





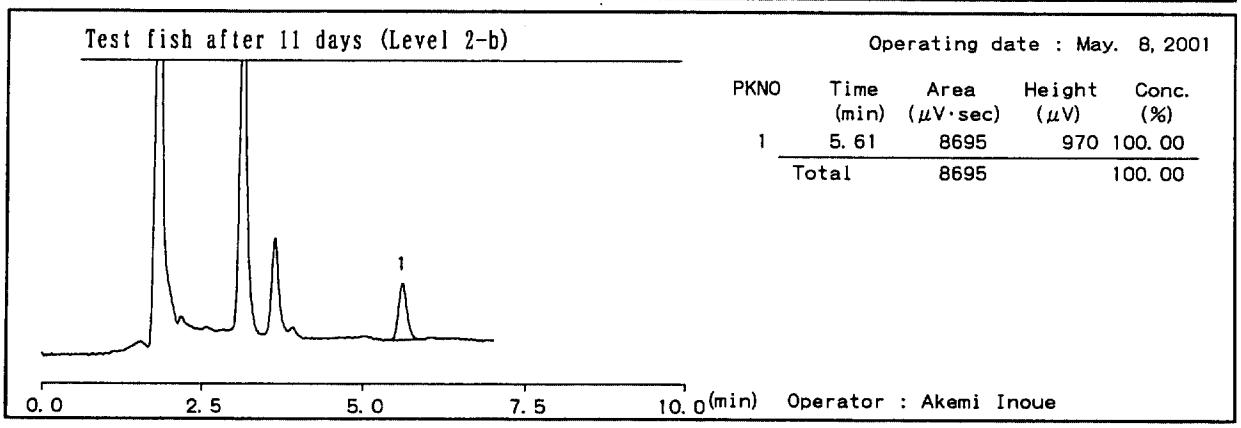
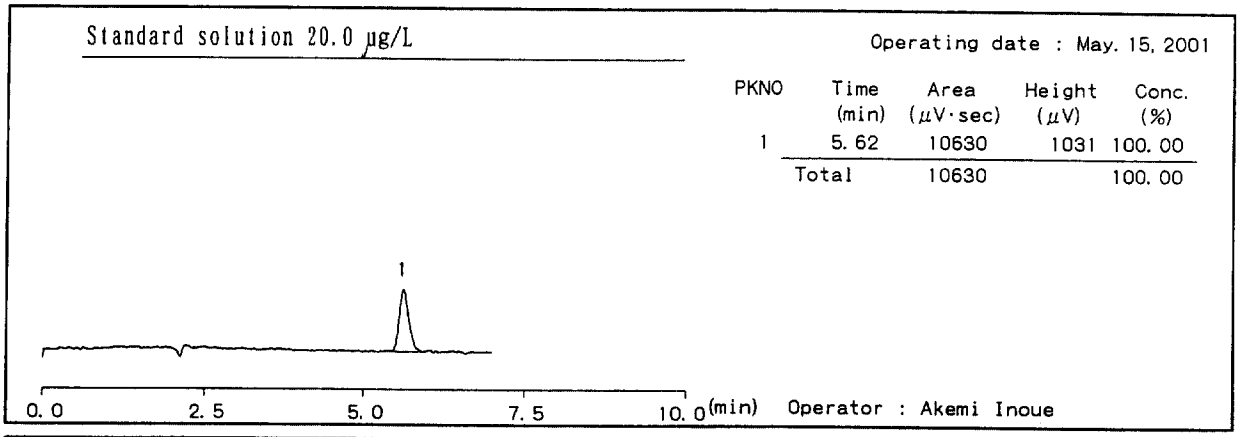
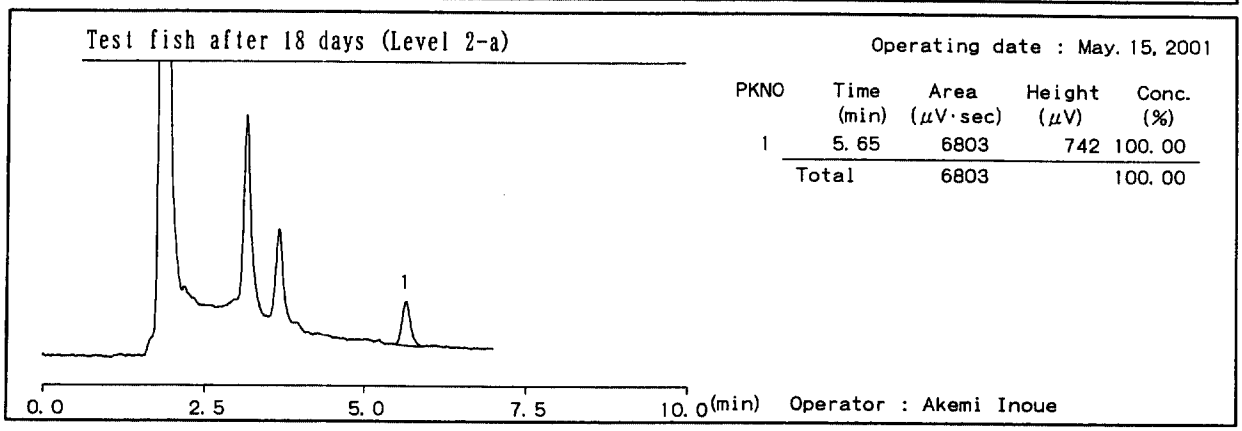


Fig. 9 - 1 Chromatogram of HPLC analysis for test fish (Level 2).

Date : May. 8, 2001 Name : A. INOUE

Test No. 43705





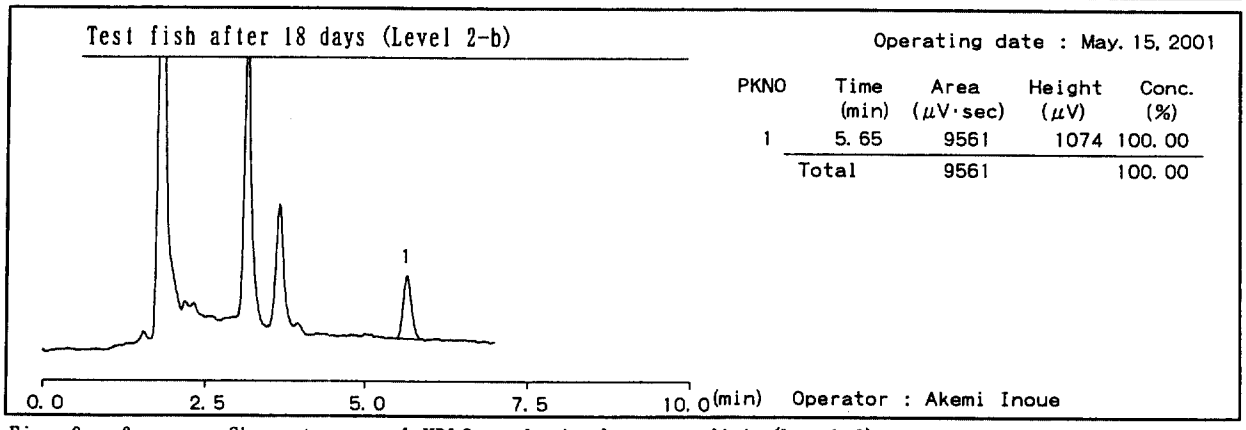


Fig. 9 - 2 Chromatogram of HPLC analysis for test fish (Level 2).

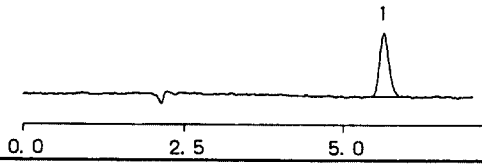
Date : May. 15, 2001 Name : A. INOUE

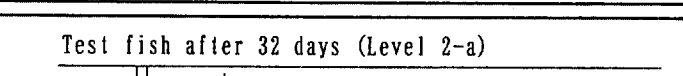
Test No. 43705

Standard solution 20.0 μg/L

Operating date : May. 29, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5. 63	10584	1059	100. 00
Total		10584		100. 00



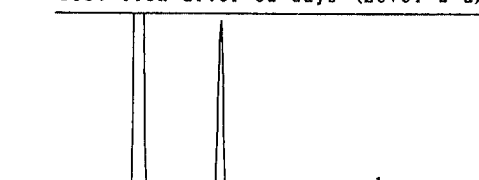


Operator : Akemi Inoue

Test fish after 32 days (Level 2-a)

Operating date : May. 29, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5. 65	14613	1628	100. 00
Total		14613		100. 00

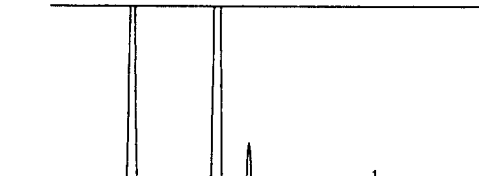


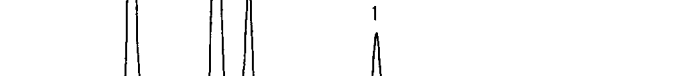
Operator : Akemi Inoue

Test fish after 32 days (Level 2-b)

Operating date : May. 29, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5. 66	15383	1690	100. 00
Total		15383		100. 00



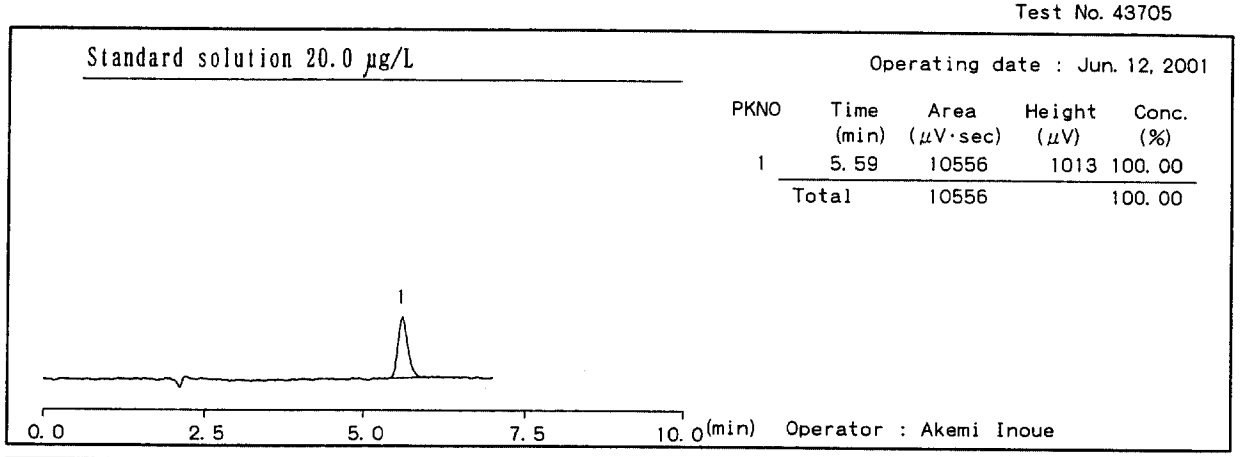


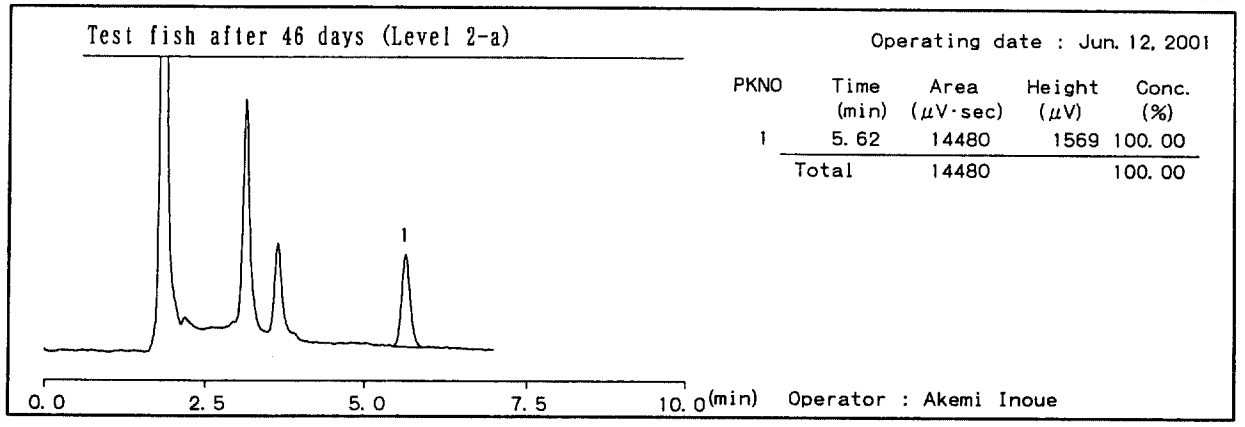
Operator : Akemi Inoue

Fig. 9 - 3 Chromatogram of HPLC analysis for test fish (Level 2).

Date : May. 29, 2001 Name : A. INOUE

Test No. 43705





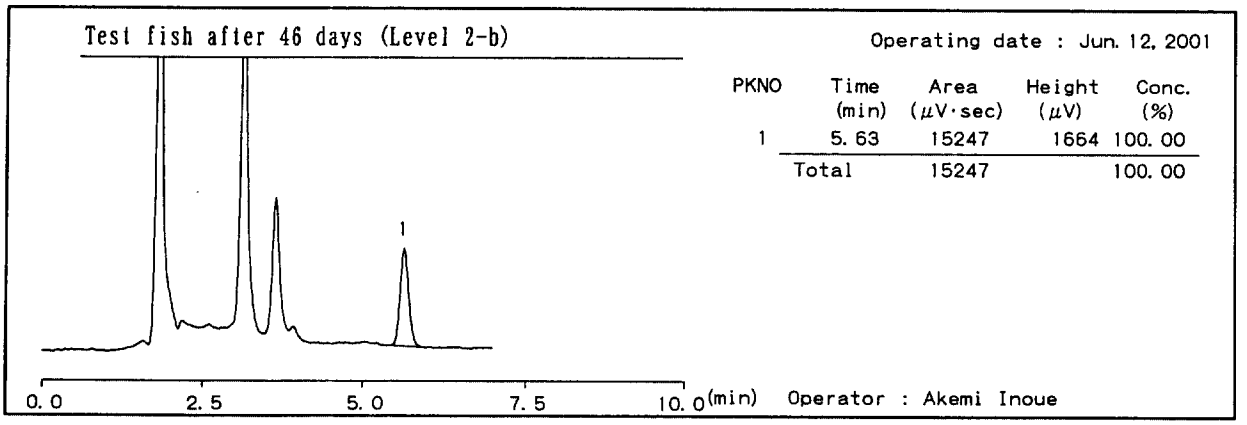


Fig. 9 - 4 Chromatogram of HPLC analysis for test fish (Level 2).

Date : Jun. 12, 2001 Name : A. INOUE

Test No. 43705

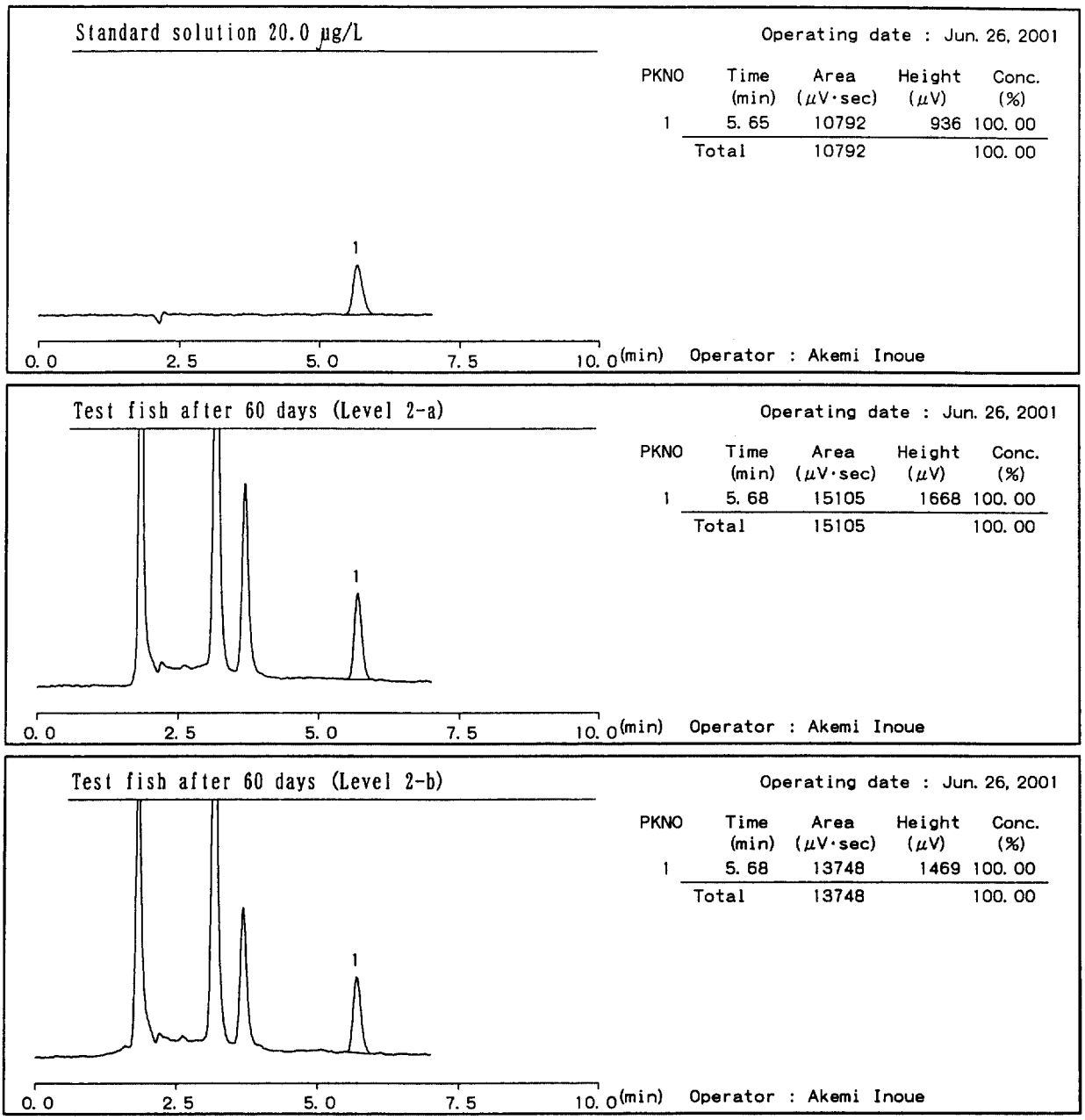


Fig. 9 - 5 Chromatogram of HPLC analysis for test fish (Level 2).

Date : Jun. 26, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jun. 29, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	10733	943	100.00
Total		10733		100.00

Operator : Akemi Inoue

Test fish after 2 days(depuration test)(Level 1-a) D·10

Operating date : Jun. 29, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.59	10745	1048	100.00
Total		10745		100.00

Operator : Akemi Inoue

Test fish after 2 days(depuration test)(Level 1-b) D·10

Operating date : Jun. 29, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.59	11663	1149	100.00
Total		11663		100.00

Operator : Akemi Inoue

Fig. 10 - 1 Chromatogram of HPLC analysis for test fish(depuration test, Level 1).

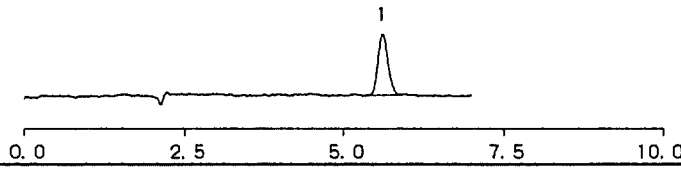
Date : Jun. 29, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jul. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.61	10970	1023	100.00
Total		10970		100.00

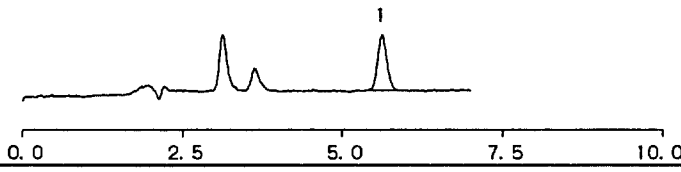


Operator : Akemi Inoue

Test fish after 15 days (depuration test) (Level 1-a) D·5

Operating date : Jul. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.62	9272	949	100.00
Total		9272		100.00

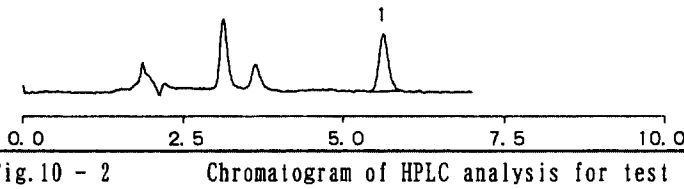


Operator : Akemi Inoue

Test fish after 15 days (depuration test) (Level 1-b) D·5

Operating date : Jul. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.62	9842	975	100.00
Total		9842		100.00



Operator : Akemi Inoue

Fig. 10 - 2 Chromatogram of HPLC analysis for test fish (depuration test, Level 1).

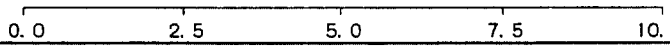
Date : Jul. 12, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jul. 19, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.43	10881	1036	100.00
Total		10881		100.00

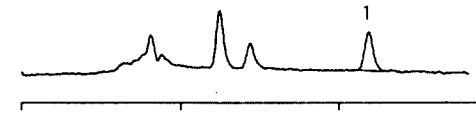


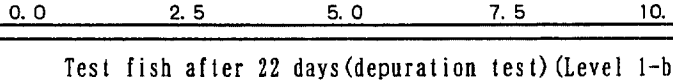
Operator : Akemi Inoue

Test fish after 22 days (depuration test) (Level 1-a) D·5

Operating date : Jul. 19, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.45	6125	649	100.00
Total		6125		100.00



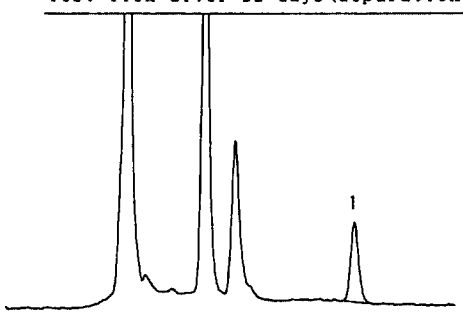


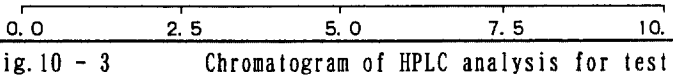
Operator : Akemi Inoue

Test fish after 22 days (depuration test) (Level 1-b)

Operating date : Jul. 19, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.46	11955	1360	100.00
Total		11955		100.00





Operator : Akemi Inoue

Fig. 10 - 3 Chromatogram of HPLC analysis for test fish (depuration test, Level 1).

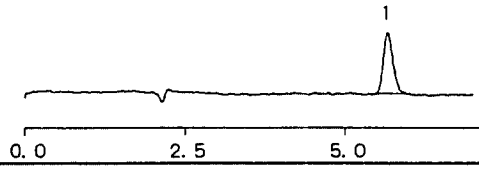
Date : Jul. 19, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Aug. 2, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.65	10683	1015	100.00
Total		10683		100.00

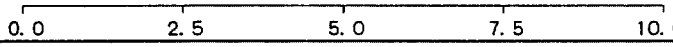


Operator : Akemi Inoue

Test fish after 36 days (depuration test) (Level 1-a) D·2

Operating date : Aug. 2, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.77	13800	1417	100.00
Total		13800		100.00

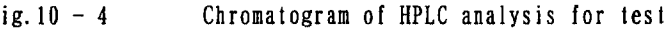


Operator : Akemi Inoue

Test fish after 36 days (depuration test) (Level 1-b) D·2

Operating date : Aug. 2, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.70	10905	1157	100.00
Total		10905		100.00

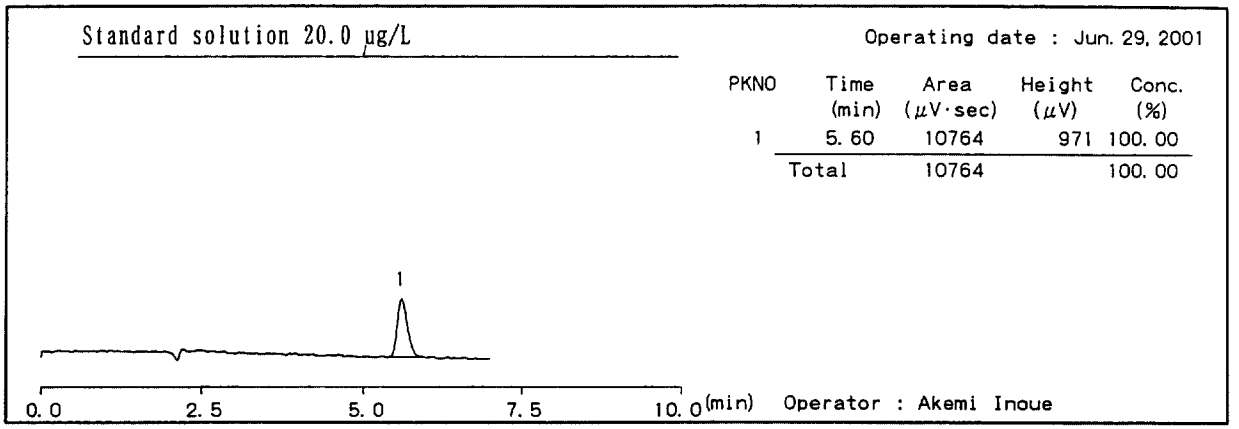


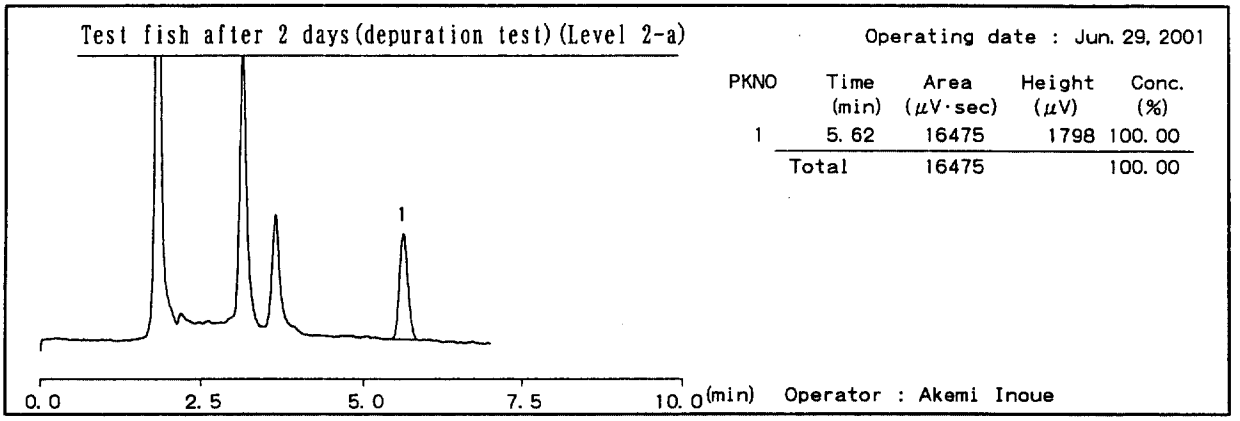
Operator : Akemi Inoue

Fig. 10 - 4 Chromatogram of HPLC analysis for test fish (depuration test, Level 1).

Date : Aug. 2, 2001 Name : A. INOUE

Test No. 43705





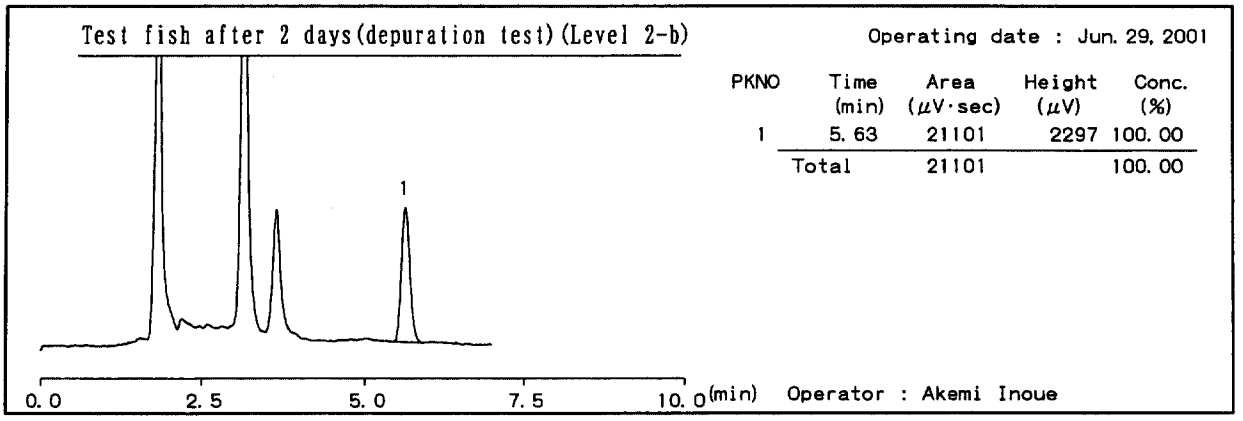


Fig. 11 - 1 Chromatogram of HPLC analysis for test fish (depuration test, Level 2).

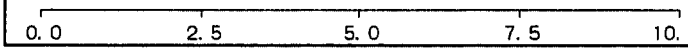
Date : Jun. 29, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jul. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	10810	981	100.00
Total		10810		100.00

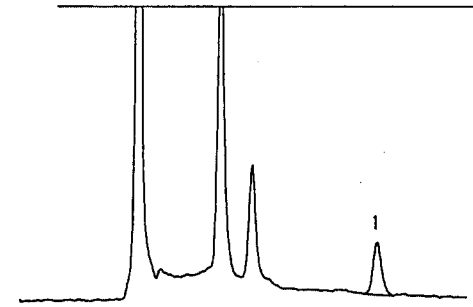


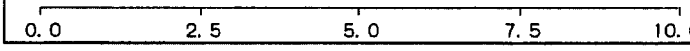
Operator : Akemi Inoue

Test fish after 15 days(depuration test)(Level 2-a)

Operating date : Jul. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.61	8397	899	100.00
Total		8397		100.00



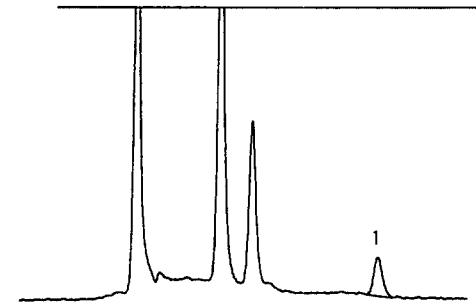


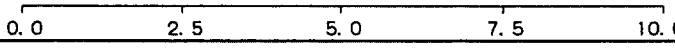
Operator : Akemi Inoue

Test fish after 15 days(depuration test)(Level 2-b)

Operating date : Jul. 12, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.61	6175	665	100.00
Total		6175		100.00





Operator : Akemi Inoue

Fig. 11 - 2 Chromatogram of HPLC analysis for test fish(depuration test, Level 2).

Date : Jul. 12, 2001 Name : A. INOUE

Test No. 43705

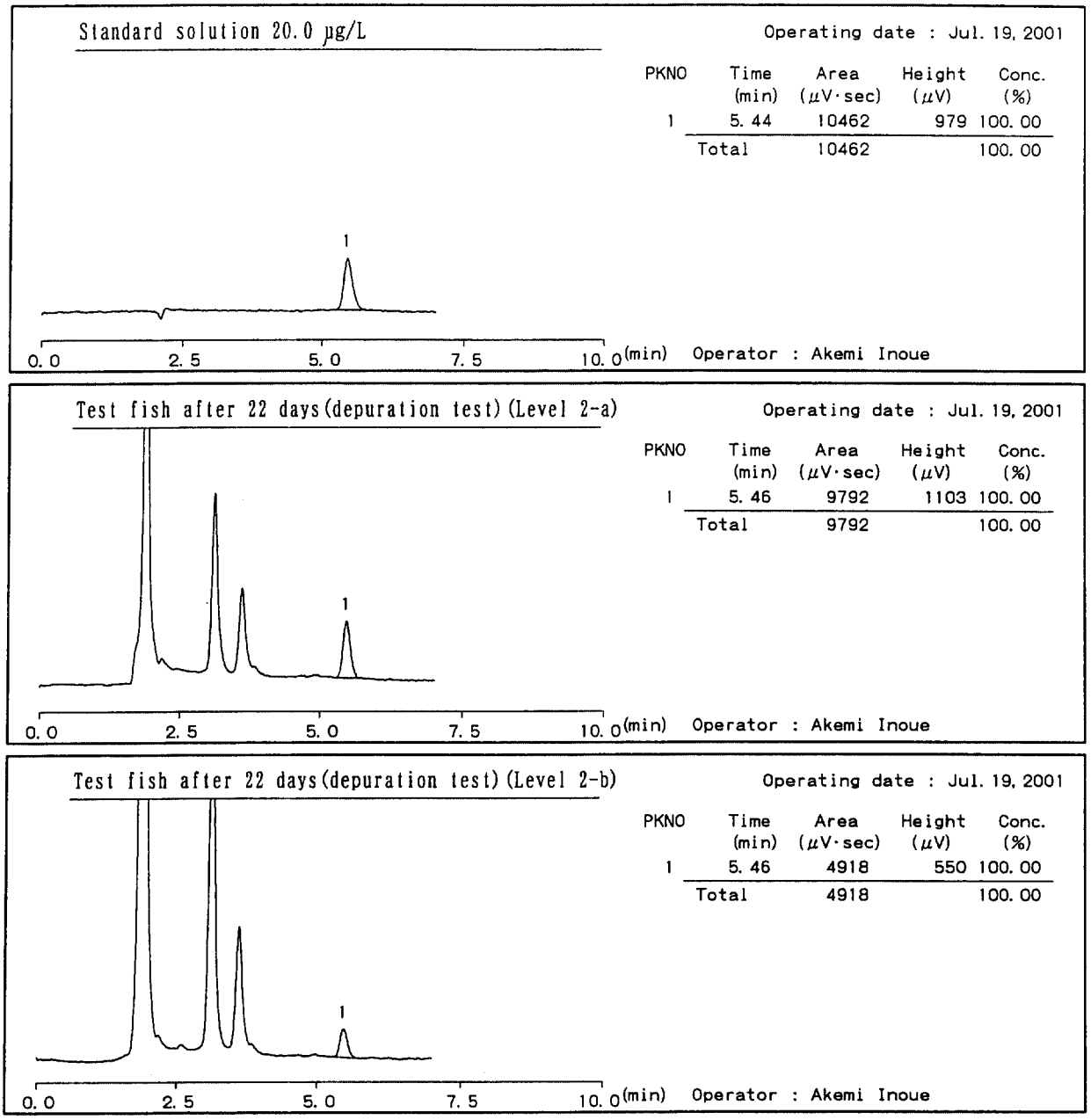


Fig. 11 - 3 Chromatogram of HPLC analysis for test fish (depuration test, Level 2).

Date : Jul. 19, 2001 Name : A. INOUE

Test No. 43705

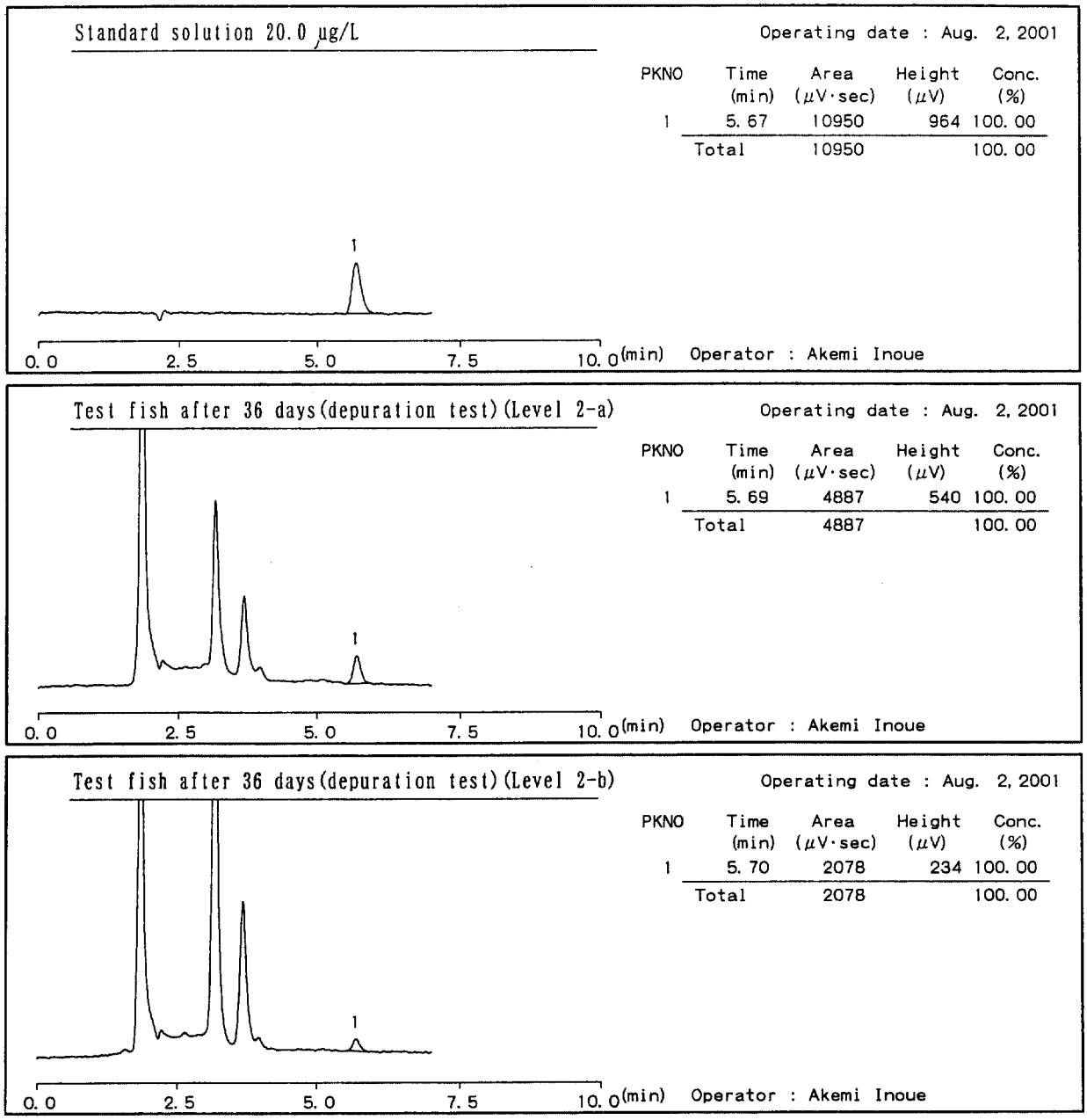


Fig. 11 - 4 Chromatogram of HPLC analysis for test fish (depuration test, Level 2).

Date : Aug. 2, 2001 Name : A. INOUE

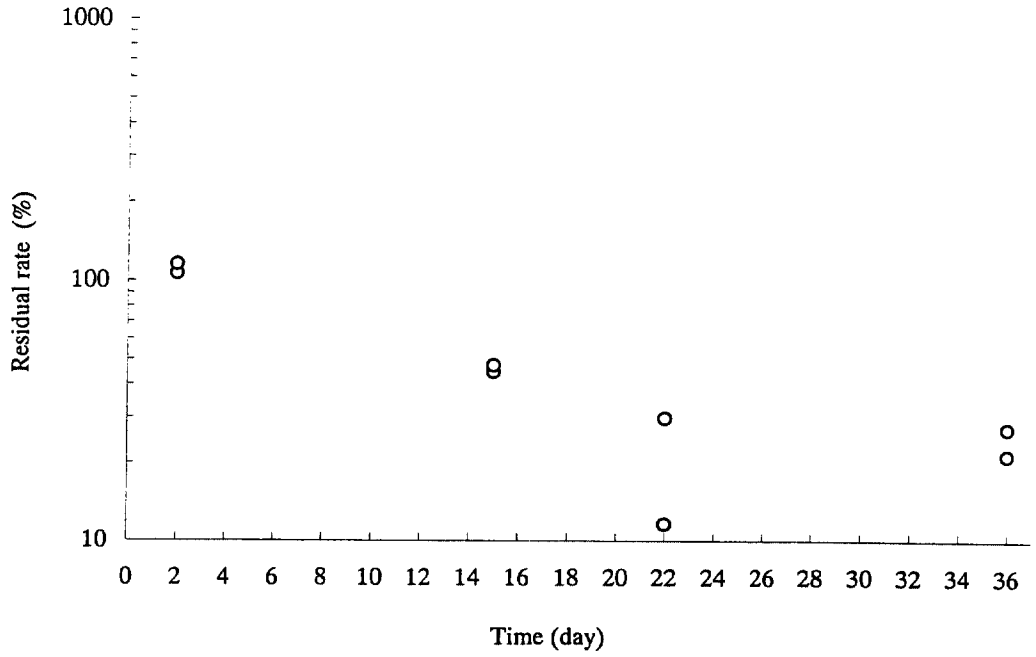


Fig. 12 Depuration curve (Level 1)

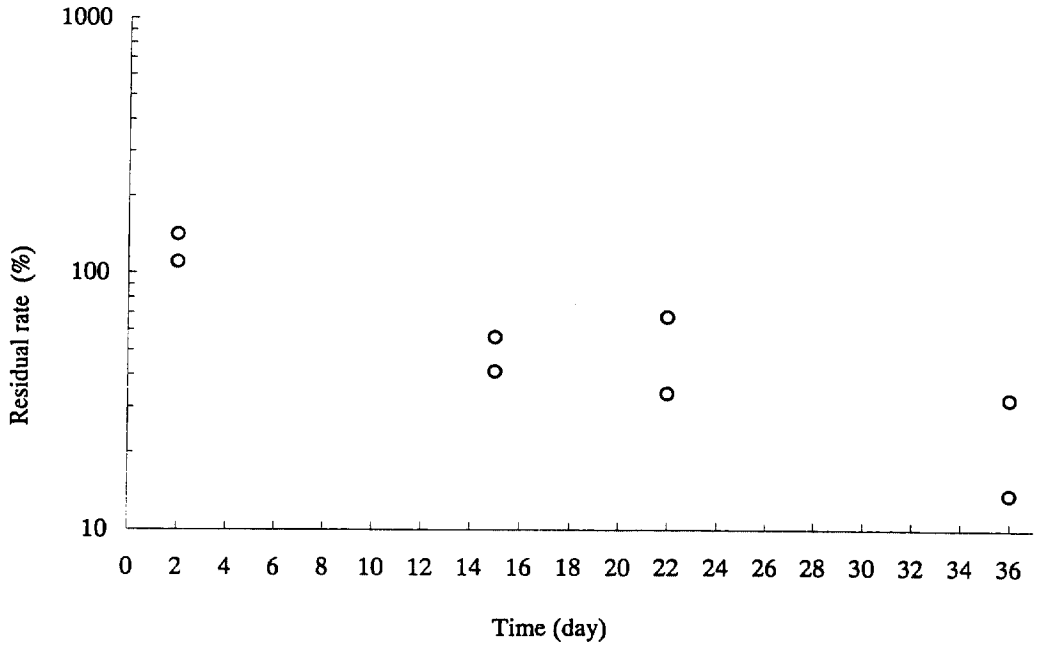


Fig. 13 Depuration curve (Level 2)

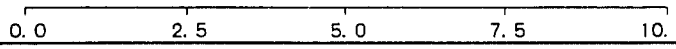
August 8, 2001 Name A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Apr. 26, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.68	10922	1048	100.00
Total		10922		100.00

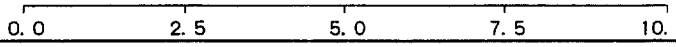


Operator : Akemi Inoue

Before the experimental start (Control-a)

Operating date : Apr. 26, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
Total		0		0.00

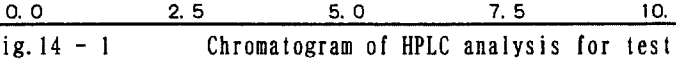


Operator : Akemi Inoue

Before the experimental start (Control-b)

Operating date : Apr. 26, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
Total		0		0.00

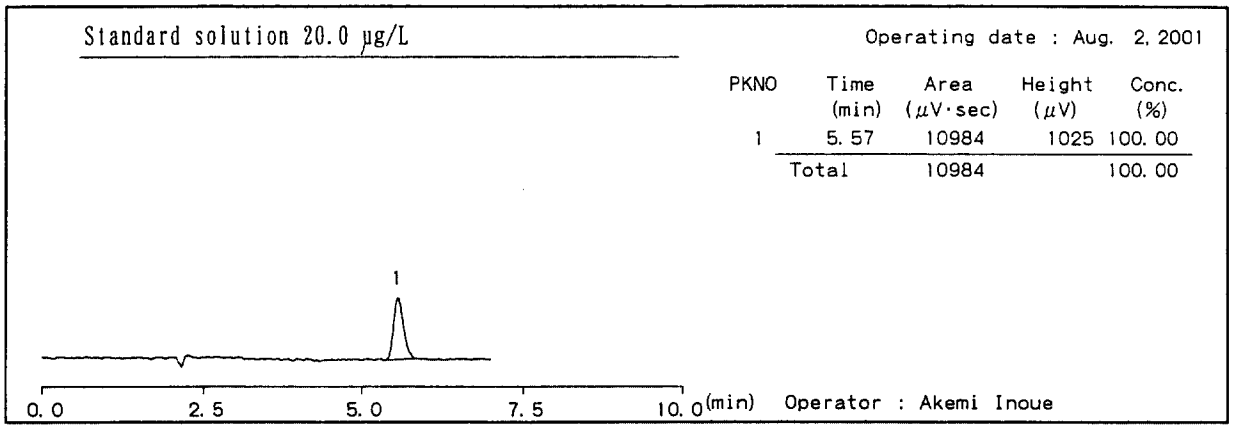


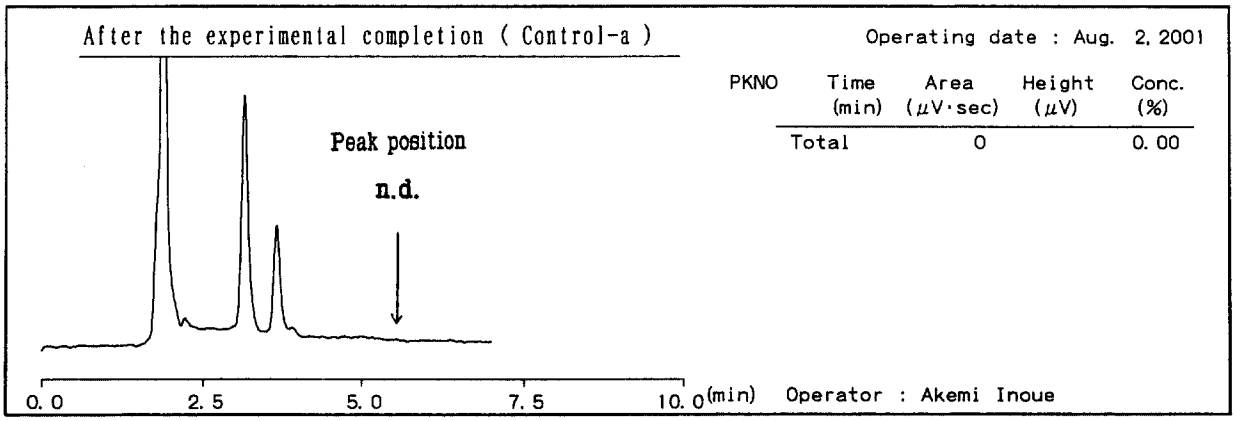
Operator : Akemi Inoue

Fig. 14 - 1 Chromatogram of HPLC analysis for test fish (Control).

Date : Apr. 26, 2001 Name : A. INOUE

Test No. 43705





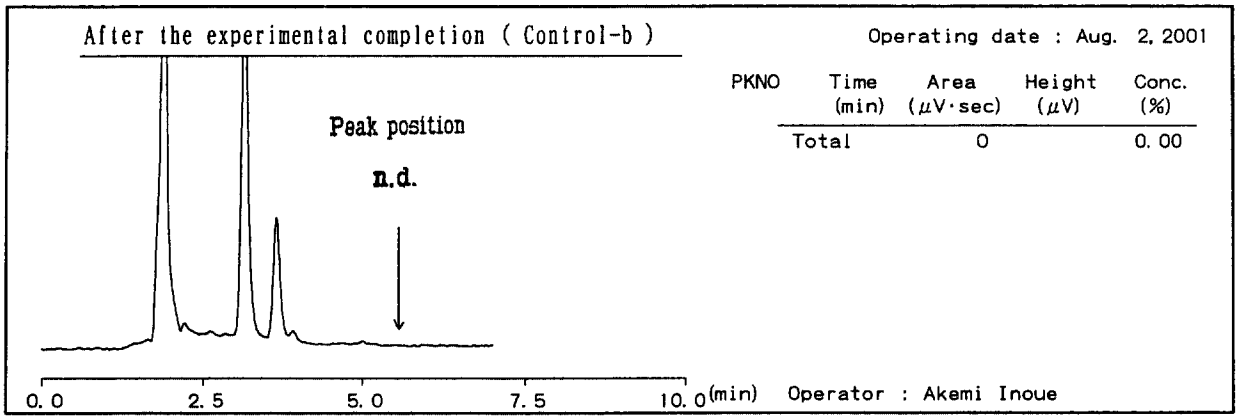


Fig. 14 - 2 Chromatogram of HPLC analysis for test fish (Control).

Date : Aug. 2, 2001 Name : A. INOUE

Test No. 43705

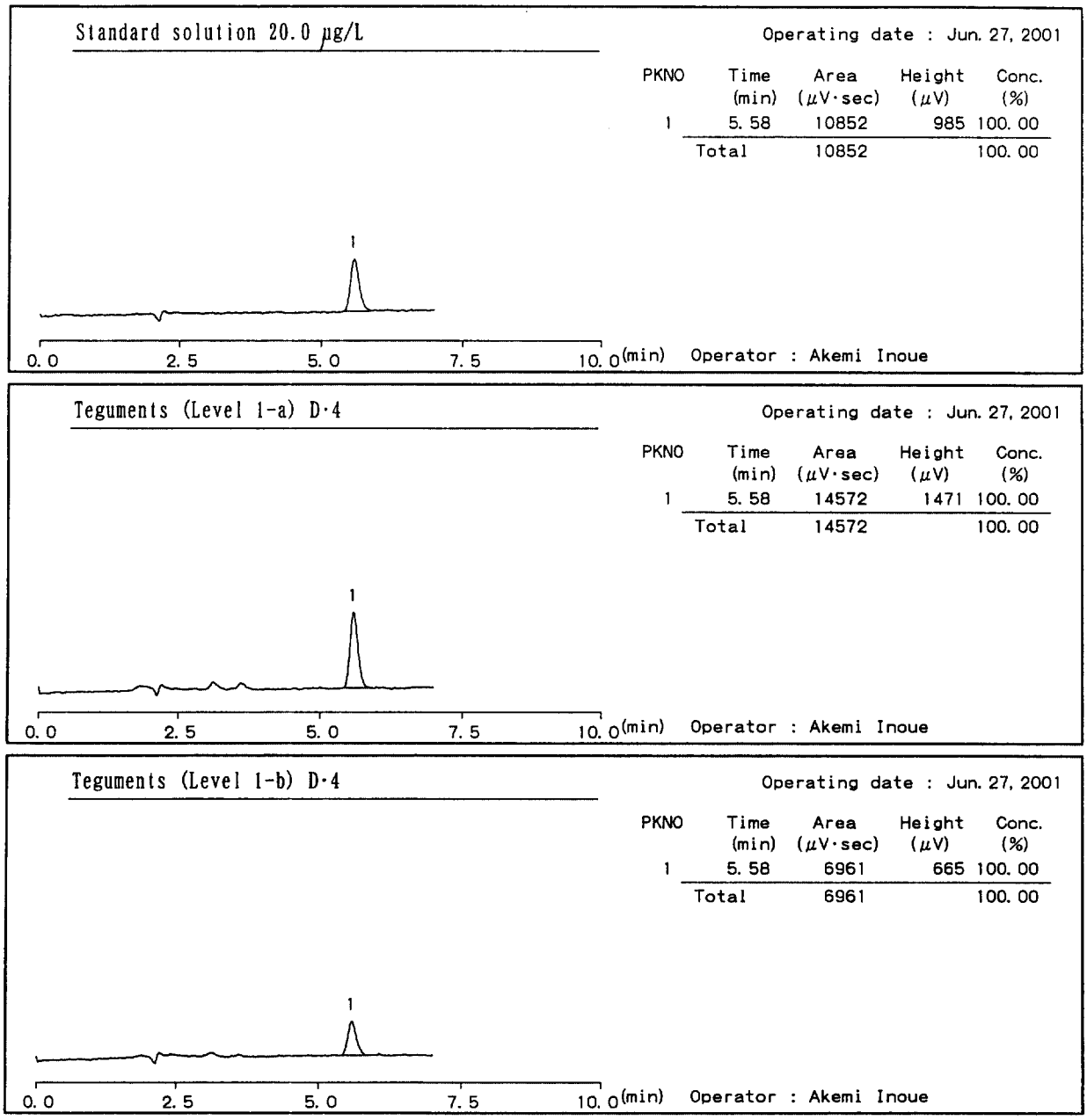


Fig. 15 - 1 Chromatogram of HPLC analysis for test fish(analysis in parts of test fish, Level 1).

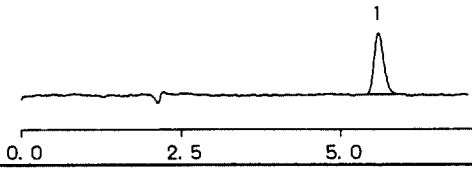
Date : Jul. 6, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	10789	1024	100.00
	Total	10789		100.00



Operator : Akemi Inoue

Head (Level 1-a) D·10

Operating date : Jun. 27, 2001

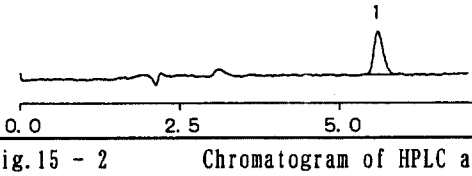
PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	12145	1197	100.00
	Total	12145		100.00

Operator : Akemi Inoue

Head (Level 1-b) D·10

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	7454	736	100.00
	Total	7454		100.00



Operator : Akemi Inoue

Fig. 15 - 2 Chromatogram of HPLC analysis for test fish(analysis in parts of test fish, Level 1).

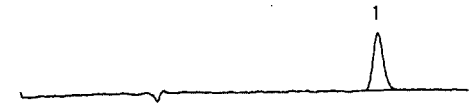
Date : Jul. 6, 2001 Name : A. INOUE

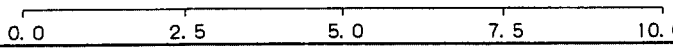
Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	10506	959	100.00
Total		10506		100.00



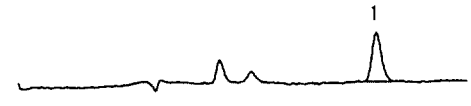


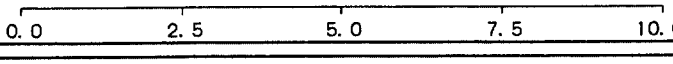
Operator : Akemi Inoue

Viscera (Level 1-a) D·4

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	8493	830	100.00
Total		8493		100.00



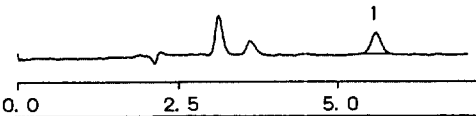


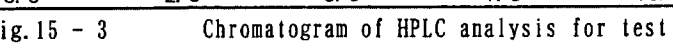
Operator : Akemi Inoue

Viscera (Level 1-b) D·4

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	3874	356	100.00
Total		3874		100.00





Operator : Akemi Inoue

Fig. 15 - 3 Chromatogram of HPLC analysis for test fish (analysis in parts of test fish, Level 1).

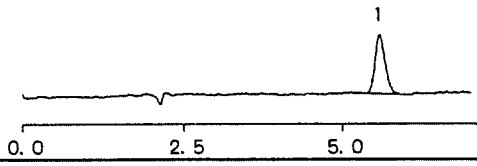
Date : Jul. 6, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	10917	989	100.00
Total		10917		100.00

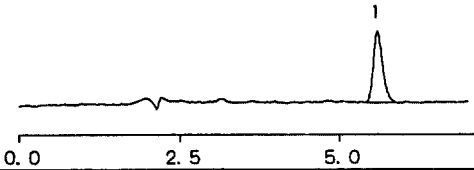


Operator : Akemi Inoue

Remainder Parts (Level 1-a) D·10

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.59	12516	1218	100.00
Total		12516		100.00

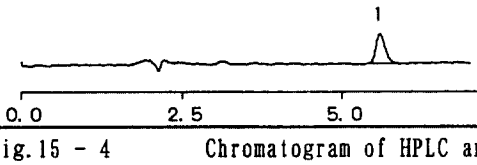


Operator : Akemi Inoue

Remainder Parts (Level 1-b) D·10

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.59	5145	511	100.00
Total		5145		100.00



Operator : Akemi Inoue

Fig. 15 - 4 Chromatogram of HPLC analysis for test fish(analysis in parts of test fish, Level 1).

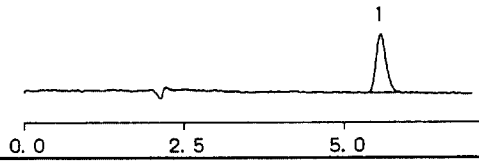
Date : Jul. 6, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.56	10795	977	100.00
	Total	10795		100.00

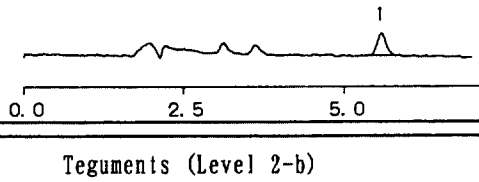


Operator : Akemi Inoue

Teguments (Level 2-a)

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	3755	377	100.00
	Total	3755		100.00

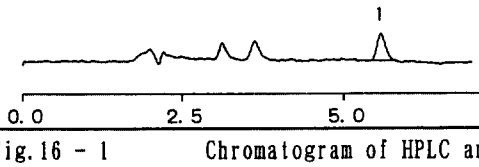


Operator : Akemi Inoue

Teguments (Level 2-b)

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	4348	461	100.00
	Total	4348		100.00



Operator : Akemi Inoue

Fig. 16 - 1 Chromatogram of HPLC analysis for test fish (analysis in parts of test fish, Level 2).

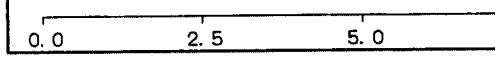
Date : Jul. 6, 2001 Name : A. INOUE

Test No. 43705

Standard solution 20.0 μg/L

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.56	10561	1001	100.00
Total		10561		100.00

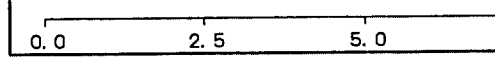


Operator : Akemi Inoue

Head (Level 2-a)

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.58	10697	1136	100.00
Total		10697		100.00

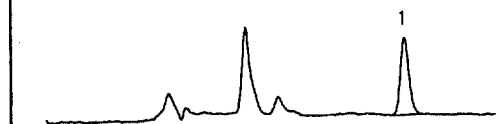


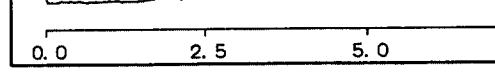
Operator : Akemi Inoue

Head (Level 2-b)

Operating date : Jun. 27, 2001

PKNO	Time (min)	Area (μV·sec)	Height (μV)	Conc. (%)
1	5.57	12431	1324	100.00
Total		12431		100.00



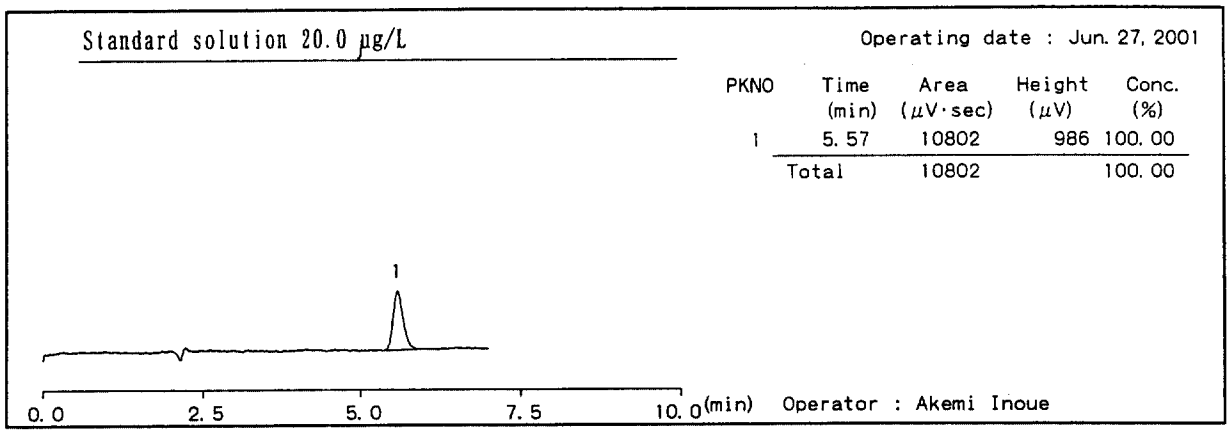


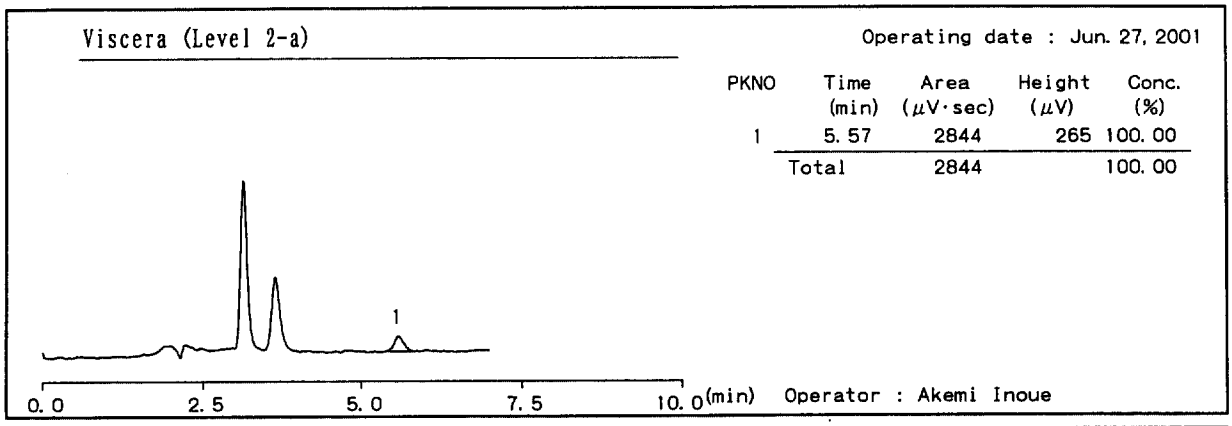
Operator : Akemi Inoue

Fig. 16 - 2 Chromatogram of HPLC analysis for test fish(analysis in parts of test fish, Level 2).

Date : Jul. 6, 2001 Name : A. INOUE

Test No. 43705





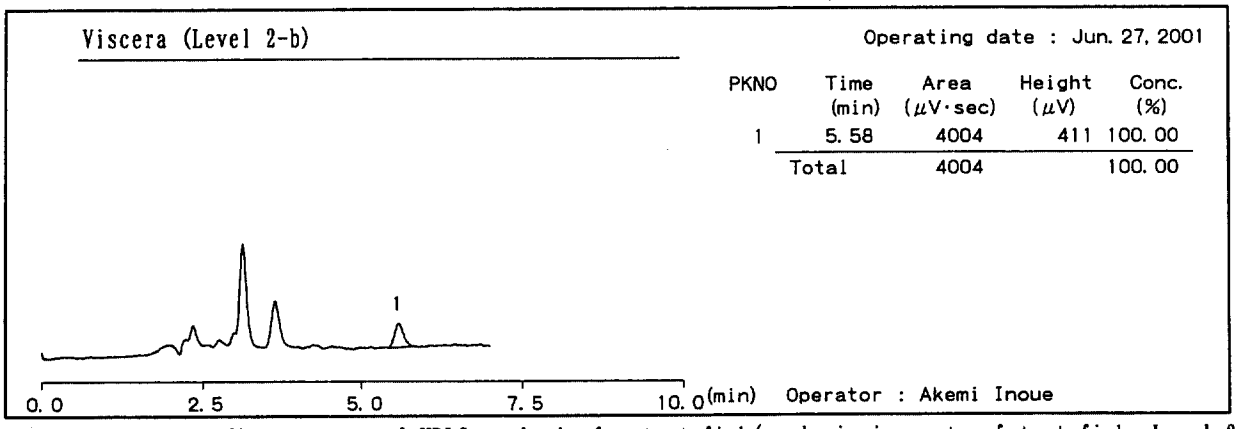


Fig. 16 - 3 Chromatogram of HPLC analysis for test fish(analysis in parts of test fish, Level 2).

Date : Jul. 6, 2001 Name : A. INOUE

Test No. 43705

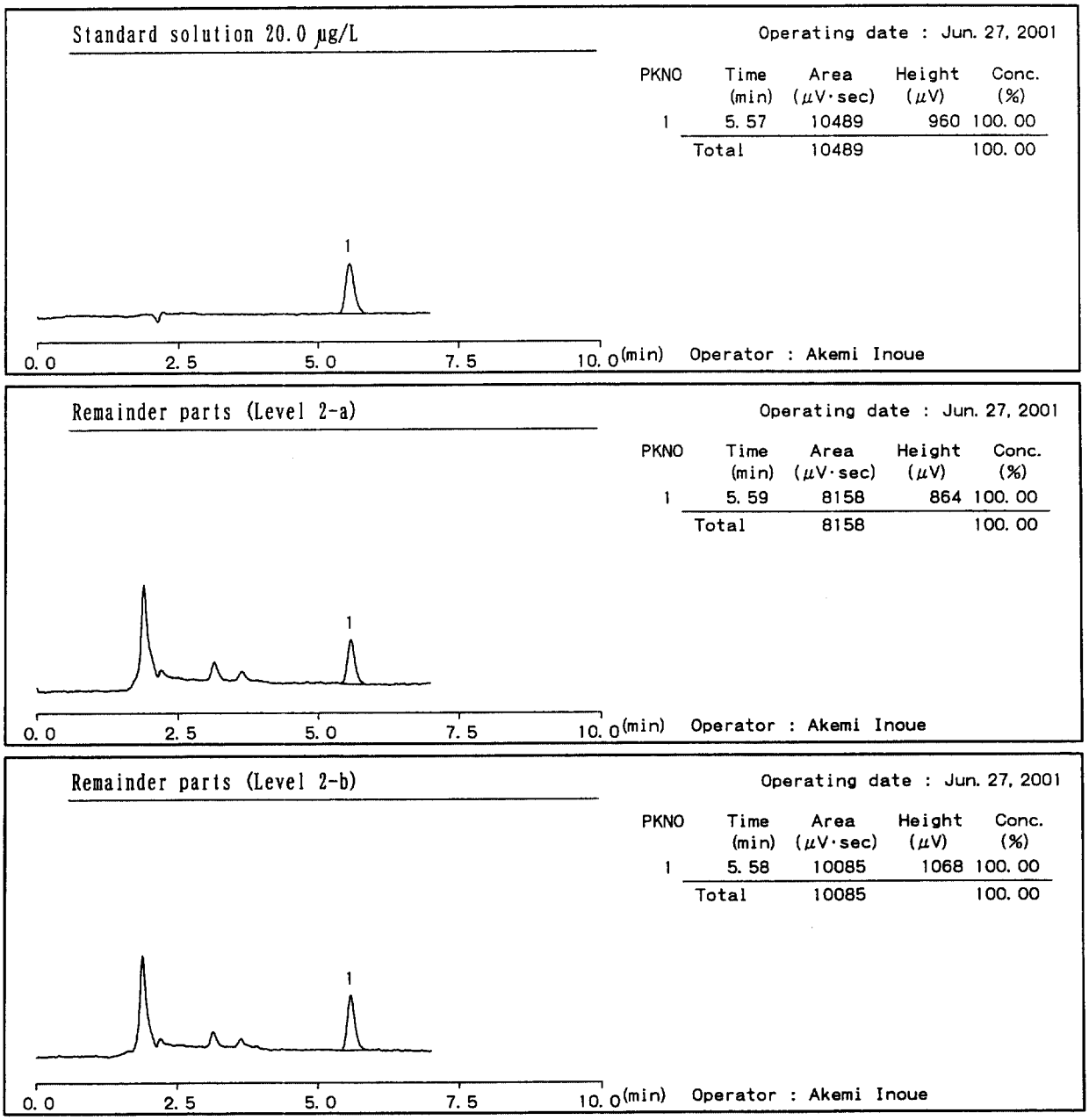


Fig. 16 - 4 Chromatogram of HPLC analysis for test fish(analysis in parts of test fish, Level 2).

Date : Jul. 6, 2001 Name : A. INOUE

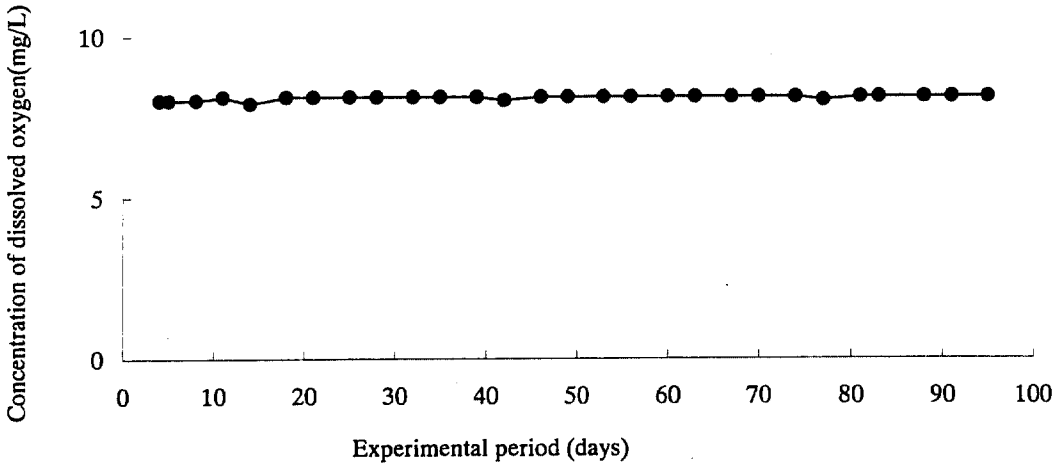


Fig. 17 Concentration of dissolved oxygen (Level 1).

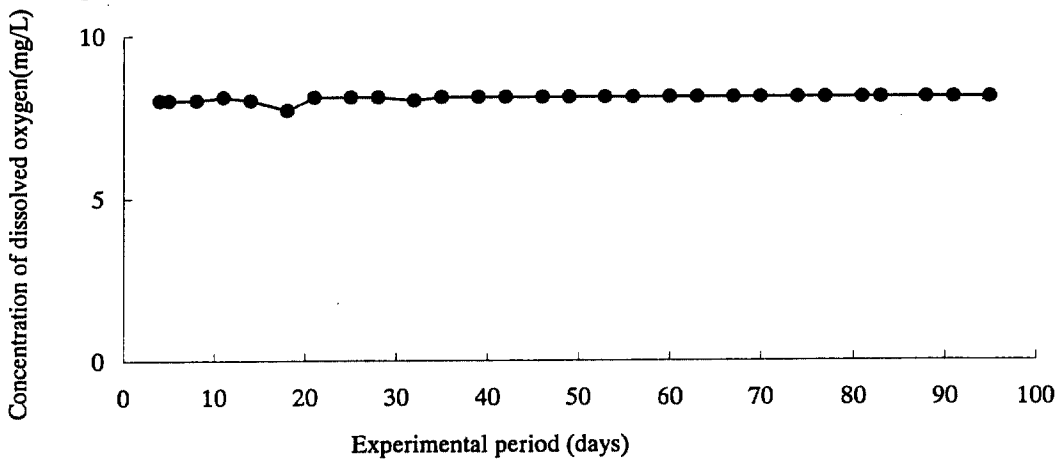


Fig. 18 Concentration of dissolved oxygen (Level 2).

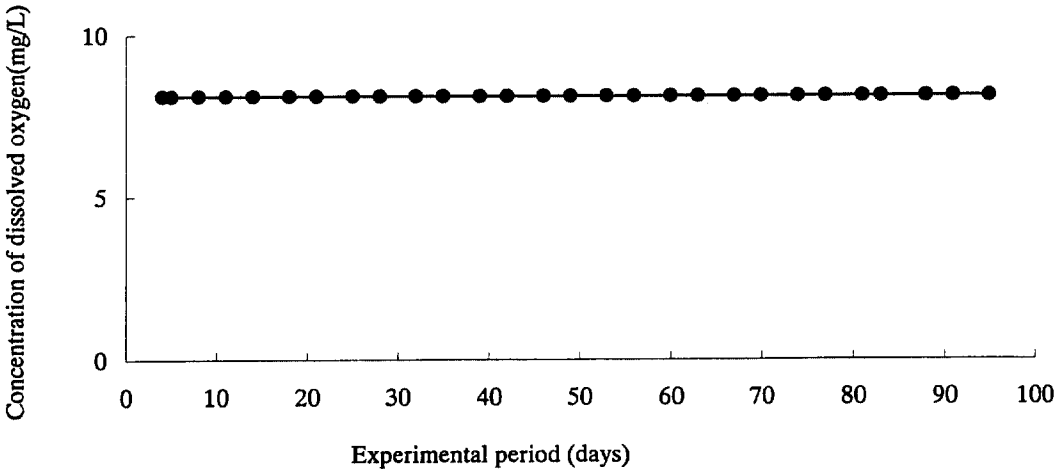


Fig. 19 Concentration of dissolved oxygen (Control).

August 13, 2001 Name A. INOUE

Fig. 20

UV-VIS

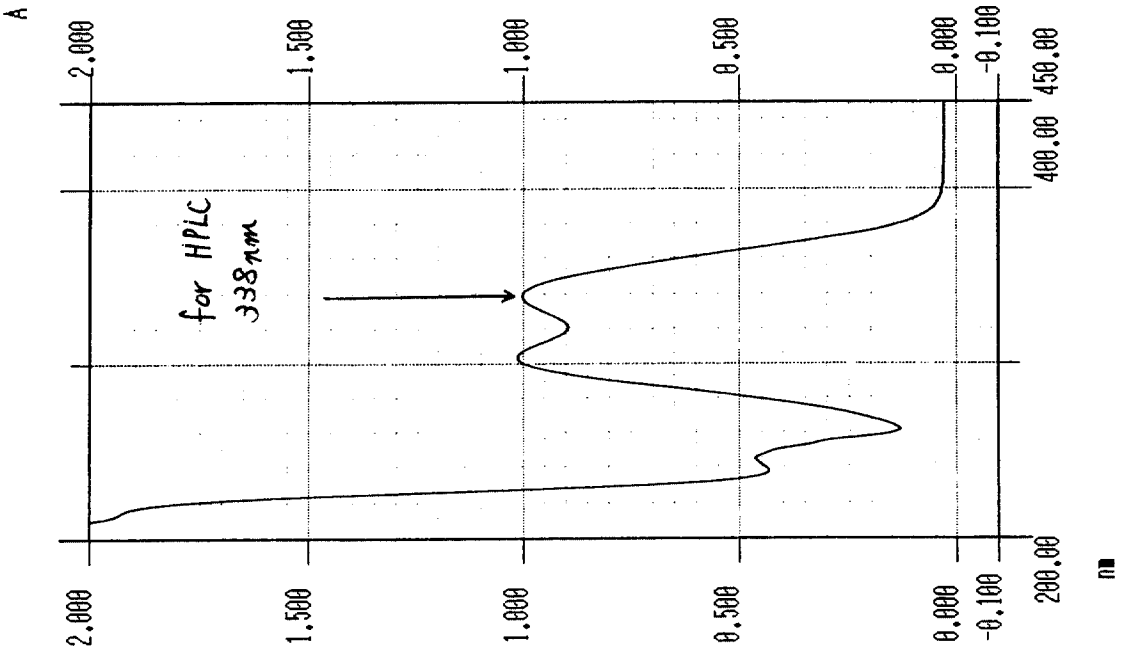
Test No.	43705	Date	2000. 12. 15
Sample	TINUVIN 343		
Solvent	Acetonitrile		
Reference	—		
Photometric Mode	Abs		
Cell	10mm×10mm(quartz)		
Wavelength	200 ~ 400		
Scale Limit	-0.100 ~ 2.000		

Instrument	Shimadzu UV-2200A
Slit(Spectral Band)Width	2.0 nm
Scan Speed	MIDDLE
Sampling Pitch	AUTO
Note.	UV-VIS absorption spectrum

Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan (1999. 10. 500)

43705 2000. 12. 15 A. INOUE

```
------------------------ PARAMETERS OF SPECTRUM -- 2000/12/15 16:40:29 ---
                MEASURING MODE ;  ABS
        SAMPLING PITCH(Delta λ) ;  AUTO(0.2 nm)
                    SCAN SPEED ;  MIDDLE
                    SLIT WIDTH ;  2.0 nm
                   SAMPLE NAME ;  43705
                       ANALYST ;  A.Inoue
                       COMMENT ;  20.0mg/L(ACN)
-----------------------------------------------------------------------
```



NO.	ABSCISSA	PEAK	HEIGHT	ABSCISSA	VALLEY	HEIGHT
1	338.2	1.0007	0.2740	320.8	0.8932	-0.1135
2	303.4	1.0126	0.3428	261.4	0.1306	-0.4885
3	244.6	0.4617	0.1201	237.4	0.4319	-0.3337
4	203.0	2.2179	0.1837			

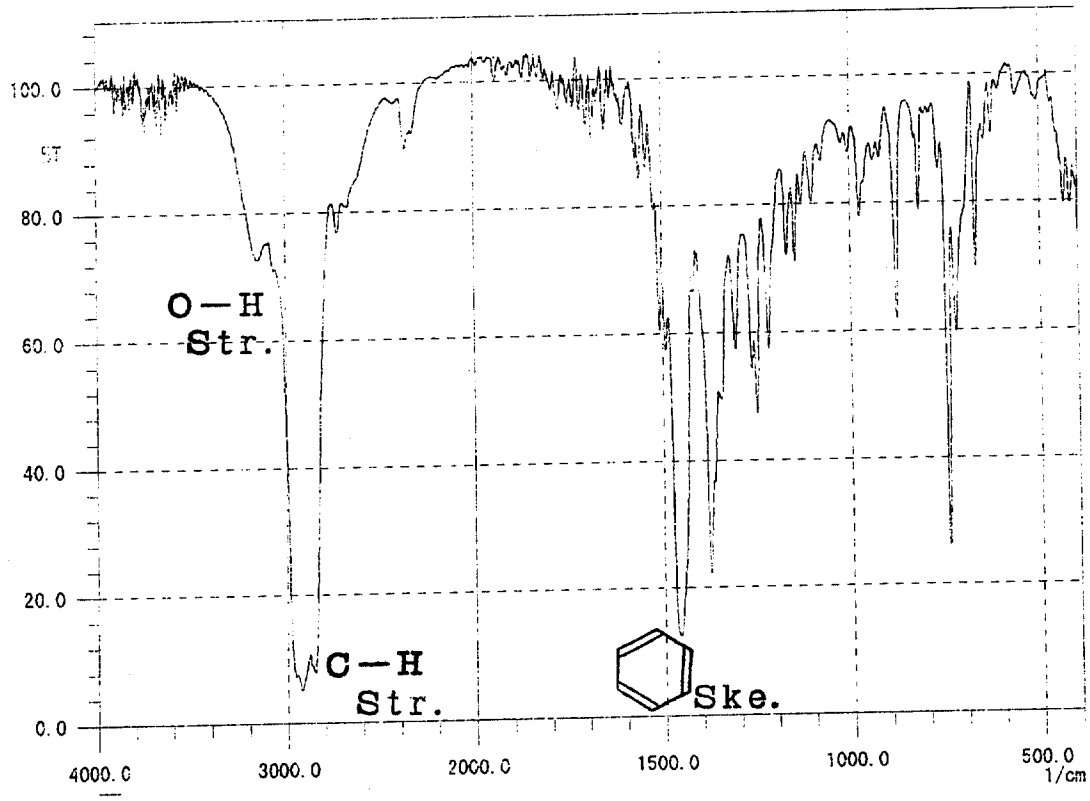


Fig. **21** -1 IR spectrum of test substance measured before the experimental start.

Test No. : 43705
Sample : TINUVIN 343
Method : Nujol
Date : 2001/03/12
Name : A. INOUE

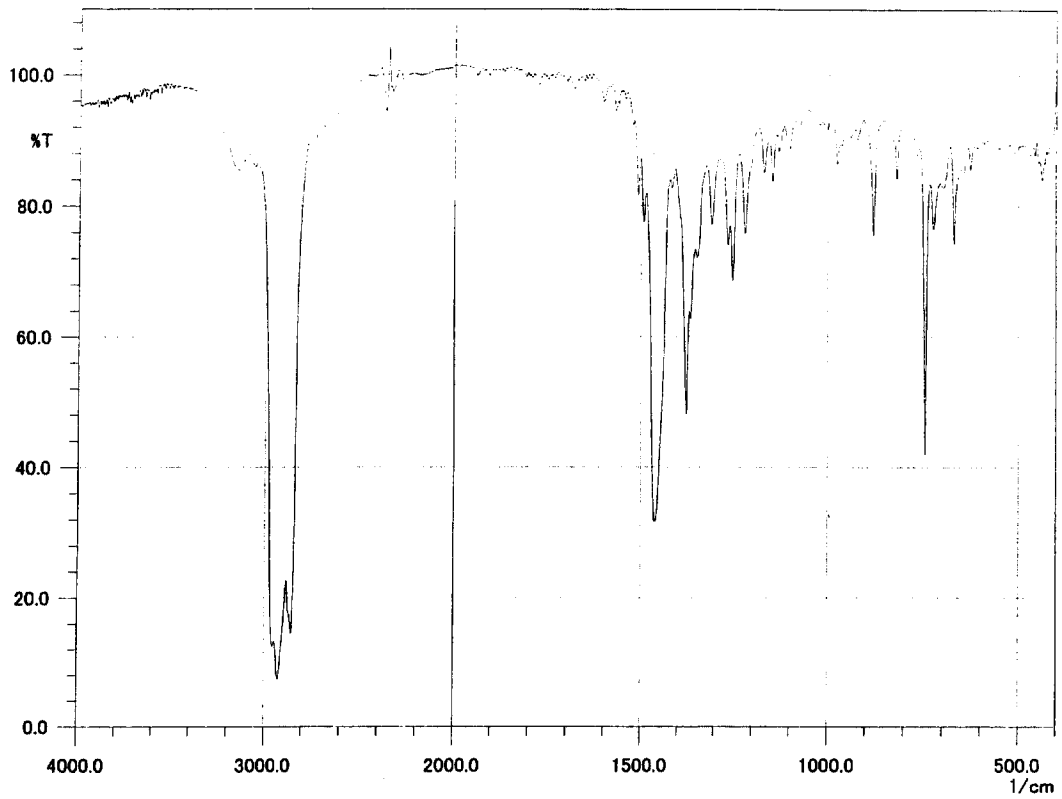
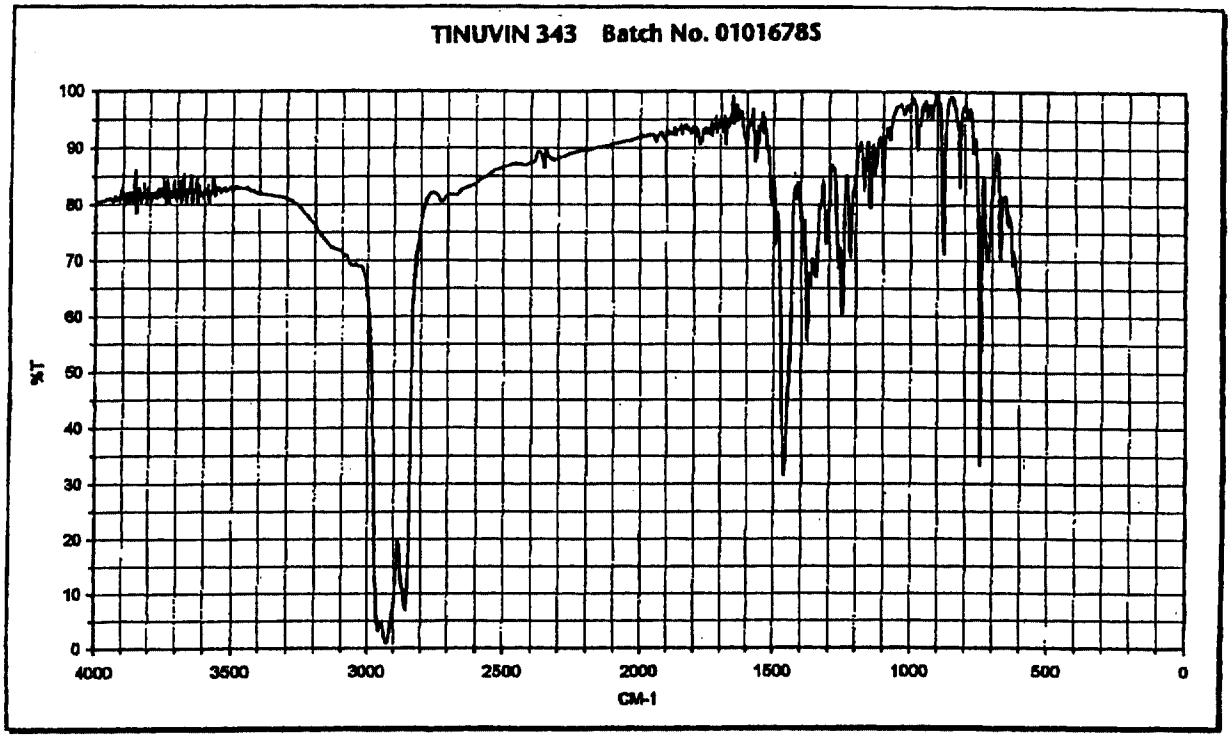


Fig.21-2 IR spectrum of test substance measured after the experimental completion.

Test No. : 43705
Sample : TINUVIN 343
Method : Nujol
Date : 2001/08/09
Name : A. INOUE



Reference 2 IR spectrum supplied by the sponsor