



Receipt number	642-06-S-4807
Study number	44807

FINAL REPORT

Bioconcentration study of ^{13}F -EtOH in carp

March 19, 2007

Kurume Laboratory
Chemicals Evaluation and Research Institute, Japan

STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor DAIKIN INDUSTRIES, LTD.

Title Bioconcentration study of 13F-EtOH in carp

Study number 44807

I, the undersigned, hereby declare that this report provides a correct English translation of the Final Report (Study No. 44807, issued on March 19, 2007).

Date

Study Director

July 16, 2009

GLP STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor DAIKIN INDUSTRIES, LTD.

Title Bioconcentration study of 13F-EtOH in carp

Study number 44807

The study described in this report was conducted in compliance with the following GLP principles:

- (1) "Standard Concerning Testing Facility Relating to New Chemical Substances" (November 21, 2003; No. 1121003, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; November 17, 2003, No. 3, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 031121004, Environmental Policy Bureau, Ministry of the Environment)
- (2) "OECD Principles of Good Laboratory Practice (November 26, 1997, ENV/MC/CHEM(98)17)"

This final report reflects the raw data accurately and it has been confirmed that the test data is valid.

Date March 19, 2007

Study Director Signed in original

QUALITY ASSURANCE STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor DAIKIN INDUSTRIES, LTD.

Title Bioconcentration study of 13F-EtOH in carp

Study number 44807

I assure that the final report accurately describes the test methods and procedures, and that the reported results accurately reflect the raw data of the study.

The inspections and audits of this study were carried out and the results were reported to the Study Director and the Test Facility Management by Quality Assurance Unit as follows.

Item of inspection / audit	Date of inspection / audit	Date of report to Study Director and Test Facility Management
Study plan draft	January 24, 2007	January 25, 2007
Study plan	January 25, 2007	January 25, 2007
Amendment to study plan	March 6, 2007	March 6, 2007
Acute toxicity test	February 6, 2007	February 6, 2007
Measurement of lipid content	February 13, 2007	February 14, 2007
	February 14, 2007	February 14, 2007
Preparation of stock solution	February 20, 2007	February 21, 2007
Analysis of test water	February 15, 2007	February 15, 2007
Analysis of test fish	February 23, 2007	February 23, 2007
Raw data and final report draft	March 19, 2007	March 19, 2007
Final report	March 19, 2007	March 19, 2007

Date March 19, 2007

Head of Quality Assurance Unit Signed in original

CONTENTS

	page
Title	1
Sponsor	1
Test facility	1
Objective	1
Test method	1
Applied GLP	2
Dates	2
Storage of test item, raw data, etc.	2
Personnel	3
Approval of final report	3
SUMMARY	4
1. Test item	5
2. Test sample	6
3. Performance of acute toxicity test	7
4. Performance of bioconcentration test	10
5. Factors affected reliability of test	25
6. Results	26
7. Remarks	28

Contents of tables and figures

Contents of tables

Table-1	Measured concentrations of test item in test water (see text)
Table-2	BCFs (see text)
Table-3	Variation of BCFs (the values were indicated for five figures) (see text)
Table-4	Concentrations of test item in test water at a steady-state (see text)
Table-5	Calculation table for recovery and blank test (analysis of test water)
Table-6	Calculation table for analysis of test water (Level 1)
Table-7	Calculation table for analysis of test water (Level 2)
Table-8	Calculation table for recovery and blank test (analysis of test fish)
Table-9	Calculation table for analysis of test fish (Level 1)
Table-10	Calculation table for analysis of test fish (Level 2)
Table-11	Calculation table for analysis of test fish (Control)
Reference 1	Analytical results of dilution water

Contents of figures

Fig.1	Correlation between exposure period and bioconcentration factor (Level 1)
Fig.2	Correlation between exposure period and bioconcentration factor (Level 2)
Fig.3	Concentration-mortality curve
Fig.4-1	Mass fragmentograms of GC-MS analysis for calibration curve (analysis of test water)
Fig.4-2	Calibration curve of test item (analysis of test water)
Fig.5	Mass fragmentograms of GC-MS analysis for recovery and blank test (analysis of test water)
Fig.6	Mass fragmentograms of GC-MS analysis for test water
Fig.7-1	Mass fragmentograms of GC-MS analysis for calibration curve (analysis of test fish)
Fig.7-2	Calibration curve of test item (analysis of test fish)
Fig.8	Mass fragmentograms of GC-MS analysis for recovery and blank test (analysis of test fish)
Fig.9	Mass fragmentograms of GC-MS analysis for test fish (Level 1)
Fig.10	Mass fragmentograms of GC-MS analysis for test fish (Level 2)
Fig.11	Mass fragmentograms of GC-MS analysis for test fish (Control)
Fig.12	Mass spectrum of test item
Fig.13-1	IR spectrum of test item measured before experimental start
Fig.13-2	IR spectrum of test item measured after experimental completion
Reference 2	IR spectrum supplied by sponsor

Title

Bioconcentration study of 13F-EtOH in carp

Sponsor

DAIKIN INDUSTRIES, LTD.
1-1, Nishihitotsuya, Settsu, Osaka 566-8585, Japan

Test facility

Kurume Laboratory
Chemicals Evaluation and Research Institute, Japan
2-7, 3-chome, Miyanojin, Kurume-shi, Fukuoka 839-0801, Japan

Objective

This study was performed to evaluate the bioconcentration potential of 13F-EtOH in carp.

Test method

This study was performed according to the following test methods.

- (1) "Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body" stipulated in the "Testing Methods for New Chemical Substances" (November 21, 2003, No.1121002, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; November 13, 2003, No.2, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No.031121002, Environmental Policy Bureau, Ministry of the Environment)
- (2) "Bioconcentration : Flow-through Fish Test (Guideline 305, June 14, 1996)" in the OECD Guidelines for Testing of Chemicals

Applied GLP

This study was conducted in compliance with the following GLP principles:

- (1) "Standard Concerning Testing Facility Relating to New Chemical Substances" (November 21, 2003; No. 1121003, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; November 17, 2003, No. 3, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 031121004, Environmental Policy Bureau, Ministry of the Environment)
- (2) "OECD Principles of Good Laboratory Practice (November 26, 1997, ENV/MC/CHEM(98)17)"

Dates

Study initiation date	January 25, 2007
Experimental starting date	February 13, 2007
Experimental completion date	March 13, 2007
Study completion date	March 19, 2007

Storage of test item, raw data, etc.

(1) Test item

The test sample is sealed in a storage vessel and stored in archives in this laboratory for 10 years after the receipt of notice specified under Clause 1 or Clause 2 in Article 4, Clause 2 or Clause 3 or Clause 8 in Article 4-2, and Clause 2 in Article 5-4 or Clause 2 in Article 24 or Clause 2 in Article 25-3 of "Law Concerning Examination and Regulation of Manufacture, etc. of Chemical Substances". If it is not stable for the storage period, it is stored as long while it is kept stable. Treatment of the test sample after the storage period will be discussed with sponsor.

(2) Raw data and materials, etc.

Raw data, the study plan, documents concerning the study presented by the sponsor, the final report and necessary materials are stored in archives in this laboratory for 10 years after the receipt of the notice specified under Clause 1 or Clause 2 in Article 4, Clause 2 or Clause 3 or Clause 8 in Article 4-2, and Clause 2 in Article 5-4 or Clause 2 in Article 24 or Clause 2 in Article 25-3 of "Law Concerning Examination and Regulation of Manufacture, etc. of Chemical Substances". Treatment of raw data and materials, etc. after the storage period will be discussed with sponsor.

Personnel

Study Director

(2nd Chemical Safety Section)Study personnel
(Operation of bioconcentration test)

Staff for fish care

Person to conduct of fish acute toxicity test

Approval of final report

Study Director

Date March 19, 2007

Signature Signed in original

SUMMARY

Title

Bioconcentration study of 13F-EtOH in carp

Test conditions

Acute toxicity test

Test fish	Orange-red killifish (<i>Oryzias latipes</i>)
Duration of exposure	96 hours
Exposure method	Semi static system (Renewal of test water, at every 8 - 16 hours)

Bioconcentration test

Test fish	Carp (<i>Cyprinus carpio</i>)
Nominal concentrations of test item	High exposure level (Level 1) 10 µg/L Low exposure level (Level 2) 1 µg/L
Duration of exposure	28 days
Exposure method	Continuous flow system
Analytical method	Gas chromatography-mass spectrometry

Results

96-hour LC50 value	>3.00 mg/L
--------------------	------------

Bioconcentration factors at a steady state

Level 1	-
Level 2	29

Bioconcentration factors

Level 1	8.4 - 58
Level 2	24 - 99

1. Test item

In this report, 13F-EtOH has the following chemical name, etc.

1.1 Chemical name^{*1}

2-(Perfluorohexyl)ethanol

1.2 Chemical structure, etc. ^{*1}

Structural formula



Molecular formula $\text{C}_8\text{H}_{13}\text{F}_{13}\text{O}$

Molecular weight 364.10

CAS number 647-42-7

^{*1} Information supplied by the sponsor

2. Test sample

2.1 Supplier and lot number^{*1}

Supplier	DAIKIN INDUSTRIES, LTD.
Lot number	180804

2.2 Purity^{*1}

Test item	99.8%
Impurity	Unknown 0.2%

The test item was treated as 100% in purity.

2.3 Confirmation of test item

Two infrared (IR) spectra of the test item provided by the sponsor and measured at this laboratory were confirmed to be identical (see Fig. 13 and Reference 2).

2.4 Physicochemical properties

Appearance ^{*1}	Colorless and transparent liquid	
Boiling point ^{*1}	78°C (14 mmHg)	
Density ^{*1}	1.678g/cm ³ (20 °C)	
Solubility	Water	12.7mg/L
	Dimethylsulfoxide	Soluble (arbitrary mixable) ^{*1}
	Acetone	Soluble (arbitrary mixable) ^{*1}

*1 Information supplied by the sponsor

2.5 Storage and stability

Storage condition

Dark storage place at room temperature

Stability

The test item was stable under the storage conditions, as shown by the finding that IR spectra of the test item before the experimental start and after the experimental completion were identical (see Fig. 13).

2.6 Stability under testing conditions

Prior to the bioconcentration test, a stability of the test item 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 Kurume Laboratory Chemicals Evaluation and Research Institute, Japan
(Address : 2-7, 3-chome, Miyanojin, Kurume-shi, Fukuoka 839-0801, Japan)

Date received November 28, 2006

- (3) Conditions of acclimatization

Period

The fish were checked visually at receipt and those with any abnormalities were removed. The remainders were transferred to an acclimatizing aquarium and acclimatized there after the external disinfection. The fish showing any abnormalities during this period were removed and the remainder was reared for 8 days in a flow-through system at the temperature of $25\pm 2^{\circ}\text{C}$. The fish were checked for health conditions and reared for 60 days after the external disinfection.

External disinfection

The first external disinfection was carried out in an aqueous solution containing 50 mg/L OTC for fisheries (Oxytetracycline hydrochloride) and 6 g/L sodium chloride for 24 hours. The second external disinfection was carried out in an aqueous solution containing 6 g/L sodium chloride for 5 hours.

- (4) Weight average 0.26 g

- (5) Length average 3.0 cm

- (6) Certification

The 48-hour LC50 value of the reference substance^{*2} for the fish of the same lot (TFO-061128) was 0.577 mg/L.

^{*2} PCP-Na (pentachlorophenol sodium salt, Tokyo Kasei Kogyo Co., Ltd.)

3.3 Dilution water for test

(1) Origin

Groundwater from the premises of Kurume Laboratory.

(2) Water quality assessment

The dilution water for test was taken out on January 29, 2007, and it was analyzed and measured. The results are shown in Reference 1.

It was confirmed that the dilution water met the requirements of at least one of the following standards.

- ① Ministerial ordinance of the Ministry of Health, Labour and Welfare No.101 (Revised May 30, 2003)
- ② OECD Guidelines for Testing of Chemicals, Fish, Early-life Stage Toxicity Test (Guideline 210, July 17, 1992)
- ③ Water quality criteria for fisheries (Japan Fisheries Resource Conservation Association, 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)

3.4 Preparation of stock solution

(1) Dispersants

Dimethylsulfoxide
MEGAFACE F-443

(2) Preparation

The test sample and MEGAFACE F-443 (30 times amount of it) were mixed and kneaded. And dimethylsulfoxide was added to the mixture to prepare 1000 mg/L stock solution.

3.5 Test conditions

- | | |
|-------------------------------|-----------------------|
| (1) Test concentrations | 3.00 mg/L and Control |
| (2) Test tank | Round glass vessel |
| (3) Volume of test water | 4 L / level |
| (4) Number of fish | 10 / level |
| (5) Temperature of test water | |
| At initial exposure | 24.1 - 24.2°C |
| Before renewal of test water | 24.6 - 25.0°C |

(6) Concentration of dissolved oxygen in test water

At initial exposure	8.1 - 8.2 mg/L
Before renewal of test water	6.1 - 6.5 mg/L

(7) pH of test water

At initial exposure	8.1 - 8.2
Before renewal of test water	7.9

(8) Duration of exposure

96 hours

(9) Exposure method

Semi static system
(Renewal of test water, at every 8 - 16 hours)

3.6 Performance of test

Place	Aquatron room B
Date	February 5, 2007 – February 9, 2007

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 >3.00 mg/L^{*3} (see Fig. 3)

*3 The concentration of the dispersant (dimethylsulfoxide) used at this time was about 3000 mg/L. Taking into account of the toxicity of the dispersant, the test was not performed at a higher concentration, because the 96-hour LC50 value of the dispersant was 37400 mg/L.

4. Performance of bioconcentration test

4.1 Test fish

- | | |
|------------------------|--|
| (1) Species | Carp (<i>Cyprinus carpio</i>) |
| 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 Kurume Laboratory Chemicals Evaluation and Research Institute, Japan
(Address : 2-7, 3-chome, Miyanojin, Kurume-shi, Fukuoka 839-0801,
Japan)

Date of hatch	September 3, 2006
Starting date of acclimatization	December 7, 2006

- (3) Conditions for acclimatization
Period

After rearing to test fish size in a receiving aquarium, the fish were transferred to an acclimatizing aquarium and acclimatized there after the external disinfection. The fish showing any abnormality during this period were removed and the remainder was reared for 41 days in a flow through system at the temperature of $25\pm 2^{\circ}\text{C}$. The fish were checked for health conditions and transferred to test tanks. Thereafter the fish were reared at the same temperature in the flow through system for 25 days, following the external disinfection.

External disinfection

The external disinfection in the acclimatizing aquarium was carried out in an aqueous solution containing 50 mg/L OTC for fisheries and 7 g/L sodium chloride for 24 hours. The external disinfection in test tanks was carried out in an aqueous solution containing 50 mg/L OTC for fisheries and 7 g/L sodium chloride for 24 hours.

- (4) Length 6.3 - 8.1 cm

- (5) Lot No. TFC-061207

- (6) Age Yearling fish

- ### (7) Feeding

Feed	Feed for fry of carp
Composition	Proteins content $\geq 43.0\%$ Lipid content $\geq 3.0\%$
Manufacturer	Nippon Formula Feed Mfg. Co., Ltd.
Feeding amount and interval	

Amount corresponding to about 2 % of total body weight was fed twice a day in halves (once a day in all at holiday).

The fish were starved for 24 hours before sampling.

4.2 Dilution water for test

The same as described in Section 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

70-L glass tank

(3) Flow rate of test water

0.04 mL/min for stock solution and 2000 mL/min for dilution water, 2880 L/day of test water, were supplied.

(4) Stock solution bottle

500-mL brown glass bottle

(Frequency of renewal 1 - 2 times/week)

(5) Temperature of test water

Level 1 24.3 - 24.6°C

Level 2 24.4 - 24.7°C

Control 24.5 - 24.8°C

(6) Concentrations of dissolved oxygen in test water

Level 1 7.8 - 8.0 mg/L

Level 2 7.7 - 8.0 mg/L

Control 7.8 - 8.0 mg/L

(7) pH of test water

Level 1 7.8 - 8.0

Level 2 7.8 - 8.0

Control 7.8 - 8.0

(8) Time of irradiation with light

Artificial light of white fluorescent lamp (14 hours/day)

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

Level 1 and 2 28

Control 20

(10) Duration of exposure

28 days

Reason : A steady-state has been reached after 28 days.

(11) Place

Aquatron room A

4.4 Preparation of stock solutions

(1) Dispersants

The same as described in Section 3.4 (1).

(2) Preparation

• Level 1

1000 mg/L stock solution was prepared in the same way as described in Section 3.4 (2). 500 mg/L solution of the test item was then prepared from this stock solution by dilution with dimethylsulfoxide.

• Level 2

50.0 mg/L stock solution was prepared in the same way as described in Section 3.4 (2). 50.0 mg/L solution of the test sample was then prepared from this stock solution by dilution with dimethylsulfoxide.

• Control

MEGAFACE F-443 was dissolved in dimethylsulfoxide to prepare 15.0 g/L stock solution as MEGAFACE F-443 concentration.

4.5 Test concentrations

Test concentrations of the test item were decided as follows. The control was set as a blank test.

Level 1	10 µg/L
Level 2	1 µg/L

4.6 Observation, measurement and cleaning of test tank

(1) Observation of test fish

Condition of test fish was observed visually twice a day (once a day at holiday).

(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 week.

(4) Concentration of dissolved oxygen in test water

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

(5) pH of test water

pH of test water was measured with pH meter twice in experimental period.

(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 item in test water and test fish was performed with gas chromatography-mass spectrometry (GC-MS) 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 thereafter. The number of each sample was one.

(2) Test fish analysis

The test fish of Level 1 and 2 was analyzed five times in duration of exposure. Four fish were taken out at each sampling time and divided into two groups, then both were analyzed individually^{*4}.

The control fish was analyzed before the experimental starting and after the experimental completion. Four fish were taken out at each sampling time and divided into two groups, and then each was analyzed individually. In addition, six fish were taken out and three groups (two fish per group) were used for measurement of lipid contents.

*4 Because one fish was not enough to the analysis sensitivity for the analysis of the test item, two fish were pooled for a group.

4.7.2 Pretreatment for analysis

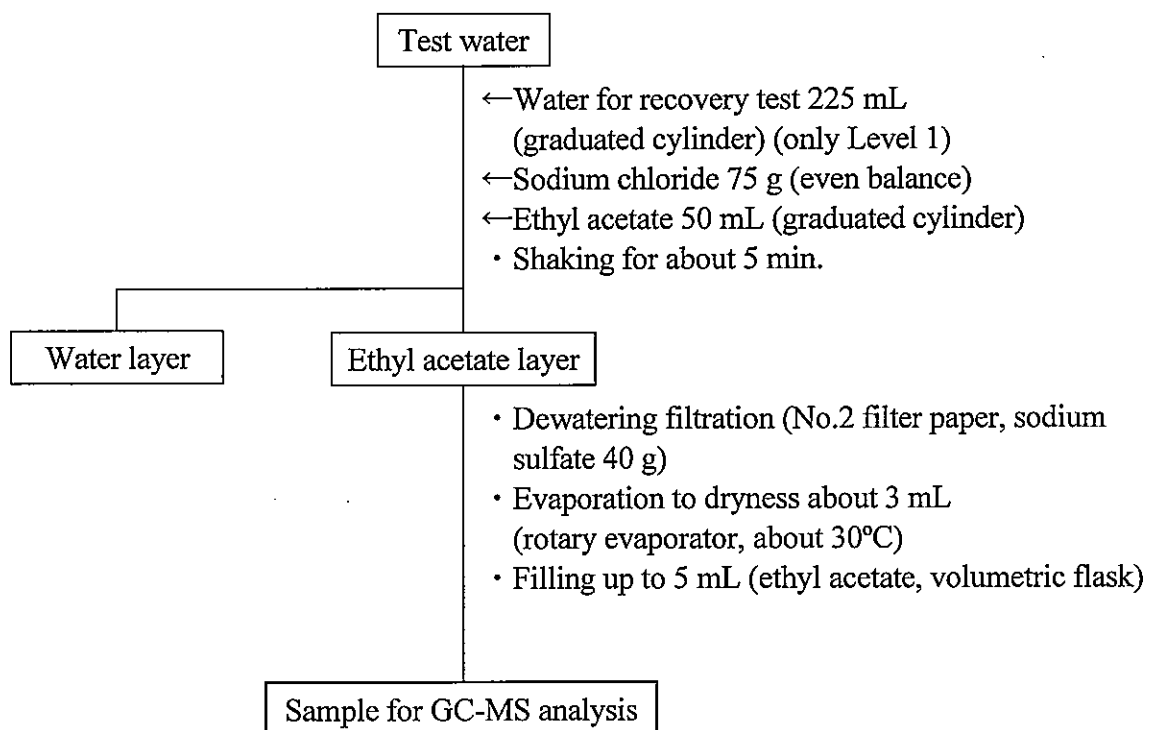
(1) Test water

An aliquot of the test water,

Level 1 25 mL

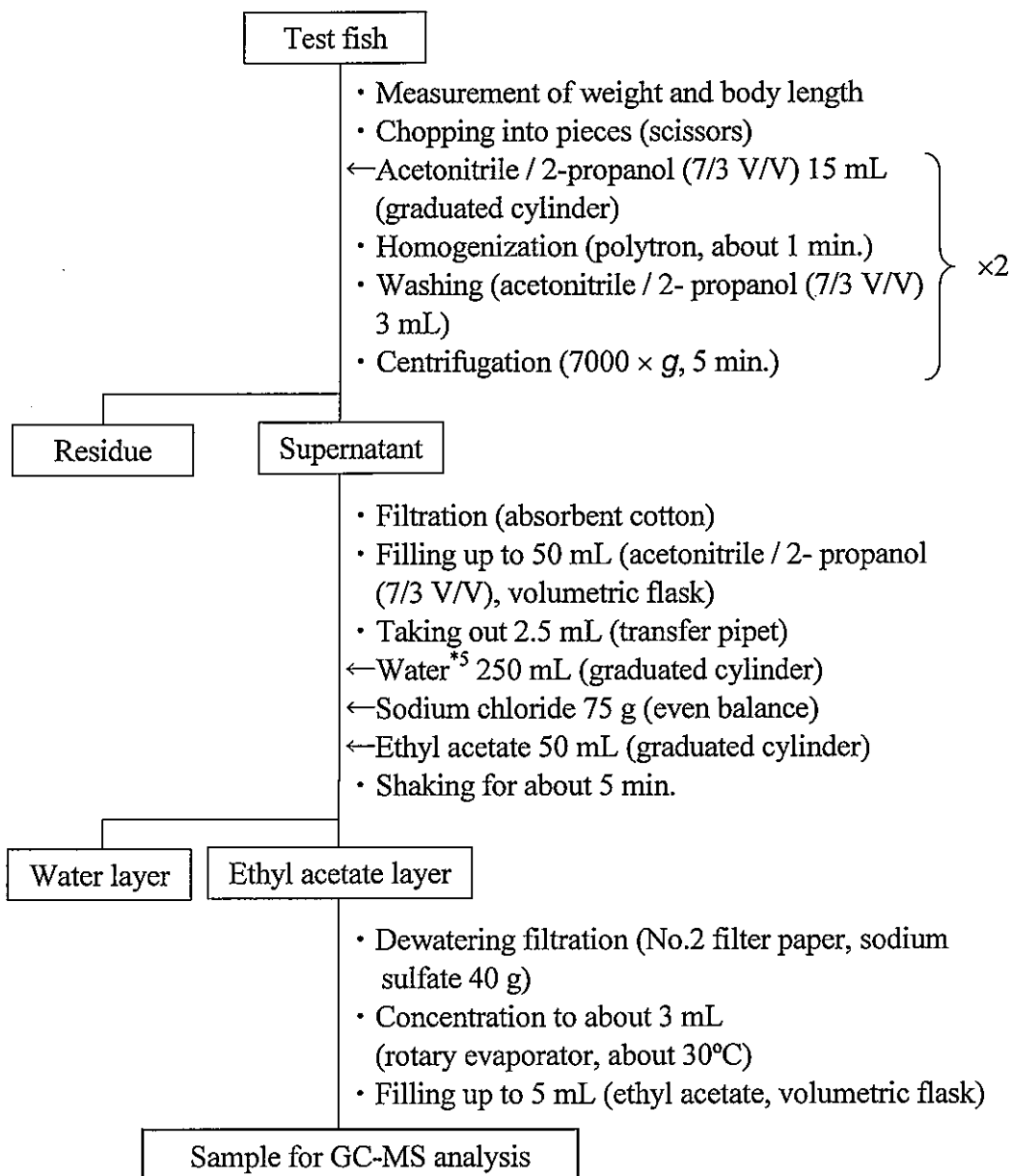
Level 2 250 mL

was taken from each test tank, and pretreated for gas chromatography-mass spectrometry (GC-MS) analysis as follows :



(2) Test fish

Test fish were taken from each test tank and pretreated for gas chromatography-mass spectrometry (GC-MS) analysis as follows :



*5 City water treated by Ultra pure water system.

4.7.3 Quantitative analysis for test item

The samples for GC-MS analysis in pretreatment were analyzed by gas chromatography-mass spectrometry under the following analytical conditions. The concentration of the test item in each sample solution was determined on the basis of a comparison of the peak area on the mass fragmentogram of the sample solution with that of a standard solution (see Tables-6, 7, Fig. 6 and Tables-9, 10, 11, Figs. 9, 10, 11).

(1) Analytical conditions

Instrument	Gas chromatograph-mass spectrometer Shimadzu Corporation type GCMS-QP2010 (No.3)
------------	---

Conditions of gas chromatograph

Column	HP-INNOWAX film thickness 0.25 μ m (Agilent Technologies) 30 m \times 0.25 mm I.D. fused silica
Column temp.	35°C (5 min) \rightarrow 135°C (0 min) \rightarrow 250°C (10 min) Temp. rate ① 20°C/min ② 50°C/min
Carrier gas	Helium
Control mode	Pressure (54.8 kPa)
Total flow	20.0 mL/min
Column flow	1.10 mL/min
Injection temp.	200°C
Sample size	1 μ L
Injection method	Split less
Sampling time	2 min

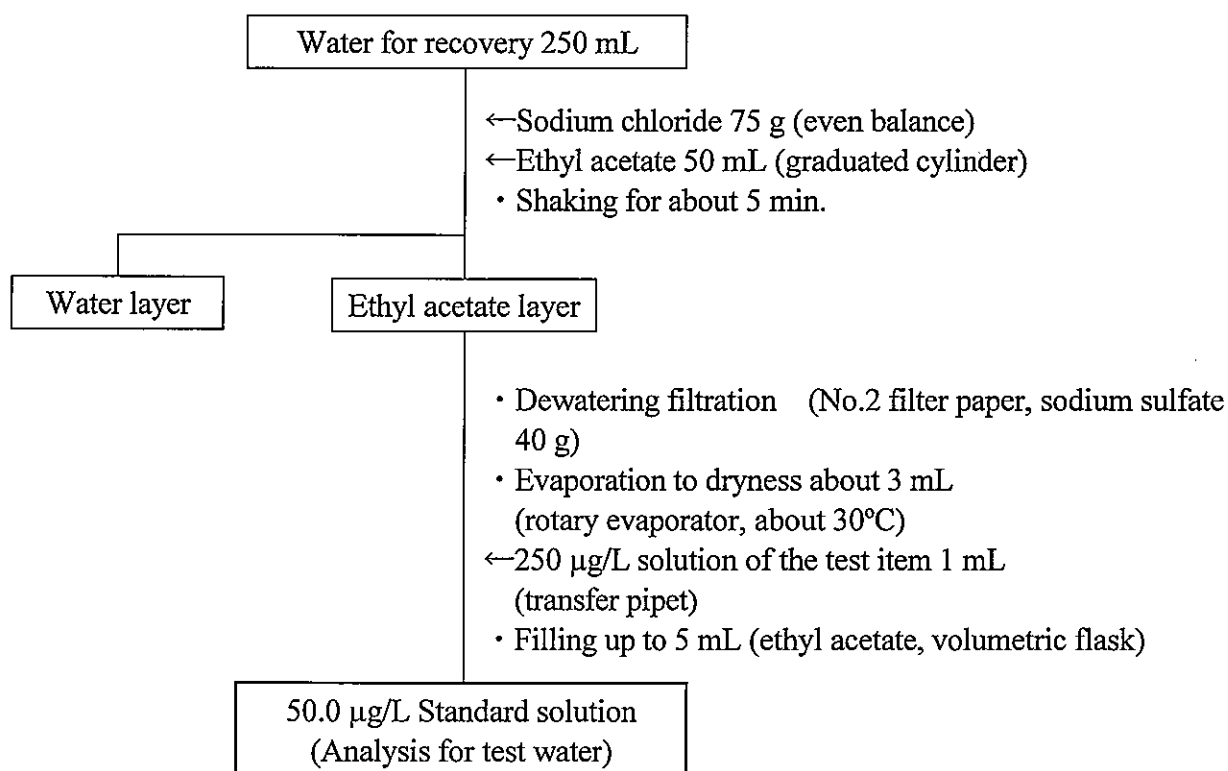
Conditions of mass spectrometer

Ionization mode	Electron ionization (EI)
Detection method	Selected ion monitoring (SIM)
Measurement (m/z)	95
Ion source temp.	230°C
Ionization voltage	70 V
Interface temp.	250°C

(2) Preparation of standard solution

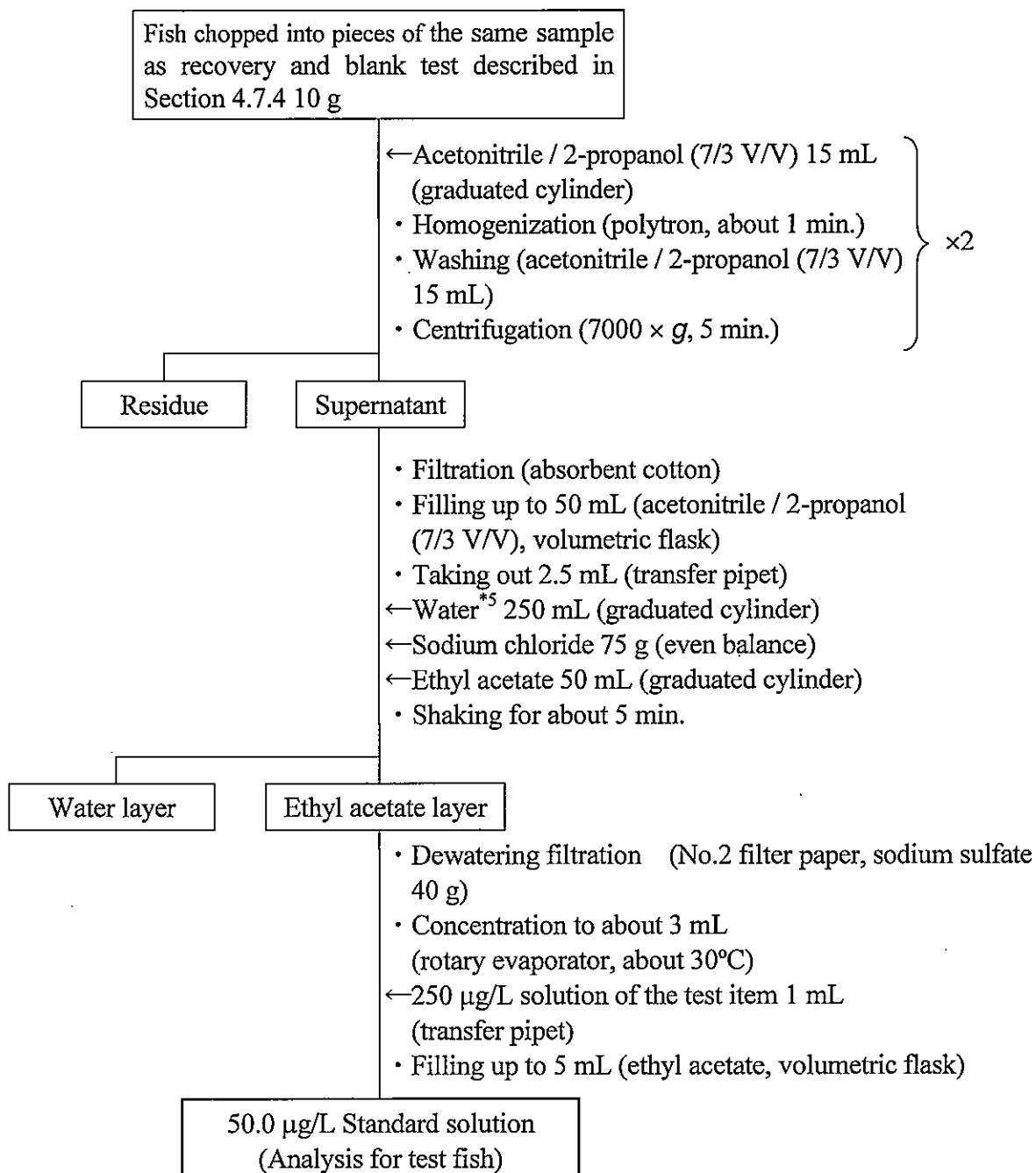
(a) Test water

100 mg of the test sample was accurately weighed and dissolved in ethyl-acetate to prepare 1000 mg/L solution of the test item. 250 µg/L solution of the test item was then prepared from this solution by dilution with ethyl acetate. Solution of the test item was pretreated as follows to prepare 50.0 µg/L standard solution.



(b) Test fish

100 mg of the test sample was accurately weighed and dissolved in ethyl-acetate to prepare 1000 mg/L solution of the test item. 250 µg/L solution of the test item was then prepared from this solution by dilution with ethyl acetate. Solution of the test item was pretreated as follows to prepare 50.0 µg/L standard solution.



(3) Calibration curve

(a) Test water

25.0, 50.0 and 100 $\mu\text{g/L}$ standard solutions were prepared by the same method as described in (2)(a). 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 mass fragmentograms and the respective concentrations.

The lowest detectable peak area of the test item was regarded as 450 considering the noise level, which corresponded to the test item concentration of 1.6 $\mu\text{g/L}$ (see Fig. 4).

(b) Test fish

25.0, 50.0 and 100 $\mu\text{g/L}$ standard solutions were prepared by the same method as described in (2)(b). 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 mass fragmentograms and the respective concentrations.

The lowest detectable peak area of the test item was regarded as 450 considering the noise level, which corresponded to the test item concentration of 1.6 $\mu\text{g/L}$ (see Fig. 7).

4.7.4 Recovery and blank test

(1) Method

Water and fish chopped into pieces (10 g) were spiked a specified amount of the test item and prepared in the same way as described in Section 4.7.2 for the recovery tests. The blank tests were also performed in the same manner without the test item. All the recovery and blank tests were performed in duplicate.

(2) Results of recovery test

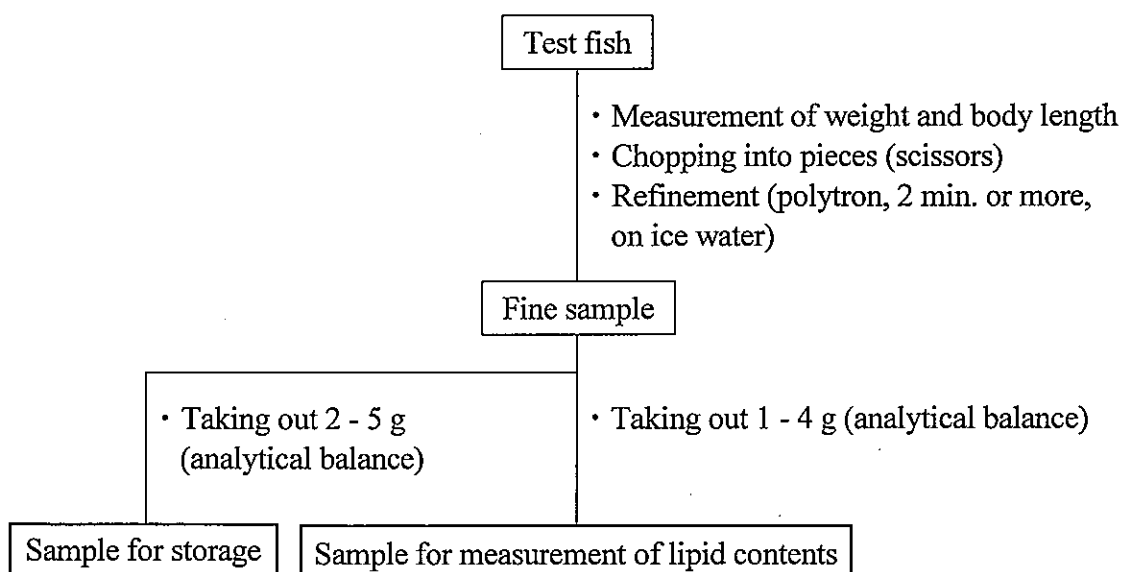
In the blank tests, the mass fragmentogram of GC-MS had no peaks interfering with determination of the test item concentration. The duplicate recovery rates and the average of them in the pretreatment are shown below (see Tables-5, 8 and Figs. 5, 8). The average recovery rate was used as correction factors for the determination of the test item concentrations in the analytical samples.

For analysis of test water (250 ng test item added)
87.3%, 83.6% average 85.5%

For analysis of test fish (5000 ng test item added)
84.0%, 81.7% average 82.8%

4.7.5 Lipid content in test fish

Lipid contents in the control test fish were determined before the experimental starting and after the experimental completion. Six fish were taken out at each sampling time and divided into three groups. Then each group was pretreated as follows, and lipid contents were determined with gravimetric analysis after chloroform-methanol extraction.



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

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

The equations in Tables-6 and 7 were used to obtain the concentrations, and they were rounded to 3 figures.

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

The determination limit^{*6} of the test item in test water was calculated on the basis of that obtained from the calibration curve in Section 4.7.3 (3)(a) as follows.

Level 1	0.37 µg/L
Level 2	0.037 µg/L

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

The equations in Tables-9, 10 and 11 were used to obtain the concentrations, and they were rounded to 3 figures.

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

Assuming the fish to be 10 g, the determination limit^{*6} of the test item in test fish was calculated to be 20 ng/g on the basis of that obtained from the calibration curve in Section 4.7.3 (3)(b).

^{*6} Minimum determination limit of the test item (µg/L or ng/g)

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

where

A : Minimum determination limit of the test item 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 to 2 figures.

4.7.7 Calculation of average concentration of the test item in test water (duration of exposure)

$$\overline{C_{wt}} = \{ C_{w(1)} + \dots + C_{w(n)} \} / n$$

where

- $\overline{C_{wt}}$: The average concentration of the test item in test water ($\mu\text{g/L}$)
- n : Number of analysis for test water (measurement times)
- $C_{w(1)}$: Concentration of the test item in 1st analysis of test water ($\mu\text{g/L}$)
- $C_{w(n)}$: Concentration of the test item in n -th analysis of test water ($\mu\text{g/L}$)

4.7.8 Calculation of bioconcentration factor (BCF)

Bioconcentration factor (BCF) was calculated as follows.

(1) Calculation of average concentration of the test item in test water for calculating BCF

$$\overline{C_w} = \{ C_{w(n-1)} + C_{w(n)} \} / 2 \quad (\text{only 1st analysis of test fish})$$

$$\overline{C_w} = \{ C_{w(n-2)} + C_{w(n-1)} + C_{w(n)} \} / 3 \quad (\text{from 2nd analysis of test fish})$$

where

- $\overline{C_w}$: The average concentration of the test item in test water for calculating BCF ($\mu\text{g/L}$)
- $C_{w(n)}$: Concentration of the test item in n -th analysis of test water ($\mu\text{g/L}$)

(2) Calculation of bioconcentration factor

$$\text{BCF} = \frac{C_f}{\overline{C_w}}$$

where

- BCF : Bioconcentration factor
- C_f : Concentration of the test item in test fish (subtract FB) (ng/g)
- $\overline{C_w}$: The average concentration of the test item in test water for calculating BCF ($\mu\text{g/L}$)
- FB : Average concentration of the test item or blank in the control test fish analyzed before and after the experiment (ng/g)

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

$$BCF_m = (BCF_a + BCF_b) / n$$

where

BCF_m : The average bioconcentration factor in m -th analysis (number of individual 2 (a,b))

$BCF_{a,b}$: Each bioconcentration factor in m -th analysis of test fish

n : Number of individual in m -th analysis of test fish

The date of the analysis for test fish containing value when were not more than calculable BCF, BCF_m is not calculated.

4.7.9 Definition of steady-state

The steady-state of BCF is defined to reach when the variation of BCFs in three successive analyses at intervals of more than 48 hours is within $\pm 20\%$. When BCFs are less than 100, it is evaluated that a steady-state has been reached after 28 days even if the variation of BCFs are over $\pm 20\%$.

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

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

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

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

$V(m-2), V(m-1), V(m)$: Variation rate of bioconcentration factor (%)
 $BCF(m-2), BCF(m-1), BCF(m)$: The average bioconcentration factor in $m-2, m-1, m$ -th analysis of individual
 \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 average concentration of the test item in test water for calculating BCFss

$$\overline{C_{ws}} = \{ C_{w(n-2)} + C_{w(n-1)} + C_{w(n)} \} / 3$$

where

$\overline{C_{ws}}$: The average concentration of the test item 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 ($\mu\text{g/L}$)

$C_{w(n)}$: Concentration of the test item in n -th analysis of test water ($\mu\text{g/L}$)

- (2) Calculation of the average concentration of the test item in test fish at a steady-state

$$\overline{C_{fs}} = \{ C_{f(m-2)} + C_{f(m-1)} + C_{f(m)} \} / 3$$

where

$\overline{C_{fs}}$: The average concentration of the test item in test fish at a steady-state (ng/g)

$C_{f(m)}$: The average concentration of the test item in m -th analysis of test fish (subtract FB) (ng/g)

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

- (3) Calculation of BCFss

$$\text{BCFss} = \overline{C_{fs}} / \overline{C_{ws}}$$

where

BCFss : Bioconcentration factor at a steady-state

$\overline{C_{fs}}$: The average concentration of the test item in test fish at a steady-state (ng/g)

$\overline{C_{ws}}$: The average concentration of the test item in test water for calculating BCFss ($\mu\text{g/L}$)

4.7.11 Calculable BCF

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

Level 1	2.0
Level 2	20

4.7.12 Calculation of lipid content

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

The concentration values of the test item in test water and fish were rounded to 3 figures. BCFs values were rounded to 2 figures.

5. Factors affected reliability of test

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

6. Results

6.1 Concentration of the test item in test water

The measured concentrations of the test item in test water are shown in Table-1. Each concentration of the test item was maintained at more than 97% of each nominated concentration. The variation of the concentrations of the test item was within $\pm 20\%$ of the average of the measured concentrations.

Table-1 Measured concentrations of test item in test water

(Unit : $\mu\text{g/L}$)

Level	After 2 days	After 10 days	After 17 days	After 20 days	After 23 days	After 28 days	Average (Standard deviation)	Table	Fig.
1	9.87	9.91	9.75	9.70	10.1	10.5	9.97 (0.279)	6	6
2	0.985	1.00	0.996	1.00	0.995	1.02	1.00 (0.013)	7	

6.2 Bioconcentration factors

BCFs are shown in Table-2.

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

These BCFs of the test item ranged from 8.4 to 58 at Level 1 and from 24 to 99 at Level 2.

Table-2 BCFs

() : average value

Level	After 10 days	After 17 days	After 20 days	After 23 days	After 28 days	Table	Fig.
1	35 27 (31)	8.4 12 (10)	38 31 (34)	29 29 (29)	58 57 (57)	9	9
2	76 99 (87)	39 34 (36)	27 25 (26)	28 24 (26)	38 30 (34)	10	10

6.3 BCFs at a steady-state (BCFss)

Confirmation whether the steady-state was reached or not was done as follows.

Table-3 Variation of BCFs (the values were indicated for five figures)

Level		After 20 days	After 23 days	After 28 days	Average of three results
1	The average BCF	34.344	29.006	57.445	40.265
	Variation rate from the average of three results (%)	14.704	27.961	42.666	
2	The average BCF	26.078	25.795	34.357	28.744
	Variation rate from the average of three results (%)	9.2719	10.257	19.529	

Level 1

Because the variation of BCFs (average value) for the last three successive analyses were over $\pm 20\%$, the BCFss was not calculated. However, because all BCFs were less than 100, it was evaluated that a steady-state was reached after 28 days.

Level 2

Because the variation of BCFs (average value) after 20, 23 and 28 days from the initiation of exposure were within $\pm 20\%$ of the average for these days' BCFs, it is evaluated that a steady-state was reached. BCFss was calculated on the basis of these results.

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

The average concentrations of the test item in test water at a steady-state are shown in Table-4. The average concentration was 101% at Level 2 of their respective nominated concentrations.

Table-4 Concentrations of test item in test water at a steady-state

(Unit : $\mu\text{g/L}$)

Level	After 20 days	After 23 days	After 28 days	Average	Table	Fig.
2	1.00	0.995	1.02	1.01	7, 10	6

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

BCFss were calculated as 29 at Level 2.

6.4 Lipid content in test fish

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

Before initiation of experiment	3.40%
After termination of experiment	2.80%

6.5 Results of test fish observation

No abnormality in behavior or appearance was noted.

7. Remarks

Instruments, apparatus, and reagents, etc. for the test

(1) Instruments for fish care

Micro quantitative pump for supplying stock solution :

NIHON SEIMITSU KAGAKU CO., LTD
type SP-D-2500S

Instrument for measuring concentration of dissolved oxygen :

Iijima Electronics Co., Ltd. type ID-100

pH meter

Toa Electronics Ltd. type HM-14P

(2) Instruments, apparatus, and reagents

Instruments and apparatus

Gas chromatograph-mass spectrometer :

see page 16

Electronic analytical balance :

Sartorius AG type BP301S

Sartorius AG type CP324S

Sartorius AG type 1404MP8

A&D type FA-2000

Infrared spectrophotometer :

Shimadzu Corporation type IRPrestige-21

Rotary evaporator :

Tokyo Rika Kikai Co., Ltd. type N-NJ

Tokyo Rika Kikai Co., Ltd. type N-1000K

Mechanical shaker :

Taitec type SR-2w

Homogenizer (polytron) :

Kinematica type PT3100

Centrifuge :

Hitachi Koki Co., Ltd. type CR21G

Reagents

Sodium chloride (extra pure) :	MANAC Incorporated
Ethyl acetate (extra pure) :	Kanto Chemical Co., Inc.
Acetonitrile (HPLC grade) :	Wako Pure Chemical Industries, Ltd.
2-propanol (extra pure)	NACALAI TESQUE, INC.
Sodium sulfate (extra pure)	Kanto Chemical Co., Inc.
Dimethylsulfoxide (extra pure) :	NACALAI TESQUE, INC.
MEGAFACE F-443	DAINIPPON INK AND CHEMICALS, INCORPORATED

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

Instruments and apparatus

Electronic analytical balance :	Sartorius type BP301S
Rotary evaporator :	Tokyo Rika Kikai Co., Ltd. type N-1000K
Homogenizer (polytron) :	Kinematica type PT3100
Homogenizer (autocellmaster) :	AS ONE CORPORATION type CM-200
Vacuum pump :	Sinku Kiko Co., Ltd. type DA-20D Sinku Kiko Co., Ltd. type DTC-21
Vacuum desiccator :	Iuchiseieido Co., Ltd. type VL

Reagents

Purified water (Japanese Pharmacopeia) :	Takasugi Pharmaceutical Co., Ltd.
Methanol (extra pure) :	Wako Pure Chemical Industries, Ltd.
Chloroform (guaranteed reagent) :	Wako Pure Chemical Industries, Ltd.
Sodium sulfate (extra pure) :	Kanto Chemical Co., Inc.

Table-5 Calculation table for recovery and blank test (analysis of test water)

Study No. 44807

Sample description	A	B	C	D	E	F
Standard 50.0µg/L	14655					
Recovery a	12797	1	5	-	218	87.3
Recovery b	12254	1	5	-	209	83.6
						Average
Standard 50.0µg/L	14950					85.5
Blank a	n.d.	1	5	-	-	
Blank b	n.d.	1	5	-	-	
				Average		
				-		
(a, b : individual sample)						
<p>A : Peak area</p> <p>A(std) : Standard solution A(t) : Sample</p> <p>B : Ratio of portion used for analysis</p> <p>C : Final volume (mL)</p> <p>D : Amount of blank in test water (ng)</p> <p>E : Amount of test item recovered (ng)</p> <p>$E = P \times (A(t) / A(std)) / B \times C - D$</p> <p>F : Recovery rate (%)</p> <p>$F = E / Q \times 100$</p> <p>P : Concentration of test item in standard solution 50.0µg/L</p> <p>Q : Amount of test item added (250ng)</p> <p>See Fig. 5</p>						

March 14, 2007

Name _____

Table-6 Calculation table for analysis of test water (Level 1)

Study No. 44807

Sample description	A	I
Standard 50.0µg/L	18511	
Test water after 2 days	15614	9.87
Standard 50.0µg/L	19844	
Test water after 10 days	16813	9.91
Standard 50.0µg/L	20895	
Test water after 17 days	17420	9.75
Standard 50.0µg/L	26696	
Test water after 20 days	22129	9.70
Standard 50.0µg/L	22230	
Test water after 23 days	19178	10.1
Standard 50.0µg/L	17340	
Test water after 28 days	15504	10.5
Average concentration of test item in test water 9.97 (S.D. 0.279)		
<p>A: Peak area A(std) : Standard solution A(t) : Sample B: Ratio of portion used for analysis 1 C: Final volume 5mL F: Recovery rate 85.5% H: Volume of test water taken out 25mL I: Concentration of test item in test water (µg/L) $I = P \times (A(t) / A(std)) / B \times C / F \times 100 / H$ J: Average concentration of test item in test water (µg/L) $J = (I(1) + \dots + I(n)) / n$ 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 item in standard solution 50.0µg/L See Fig. 6</p>		

March 14, 2007

Name _____

Table-7 Calculation table for analysis of test water (Level 2)

Study No. 44807

Sample description	A	I
Standard 50.0µg/L	18511	
Test water after 2 days	15585	0.985
Standard 50.0µg/L	19844	
Test water after 10 days	17003	1.00
Standard 50.0µg/L	20895	
Test water after 17 days	17790	0.996
Standard 50.0µg/L	26696	
Test water after 20 days	22900	1.00
Standard 50.0µg/L	22230	
Test water after 23 days	18912	0.995
Standard 50.0µg/L	17340	
Test water after 28 days	15173	1.02
Average concentration of test item in test water 1.00 (S.D. 0.013)		
<p>A: Peak area A(std) : Standard solution A(t) : Sample B: Ratio of portion used for analysis 1 C: Final volume 5mL F: Recovery rate 85.5% H: Volume of test water taken out 250mL I: Concentration of test item in test water (µg/L) $I = P \times (A(t) / A(std)) / B \times C / F \times 100 / H$ J: Average concentration of test item in test water (µg/L) $J = (I(1) + \dots + I(n)) / n$ n : Number of test water analyses (n = 6) I (1) : First analysis of test water I (n) : Last analysis of test water</p> $S.D. = \sqrt{\frac{n \times \sum_{i=1}^n I(i)^2 - \left(\sum_{i=1}^n I(i) \right)^2}{n \times (n - 1)}}$ <p>P: Concentration of test item in standard solution 50.0µg/L See Fig. 6</p>		

March 14, 2007

Name _____

Table-8 Calculation table for recovery and blank test (analysis of test fish)

Study No. 44807

Sample description	A	C	D	E	F	G
Standard 50.0 μ g/L	13583					
Recovery a	11407	2.5/50	5	-	4200	84.0
Recovery b	11098	2.5/50	5	-	4090	81.7
						Average
						82.8
Standard 50.0 μ g/L	13364					
Blank a	n.d.	2.5/50	5	-	-	
Blank b	n.d.	2.5/50	5	-	-	
				Average		
				-		
(a, b : individual sample)						
<p>A: Peak area</p> <p>A(std) : Standard solution A(t) : Sample</p> <p>B: Ratio of portion used for analysis 1</p> <p>C: Ratio of portion used for analysis (extracted solution)</p> <p>D: Final volume (mL)</p> <p>E: Amount of blank in test fish (ng)</p> <p>F: Amount of test item recovered (ng)</p> $F = P \times (A(t) / A(std)) / B / C \times D - E$ <p>G: Recovery rate (%)</p> $G = F / Q \times 100$ <p>P: Concentration of test item in standard solution 50.0μg/L</p> <p>Q: Amount of test item added (5000ng)</p> <p>See Fig. 8</p>						

March 14, 2007

Name _____

Table-9

Calculation table for analysis of test fish (Level 1)

Study No. 44807

Sample description	A	D	G	K	H	J	M
Standard 50.0µg/L	21621						
Test fish after 10 days a	11235	1	9.19	341	9.89	35	31
Test fish after 10 days b	8570	1	8.98	266	9.89	27	
Standard 50.0µg/L	21228						
Test fish after 17 days a	2705	1	9.28	82.9	9.85	8.4	10
Test fish after 17 days b	4000	1	9.64	118	9.85	12	
Standard 50.0µg/L	24134						
Test fish after 20 days a	12431	1	8.37	371	9.79	38	34
Test fish after 20 days b	10939	1	9.09	301	9.79	31	
Standard 50.0µg/L	23548						
Test fish after 23 days a	9138	1	8.24	284	9.85	29	29
Test fish after 23 days b	9410	1	8.40	287	9.85	29	
Standard 50.0µg/L	16585						
Test fish after 28 days a	13849	1	8.57	588	10.1	58	57
Test fish after 28 days b	14847	1	9.47	571	10.1	57	
(a, b : individual sample)							
<p>A: Peak area A(std) : Standard solution A(t) : Sample B: Ratio of portion used for analysis 2.5/50 C: Final volume 5mL D: Dilution factor E: Average concentration of blank in analysis of control 0ng/g F: Recovery rate 82.8% G: Weight of test fish (g) K: Concentration of test item 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 item 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 item in test water (µg/L) J: BCF $J = K / H$ M: Average value of BCF(a) and BCF(b) $M = \{ BCF(a) + BCF(b) \} / 2$ P: Concentration of test item in standard solution 50.0µg/L</p> <p>See Fig. 9</p>							

March 14, 2007

Name _____

Table-10 Calculation table for analysis of test fish (Level 2)

Study No. 44807

Sample description	A	D	G	K	H	J	M	O
Standard 50.0µg/L	21444							
Test fish after 10 days a	2260	1	8.41	75.6	0.994	76	87	-
Test fish after 10 days b	3019	1	8.67	98.0	0.994	99		
Standard 50.0µg/L	20849							
Test fish after 17 days a	1222	1	9.13	38.7	0.995	39	36	-
Test fish after 17 days b	1066	1	9.20	33.5	0.995	34		
Standard 50.0µg/L	24513							
Test fish after 20 days a	1004	1	8.99	27.5	1.00	27	26	50
Test fish after 20 days b	944	1	9.41	24.7	1.00	25		
Standard 50.0µg/L	22890							
Test fish after 23 days a	983	1	9.36	27.7	0.998	28	26	29
Test fish after 23 days b	803	1	8.89	23.8	0.998	24		
Standard 50.0µg/L	16813							
Test fish after 28 days a	1018	1	9.48	38.5	1.01	38	34	29
Test fish after 28 days b	813	1	9.51	30.7	1.01	30		
(a, b : individual sample)								
BCFss : 29								
<p>A: Peak area A(std) : Standard solution A(t) : Sample B: Ratio of portion used for analysis 2.5/50 C: Final volume .5mL D: Dilution factor E: Average concentration of blank in analysis of control 0ng/g F: Recovery rate 82.8% G: Weight of test fish (g) K: Concentration of test item 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 item 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 item 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 item in standard solution 50.0µg/L K: Average value of K (ng/g) $\bar{K} = \{ K(n-1)a + K(n-1)b \} / 2$ $BCFss = [\{ \bar{K}(n-3) + \bar{K}(n-2) + \bar{K}(n-1) \} / 3] / [\{ I(n-2) + I(n-1) + I(n) \} / 3]$</p>								
See Fig. 10								

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

Study No. 44807

Sample description	A	E	G	I
Standard 50.0µg/L	20931			
Before the experimental start a	n.d.	-	6.55	-
Before the experimental start b	n.d.	-	6.66	-
Standard 50.0µg/L	16472			
After the experimental completion a	n.d.	-	10.6	-
After the experimental completion b	n.d.	-	10.9	-
				Average
				-
(a, b : individual sample)				
<p>A: Peak area</p> <p>A(std) : Standard solution A(t) : Sample</p> <p>B: Ratio of portion used for analysis 2.5/50</p> <p>C: Final volume 5mL</p> <p>E: Amount of blank in analysis of control (ng)</p> $E = P \times (A(t) / A(std)) / B \times C$ <p>G: Weight of test fish (g)</p> <p>I: Concentration of blank in test fish (ng/g)</p> $I = E / G$ <p>P: Concentration of test item in standard solution 50.0µg/L</p> <p>See Fig. 11</p>				

March 14, 2007

Name _____

Item	Unit	Measured value	Standard value	Detection limit
Total hardness (Ca, Mg)	mg/L	14.2	< 300 ^{*1}	0.1
Suspended solid	mg/L	< 1	< 20 ^{*2}	1
pH	—	8.0	6.5 ~ 8.5 ^{*3}	—
Total organic carbon	mg/L	0.3	< 2 ^{*2}	0.1
Chemical oxygen demand	mg/L	2.3	< 5 ^{*3}	0.5
Residual chlorine	mg/L	< 0.02	< 0.02 ^{*3}	0.02
Ammonia nitrogen	mg/L	0.02	< 1 ^{*3}	0.01
Total cyan	mg/L	< 0.01	n.d. ^{*3}	0.01
Alkalinity	mg/L	101	—	1
Electric conductivity	μS/cm	278	—	—
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	< 0.01	< 0.05 ^{*3}	0.01
Lead	mg/L	< 0.005	< 0.1 ^{*3}	0.005
Arsenic	mg/L	0.004	< 0.05 ^{*3}	0.001
Iron	mg/L	0.02	< 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	< 0.005	< 1.0 ^{*1}	0.005
Aluminium	mg/L	0.007	< 0.2 ^{*1}	0.001
Nickel	mg/L	< 0.001	< 0.001 ^{*5}	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	< 0.0001	< 0.04 ^{*4}	0.0001
Propyzamide	mg/L	< 0.0001	< 0.008 ^{*4}	0.0001
Chlornitrofen	mg/L	< 0.0001	< 0.0001 ^{*1}	0.0001
Simazine	mg/L	< 0.001	< 0.003 ^{*4}	0.001
Thiobencarb	mg/L	< 0.0001	< 0.02 ^{*4}	0.0001
Organophosphorous pesticides				
Diazinon	mg/L	< 0.0001	< 0.005 ^{*4}	0.0001
Isoxathion	mg/L	< 0.0001	< 0.008 ^{*4}	0.0001
Fenitrothion	mg/L	< 0.0001	< 0.003 ^{*4}	0.0001
EPN	mg/L	< 0.0001	< 0.006 ^{*4}	0.0001
Dichlorvos	mg/L	< 0.0001	< 0.01 ^{*4}	0.0001
Iprobenfos	mg/L	< 0.0001	< 0.008 ^{*4}	0.0001
PCB	mg/L	< 0.0005	n.d. ^{*4}	0.0005
Coliform bacteria count	—	n.d.	n.d. ^{*1}	—
Fluorine compound	mg/L	1.3	< 1.5 ^{*3}	0.1
Anionic surfactant	mg/L	< 0.01	< 0.2 ^{*1}	0.01

*1 Ministerial ordinance of the Ministry of Health, Labour and Welfare No.101 (Revised May 30, 2003)

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

*3 Water quality criteria for fisheries (Japan Fisheries Resource Conservation Association, 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)

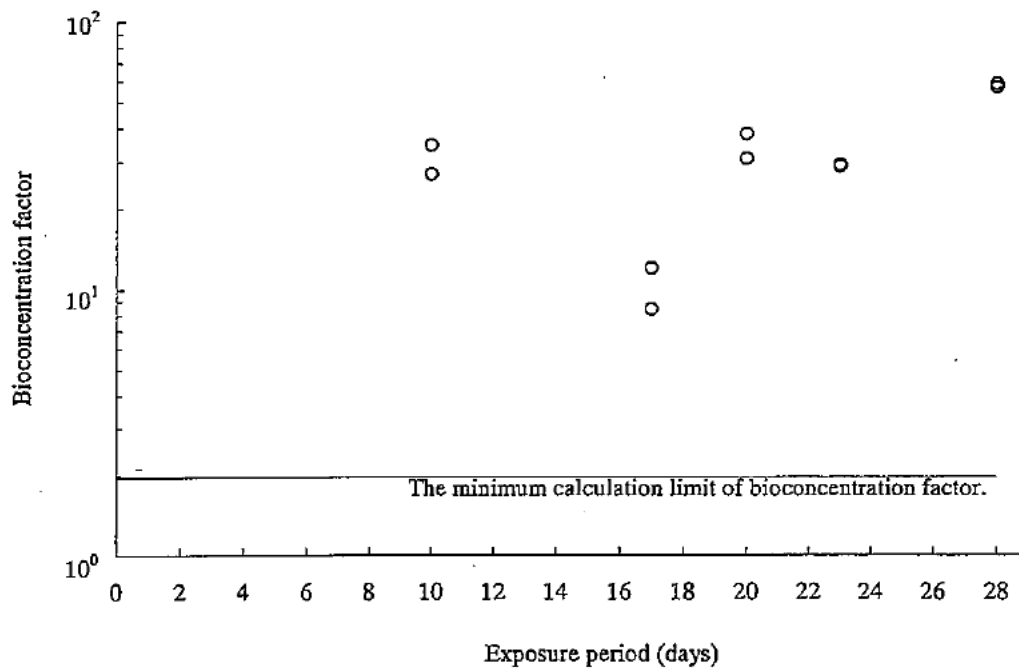


Fig.1 Correlation between exposure period and bioconcentration factor (Level 1).

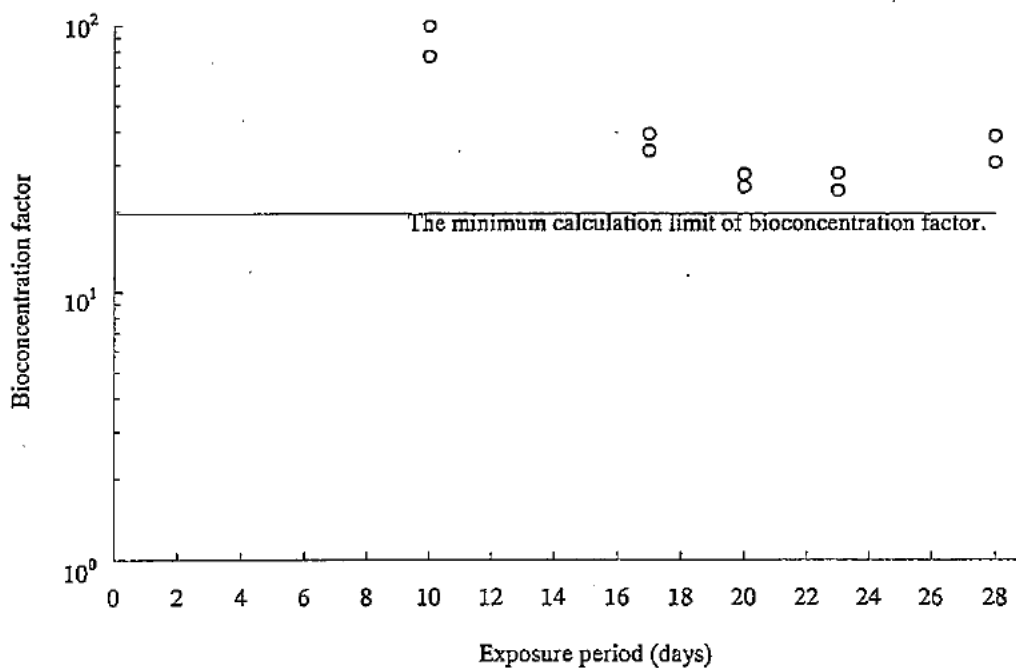
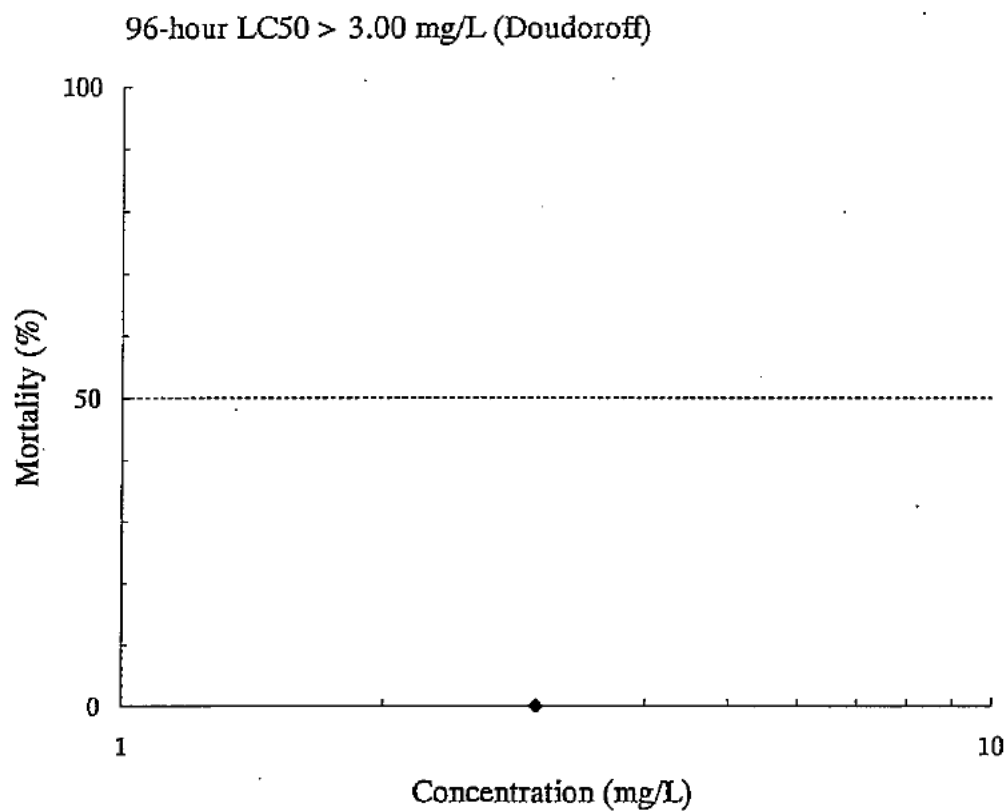


Fig.2 Correlation between exposure period and bioconcentration factor (Level 2).



Concentration (mg/L)	Cumulative Mortality (%)			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
3.00	0	0	0	0

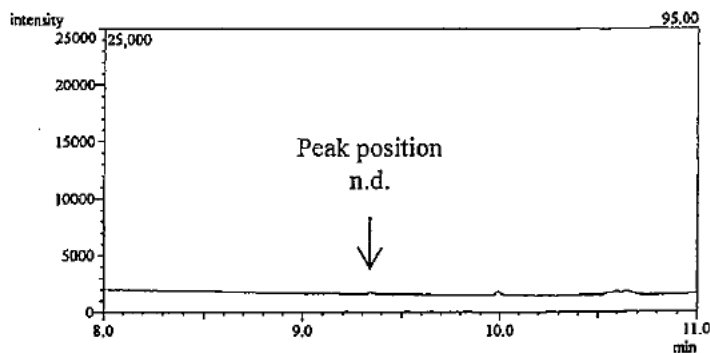
Fig. 3 Concentration - mortality curve.

Date : February 9, 2007 Name _____

Solvent (W)

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_wsol.qgd

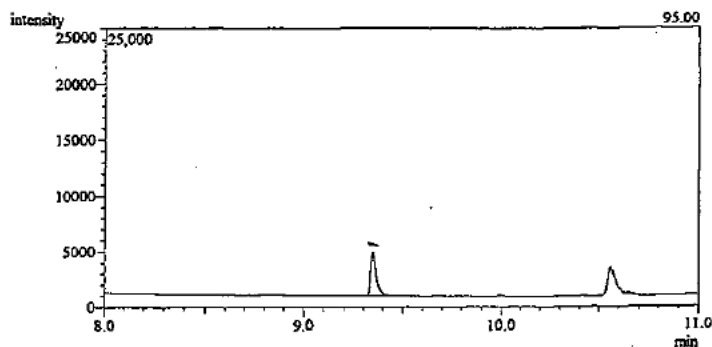


Peak No.	Time(min)	m/z	Area
1	-	95.00	---

Standard solution 25.0 ug/L (W)

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_w25.qgd

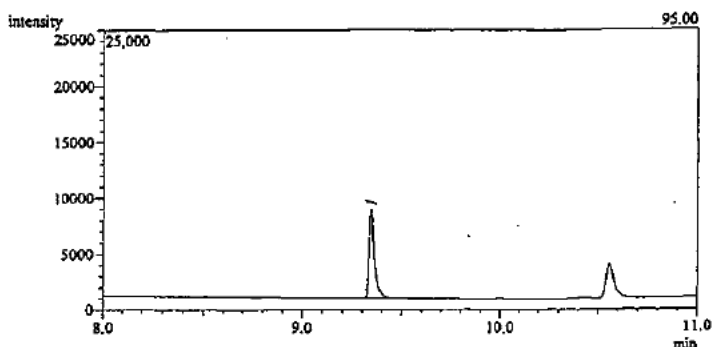


Peak No.	Time(min)	m/z	Area
1	9.35	95.00	7435

Standard solution 50.0 ug/L (W)

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_w50.qgd

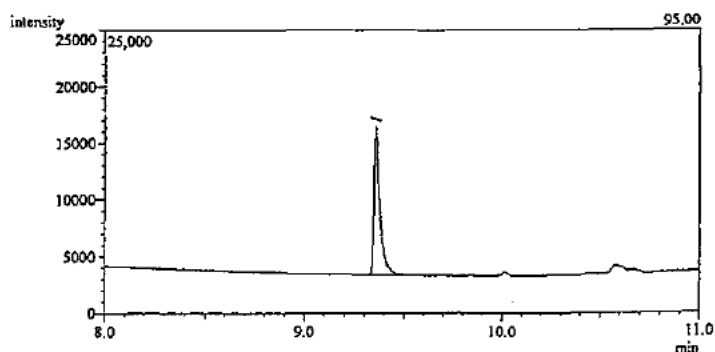


Peak No.	Time(min)	m/z	Area
1	9.35	95.00	14883

Standard solution 100 ug/L (W)

Operating date : Dec. 23, 2006

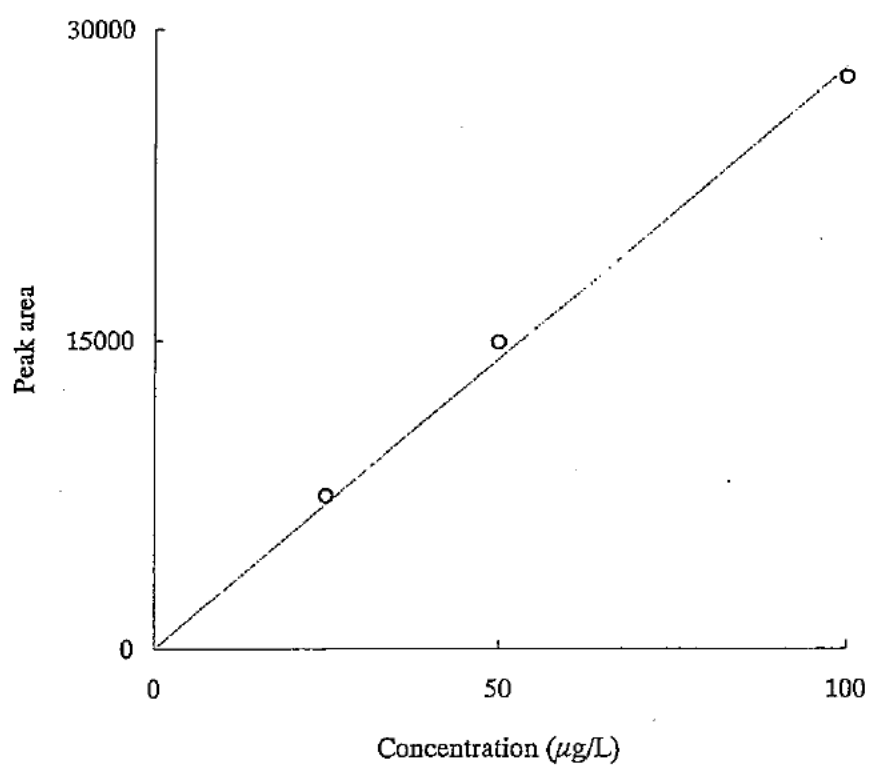
File name : C:\GCMSsolution\Data\Section2\44807\061222\1_w100.qgd



Peak No.	Time(min)	m/z	Area
1	9.36	95.00	27689

Fig. 4-1 Mass fragmentograms of GC-MS analysis for calibration curve (test water).

Date : Dec. 25, 2006 Name :



$$y = 282x$$

$$r = 0.998$$

Concentration ($\mu\text{g/L}$)	Peak area
25.0	7435
50.0	14883
100	27689

Fig. 4 - 2 Calibration curve of test item (test water).

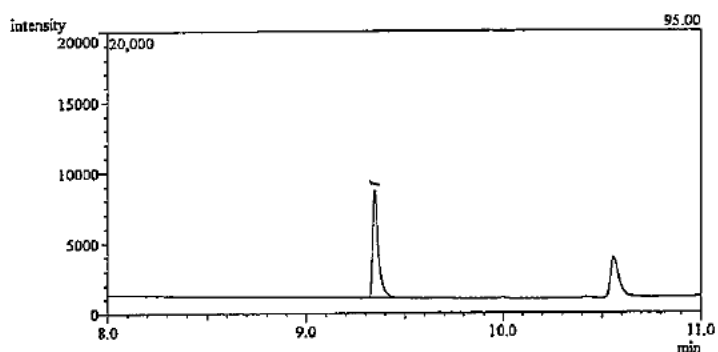
December 25, 2006

Name _____

Standard solution 50.0 ug/L (W)

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_wrs.qgd

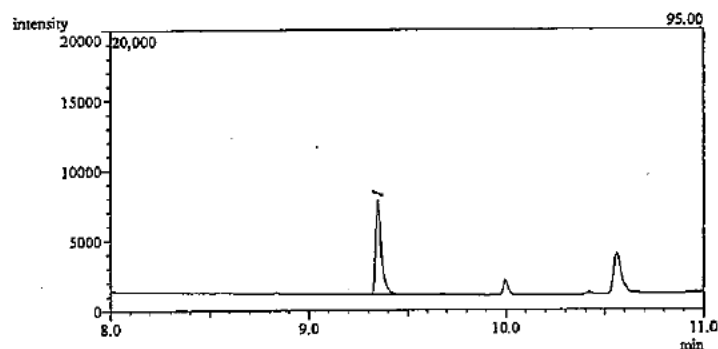


Peak No.	Time(min)	m/z	Area
1	9.35	95.00	14655

Recovery test from test water a

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_wra.qgd

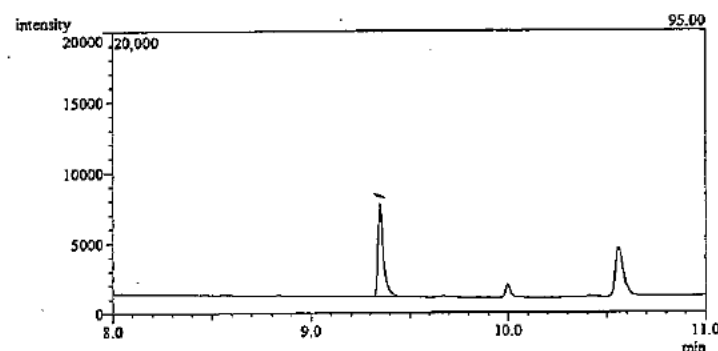


Peak No.	Time(min)	m/z	Area
1	9.35	95.00	12797

Recovery test from test water b

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_wrb.qgd



Peak No.	Time(min)	m/z	Area
1	9.35	95.00	12254

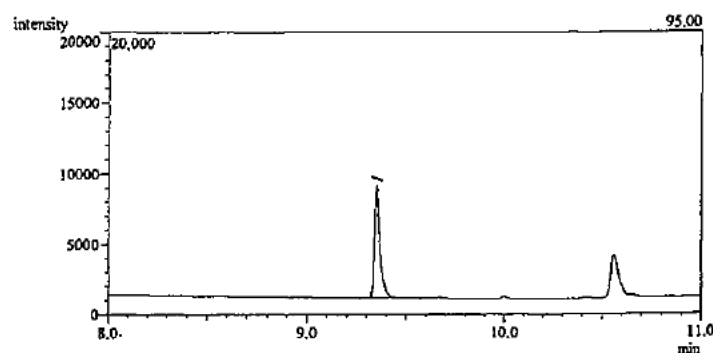
Fig. 5-1 Mass fragmentograms of GC-MS analysis for recovery and blank test (test water).

Date : Dec. 25, 2006 Name :

Standard solution 50.0 µg/L (W)

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_wbs.qgd

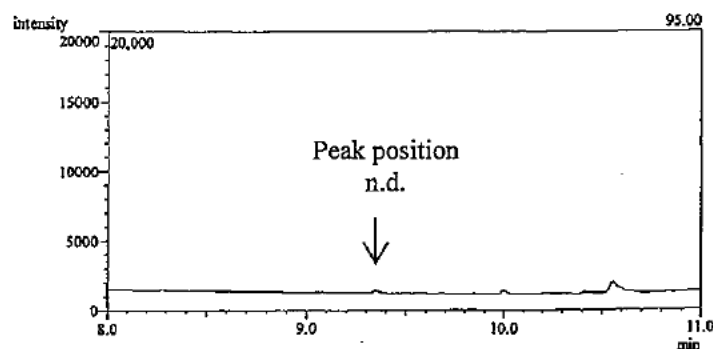


Peak No.	Time(min)	m/z	Area
1	9.35	95.00	14950

Blank test of test water a

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_wba.qgd

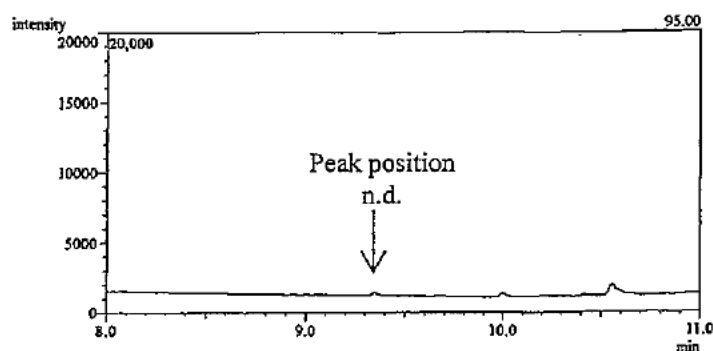


Peak No.	Time(min)	m/z	Area
1	-	95.00	---

Blank test of test water b

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_wbb.qgd



Peak No.	Time(min)	m/z	Area
1	-	95.00	---

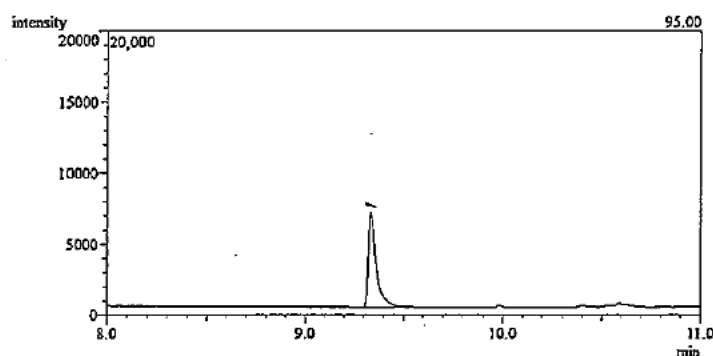
Fig. 5-2 Mass fragmentograms of GC-MS analysis for recovery and blank test (test water).

Date : Dec. 25, 2006 Name :

Standard solution 50.0 ug/L (W)

Operating date : Feb. 15, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070215\3ws.qgd

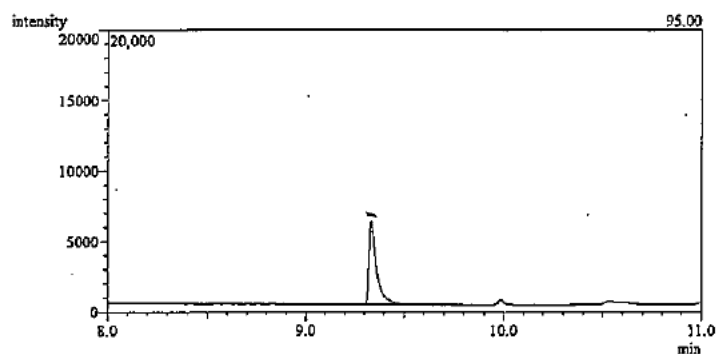


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	18511

Test water after 2 days (Level 1)

Operating date : Feb. 15, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070215\3wh.qgd

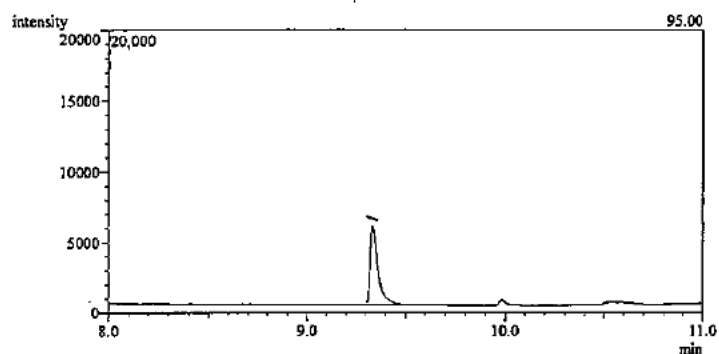


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	15614

Test water after 2 days (Level 2)

Operating date : Feb. 15, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070215\3wl.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	15585

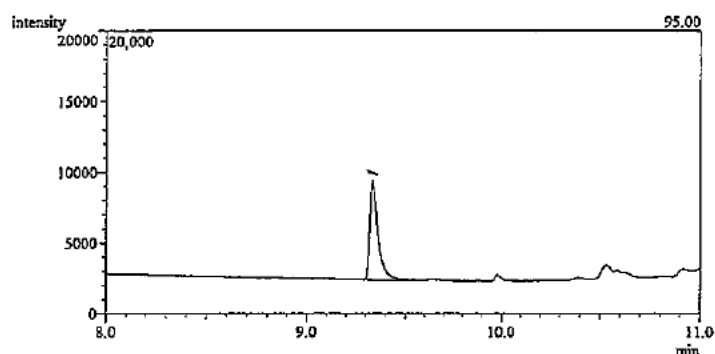
Fig. 6-1 Mass fragmentograms of GC-MS analysis for test water.

Date : Feb. 15, 2007 Name :

Standard solution 50.0 ug/L (W)

Operating date : Feb. 23, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070223\1ws.qgd

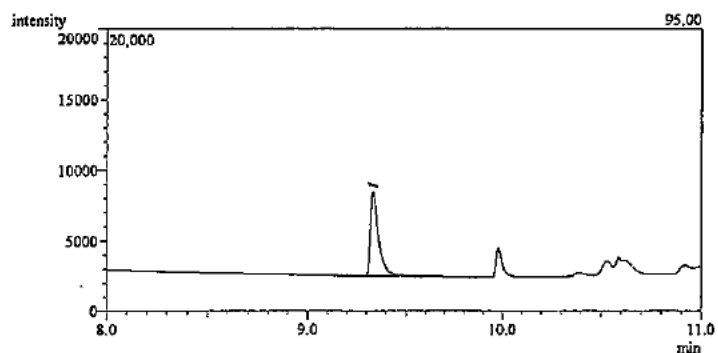


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	19844

Test water after 10 days (Level 1)

Operating date : Feb. 23, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070223\1wh.qgd

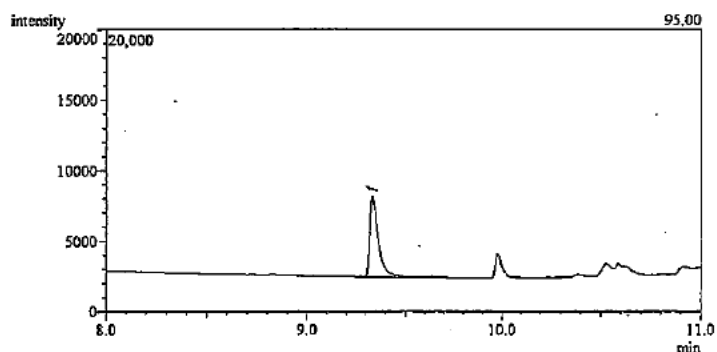


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	16813

Test water after 10 days (Level 2)

Operating date : Feb. 23, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070223\1wl.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	17003

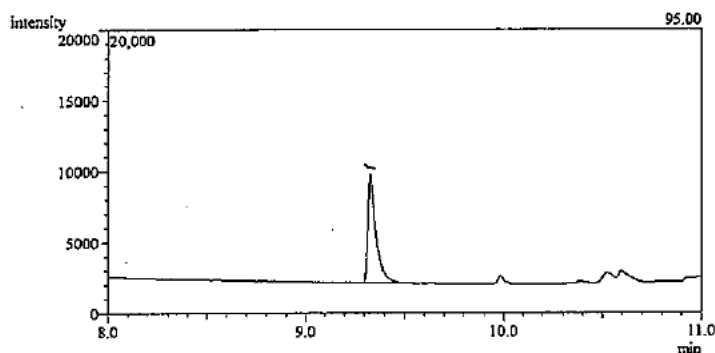
Fig. 6-2 Mass fragmentograms of GC-MS analysis for test water.

Date : Feb. 23, 2007 Name :

Standard solution 50.0 ug/L (W)

Operating date : Mar. 02, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070302\1ws..qgd

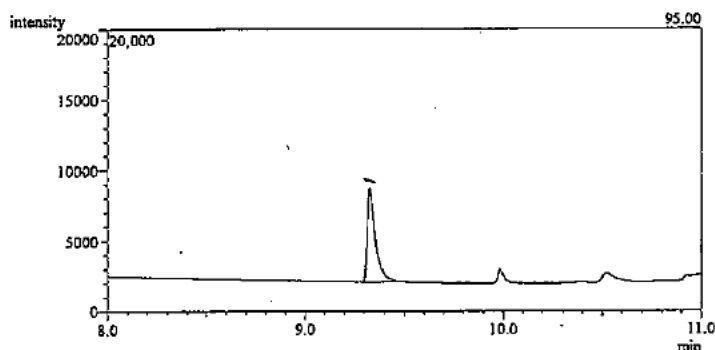


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	20895

Test water after 17 days (Level 1)

Operating date : Mar. 02, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070302\1wh.qgd

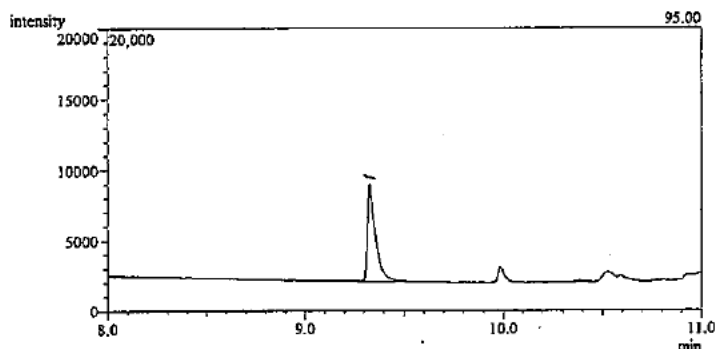


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	17420

Test water after 17 days (Level 2)

Operating date : Mar. 02, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070302\1wl.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	17790

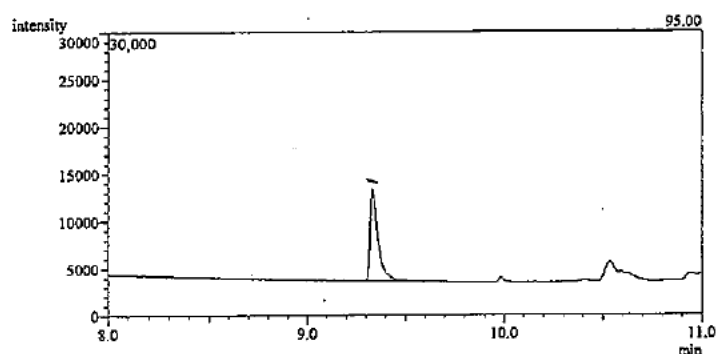
Fig. 6-3 Mass fragmentograms of GC-MS analysis for test water.

Date : Mar. 2, 2007 Name :

Standard solution 50.0 ug/L (W)

Operating date : Mar. 05, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070305\2wbs.qgd

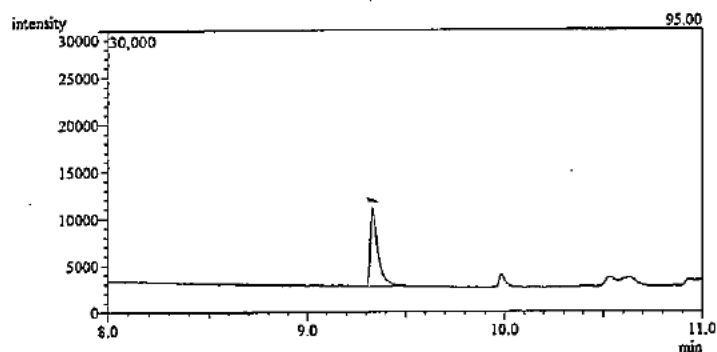


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	26696

Test water after 20 days (Level 1)

Operating date : Mar. 05, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070305\2wh.qgd

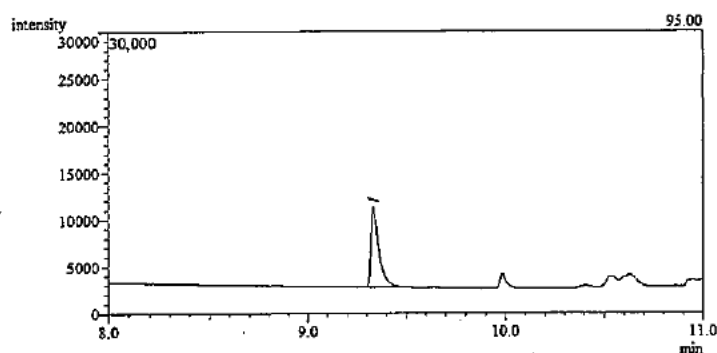


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	22129

Test water after 20 days (Level 2)

Operating date : Mar. 05, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070305\2wl.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	22900

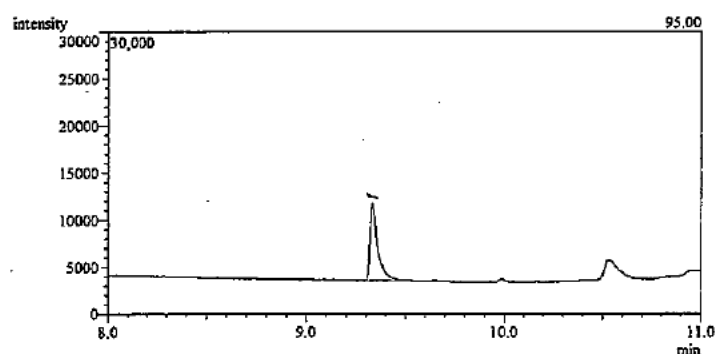
Fig. 6-4 Mass fragmentograms of GC-MS analysis for test water.

Date : Mar. 5, 2007 Name :

Standard solution 50.0 ug/L (W)

Operating date : Mar. 08, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070308\1ws.qgd

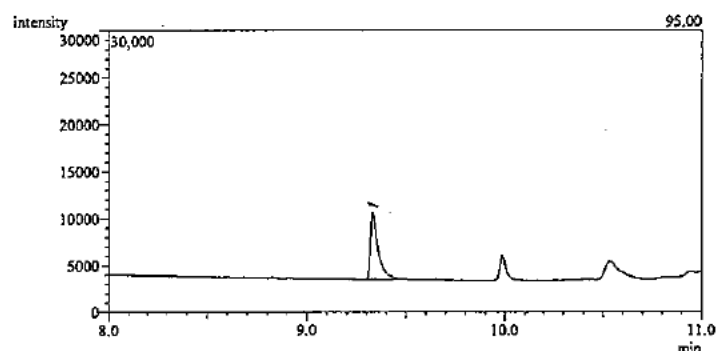


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	22230

Test water after 23 days (Level 1)

Operating date : Mar. 08, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070308\1wh.qgd

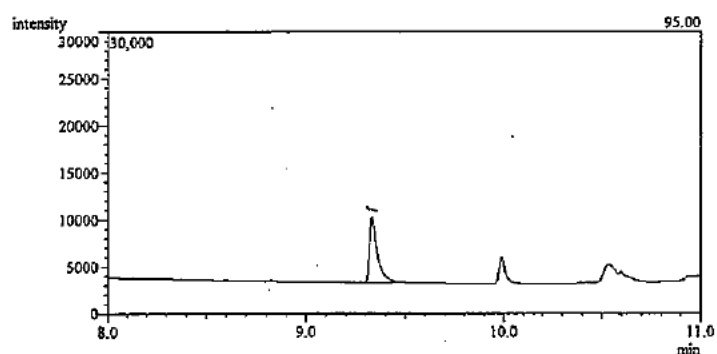


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	19178

Test water after 23 days (Level 2)

Operating date : Mar. 08, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070308\1wl.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	18912

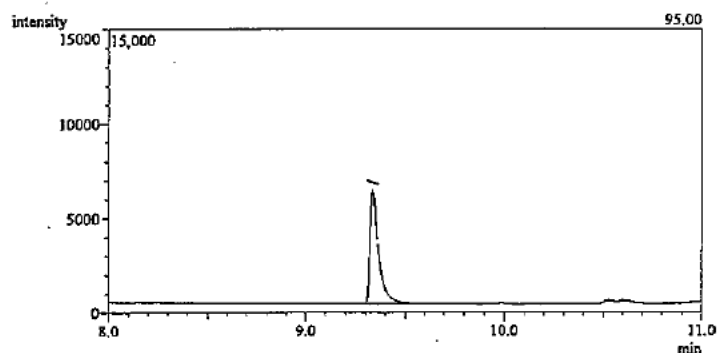
Fig. 6-5 Mass fragmentograms of GC-MS analysis for test water.

Date : Mar. 8, 2007 Name :

Standard solution 50.0 ug/L (W)

Operating date : Mar. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070313\2_ws.qgd

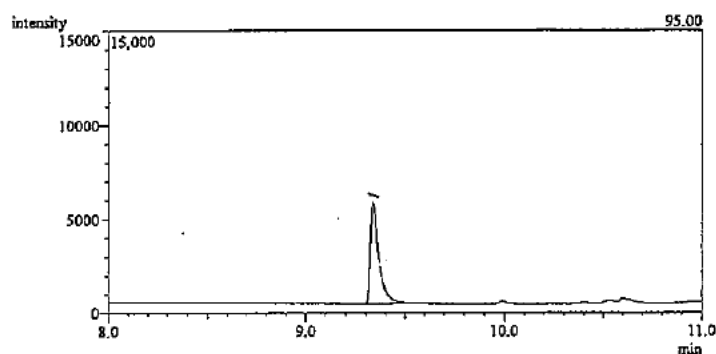


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	17340

Test water after 28 days (Level 1)

Operating date : Mar. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070313\2_wh.qgd

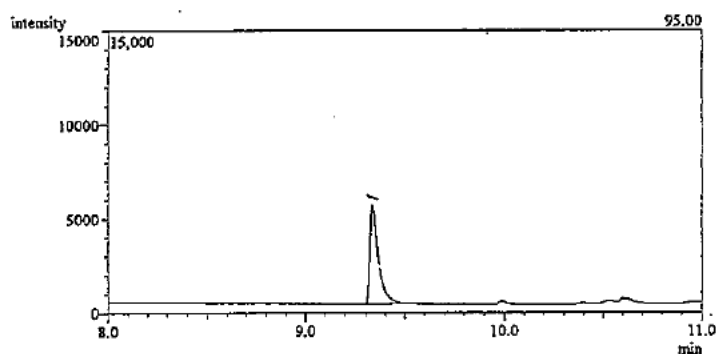


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	15504

Test water after 28 days (Level 2)

Operating date : Mar. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070313\2_wl.qgd



Peak No.	Time(min)	m/z	Area
1	9.34	95.00	15173

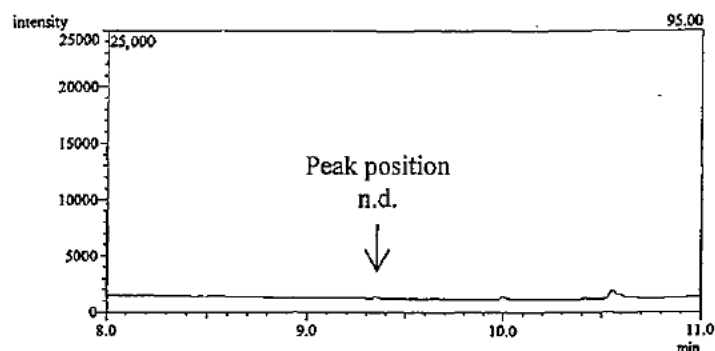
Fig. 6-6 Mass fragmentograms of GC-MS analysis for test water.

Date : Mar. 13, 2007 Name :

Solvent (F)

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_fsol_.qgd

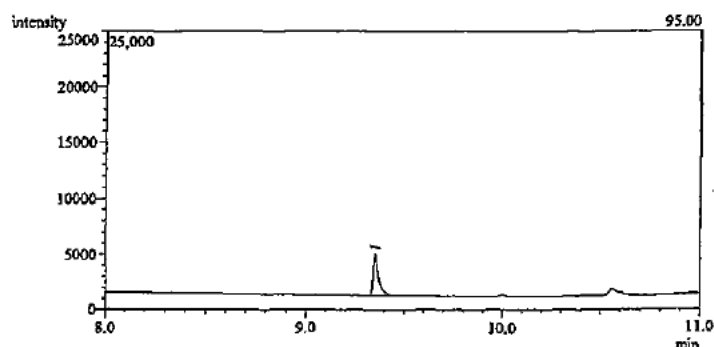


Peak No.	Time(min)	m/z	Area
1	-	95.00	—

Standard solution 25.0 ug/L (F)

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_f25_.qgd

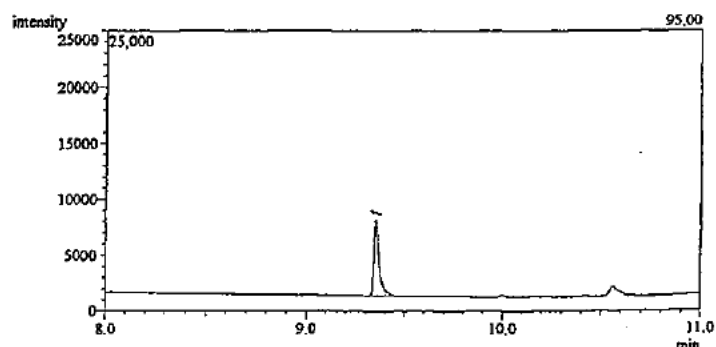


Peak No.	Time(min)	m/z	Area
1	9.35	95.00	7165

Standard solution 50.0 ug/L (F)

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_f50_.qgd

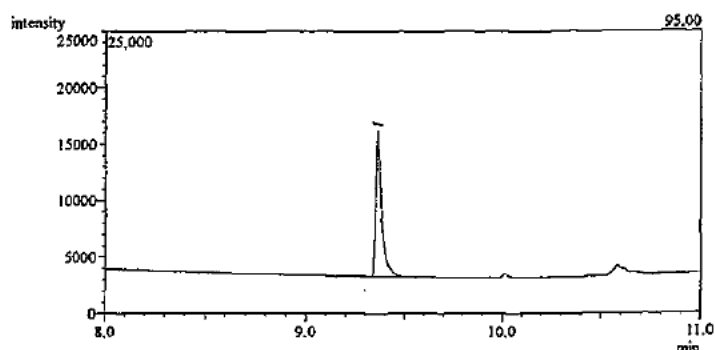


Peak No.	Time(min)	m/z	Area
1	9.35	95.00	13322

Standard solution 100 ug/L (F)

Operating date : Dec. 23, 2006

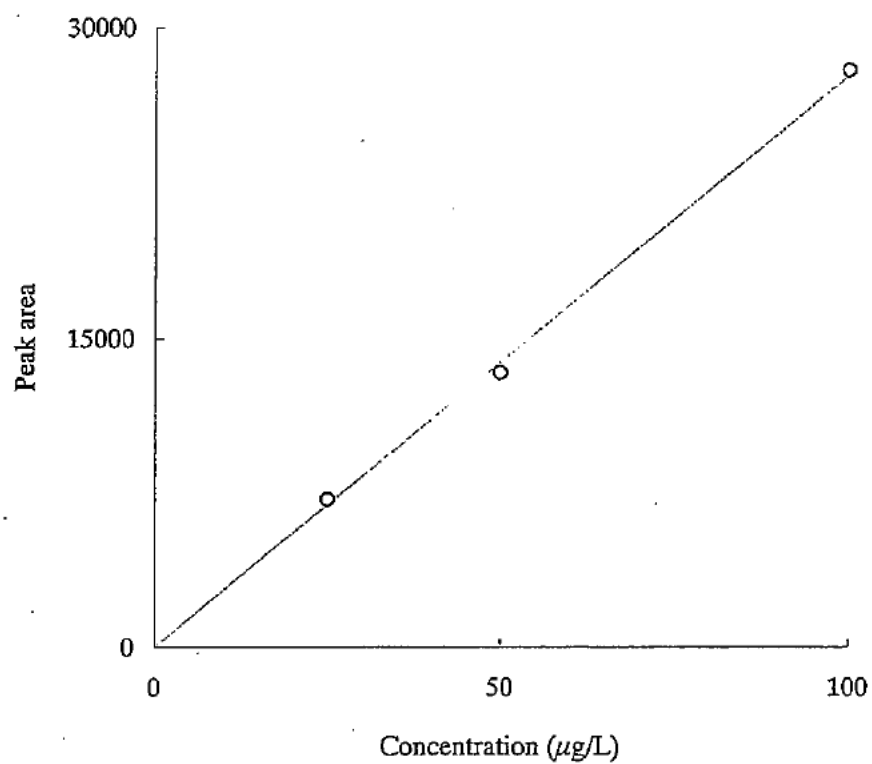
File name : C:\GCMSsolution\Data\Section2\44807\061222\1_f100_.qgd



Peak No.	Time(min)	m/z	Area
1	9.36	95.00	27914

Fig. 7-1 Mass fragmentograms of GC-MS analysis for calibration curve (test fish).

Date : Dec. 25, 2006 Name :



$$y = 277x$$

$$r = 0.999$$

Concentration ($\mu\text{g/L}$)	Peak area
25.0	7165
50.0	13322
100	27914

Fig. 7 - 2 Calibration curve of test item (test fish).

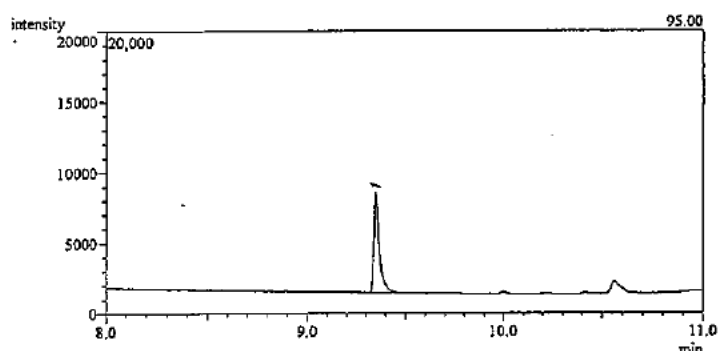
December 25, 2006

Name _____

Standard solution 50.0 ug/L (F)

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_frs_.qgd

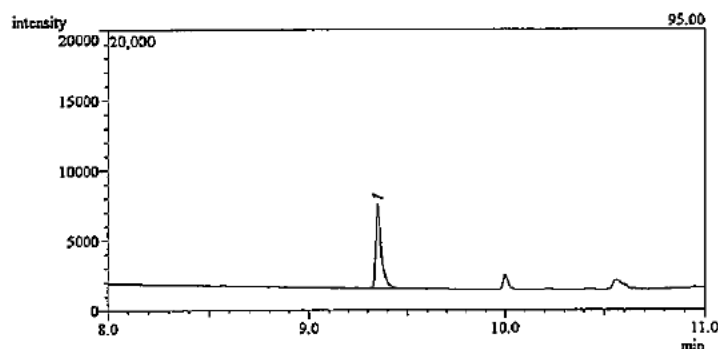


Peak No.	Time(min)	m/z	Area
1	9.35	95.00	13583

Recovery test from test fish a

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_fra_.qgd

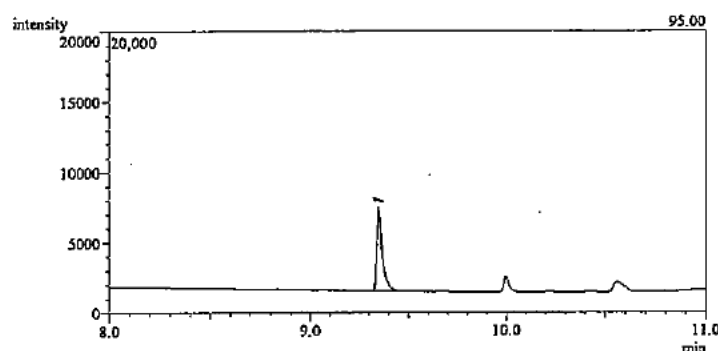


Peak No.	Time(min)	m/z	Area
1	9.35	95.00	11407

Recovery test from test fish b

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_frb_.qgd



Peak No.	Time(min)	m/z	Area
1	9.35	95.00	11098

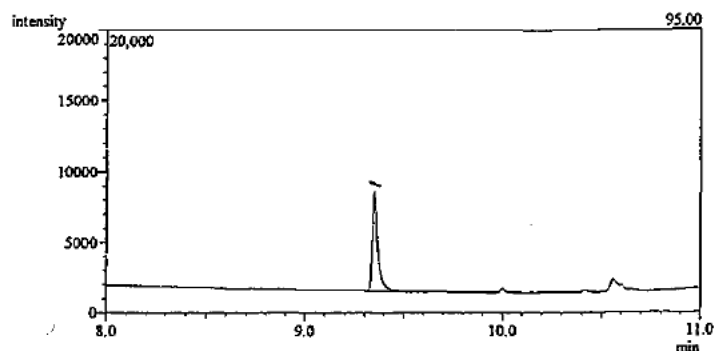
Fig. 8-1 Mass fragmentograms of GC-MS analysis for recovery and blank test (test fish).

Date : Dec. 25, 2006 Name :

Standard solution 50.0 ug/L (F)

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_fbs_.qgd

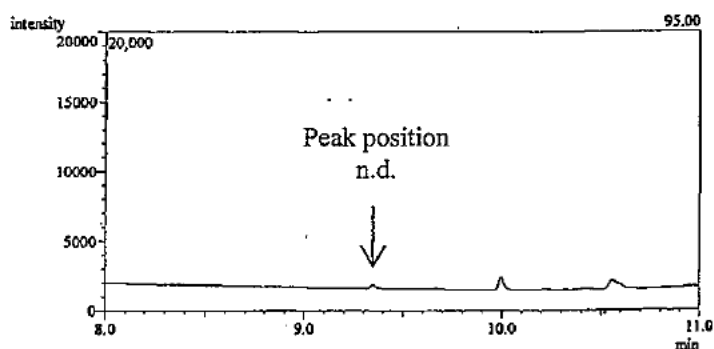


Peak No.	Time(min)	m/z	Area
1	9.35	95.00	13364

Blank test of test fish b

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_fbb_.qgd

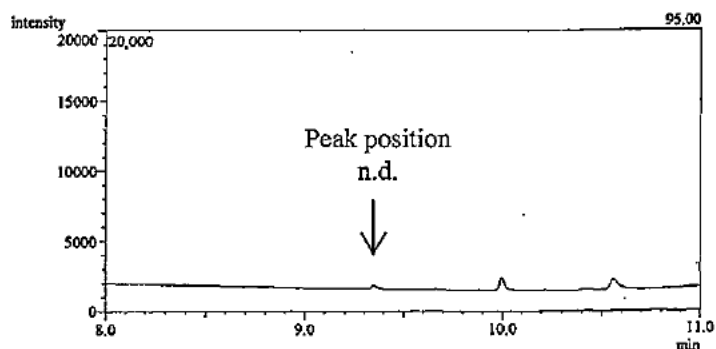


Peak No.	Time(min)	m/z	Area
1	-	95.00	—

Blank test of test fish a

Operating date : Dec. 23, 2006

File name : C:\GCMSsolution\Data\Section2\44807\061222\1_fba_.qgd



Peak No.	Time(min)	m/z	Area
1	-	95.00	—

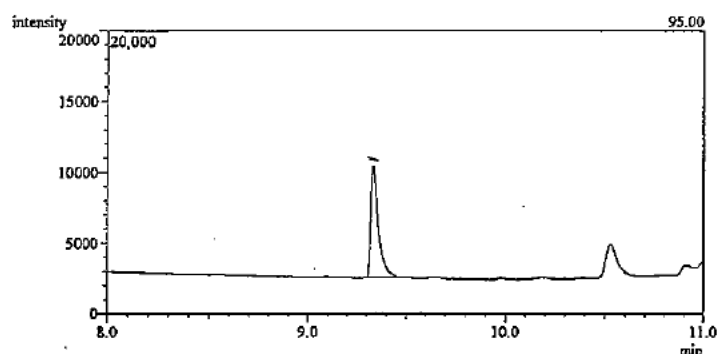
Fig. 8-2 Mass fragmentograms of GC-MS analysis for recovery and blank test (test fish).

Date : Dec. 25, 2006 Name :

Standard solution 50.0 ug/L (F)

Operating date : Feb. 23, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070223\1fhs.qgd

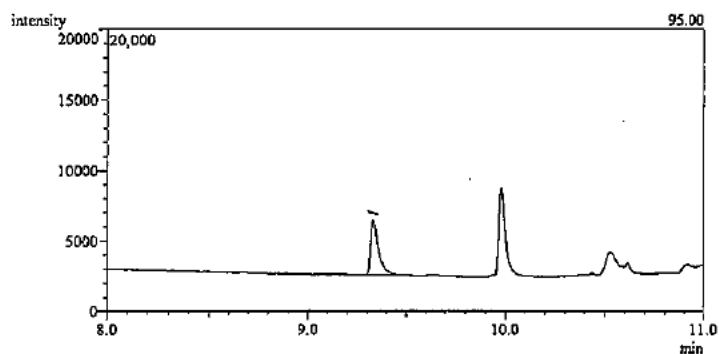


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	21621

Test fish after 10 days (Level 1-a)

Operating date : Feb. 23, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070223\1fha.qgd

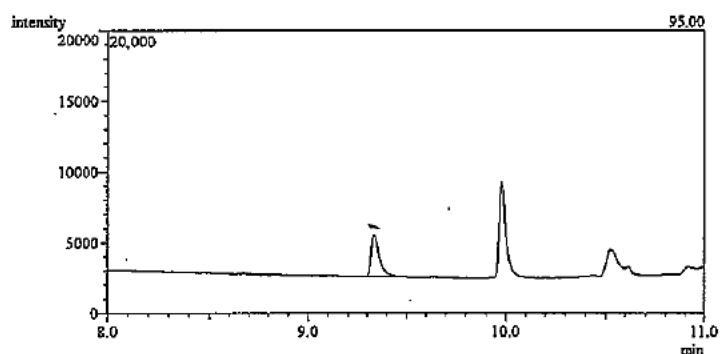


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	11235

Test fish after 10 days (Level 1-b)

Operating date : Feb. 23, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070223\1fha.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	8570

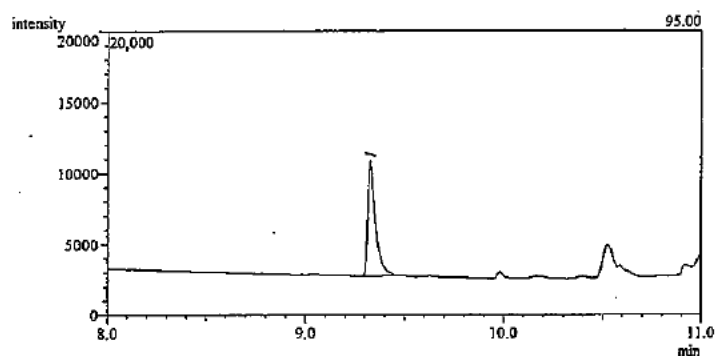
Fig. 9-1 Mass fragmentograms of GC-MS analysis for test fish (Level 1).

Date : Feb. 23, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Mar. 02, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070302\3fhs.qgd

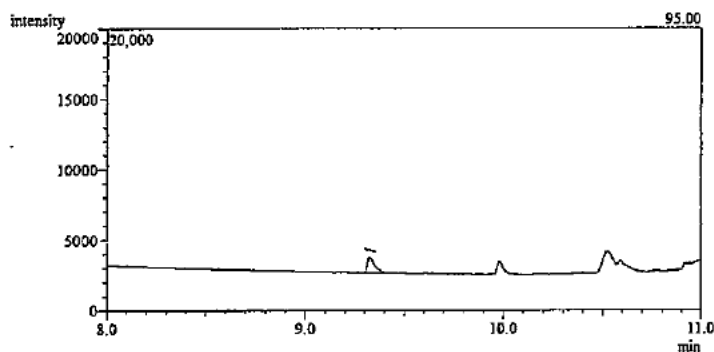


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	21228

Test fish after 17 days (Level 1-a)

Operating date : Mar. 02, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070302\3fha.qgd

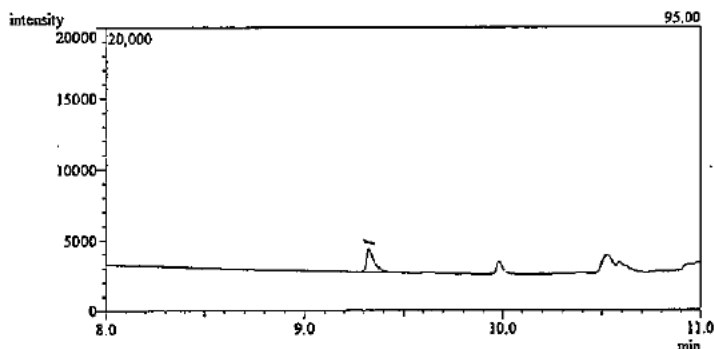


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	2705

Test fish after 17 days (Level 1-b)

Operating date : Mar. 02, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070302\3fhh.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	4000

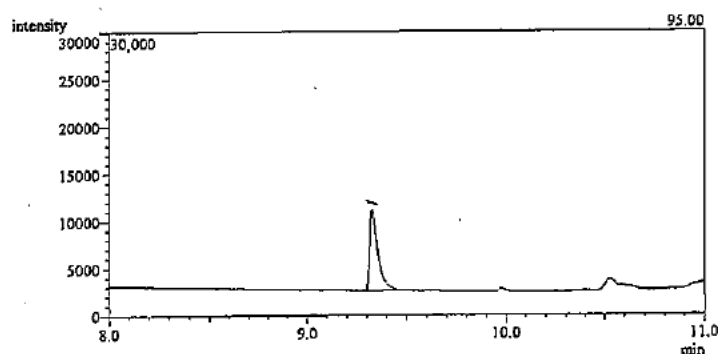
Fig. 9-2 Mass fragmentograms of GC-MS analysis for test fish (Level 1).

Date : Mar. 4, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Mar. 05, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070305\2fhs.qgd

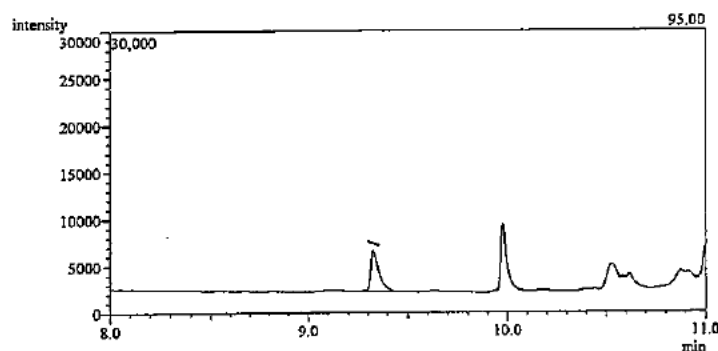


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	24134

Test fish after 20 days (Level 1-a)

Operating date : Mar. 05, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070305\2fha.qgd

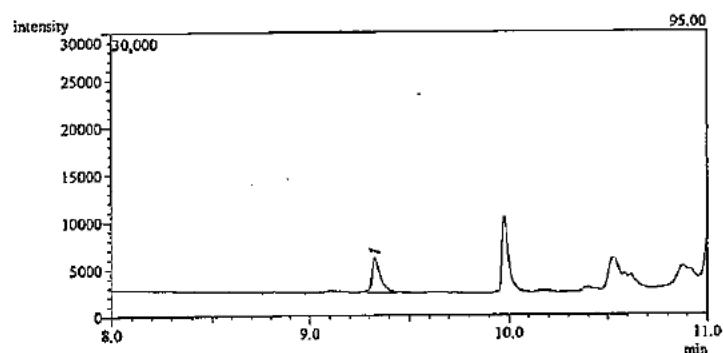


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	12431

Test fish after 20 days (Level 1-b)

Operating date : Mar. 06, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070305\2fhb.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	10939

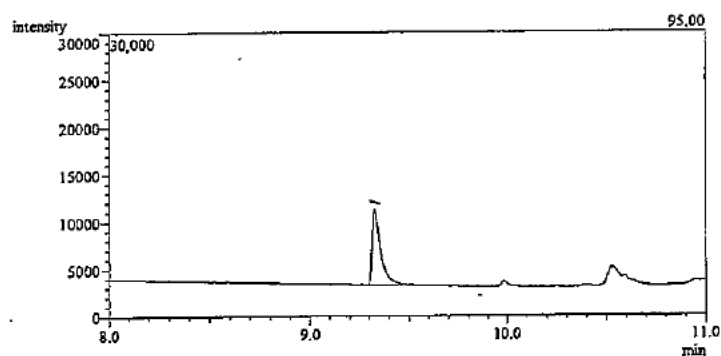
Fig. 9-3 Mass fragmentograms of GC-MS analysis for test fish (Level 1).

Date : Mar. 6, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Mar. 08, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070308\1fhs.qgd

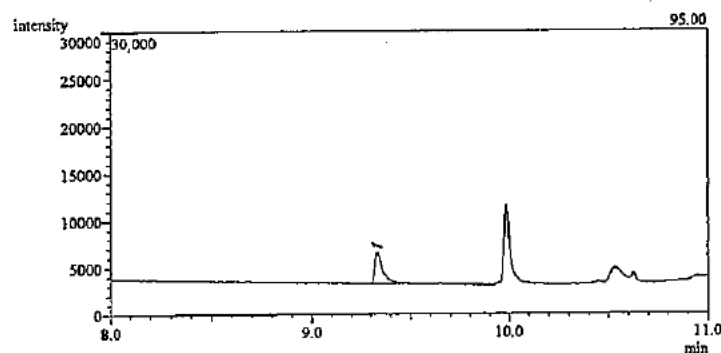


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	23548

Test fish after 23 days (Level 1-a)

Operating date : Mar. 08, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070308\1fha.qgd

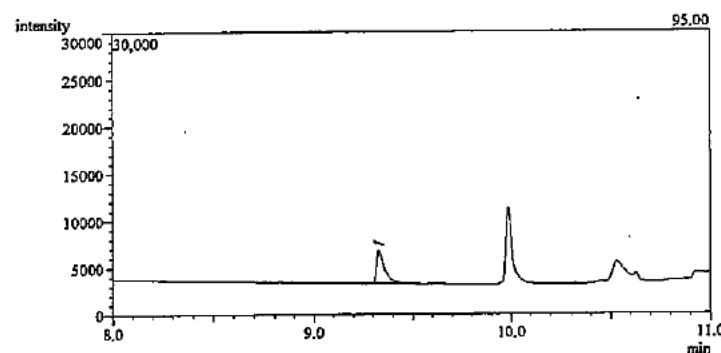


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	9138

Test fish after 23 days (Level 1-b)

Operating date : Mar. 08, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070308\1fhb.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	9410

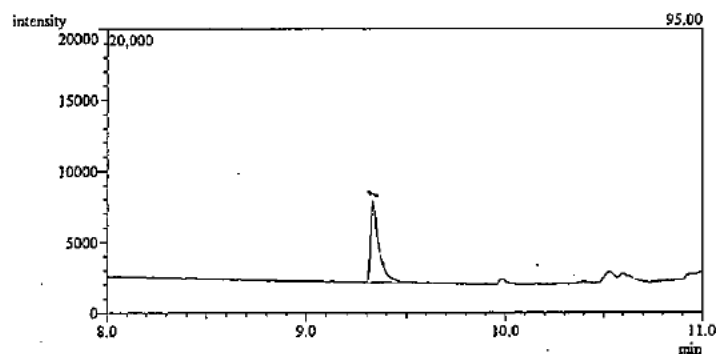
Fig. 9-4 Mass fragmentograms of GC-MS analysis for test fish (Level 1).

Date : Mar. 9, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Mar. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070313\1fhs.qgd

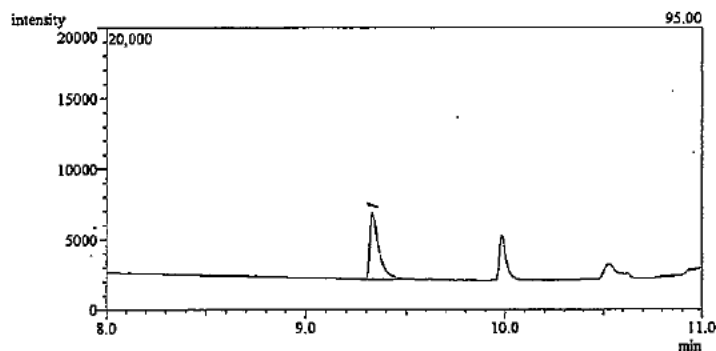


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	16585

Test fish after 28 days (Level 1-a)

Operating date : Mar. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070313\1fha.qgd

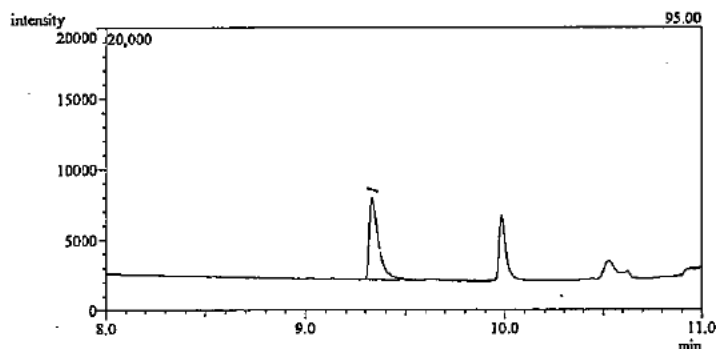


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	13849

Test fish after 28 days (Level 1-b)

Operating date : Mar. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070313\1fhb.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	14847

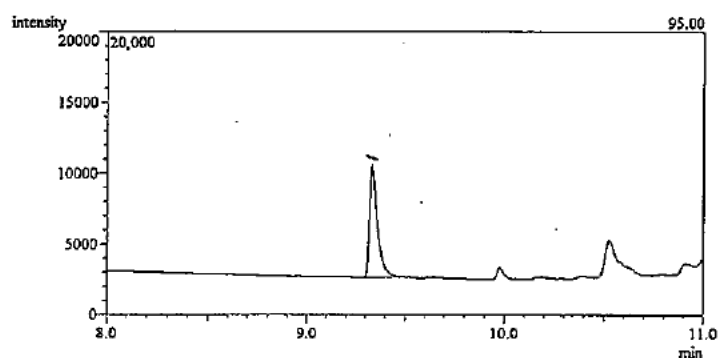
Fig. 9-5 Mass fragmentograms of GC-MS analysis for test fish (Level 1).

Date : Mar. 14, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Feb. 23, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070223\1fls.qgd

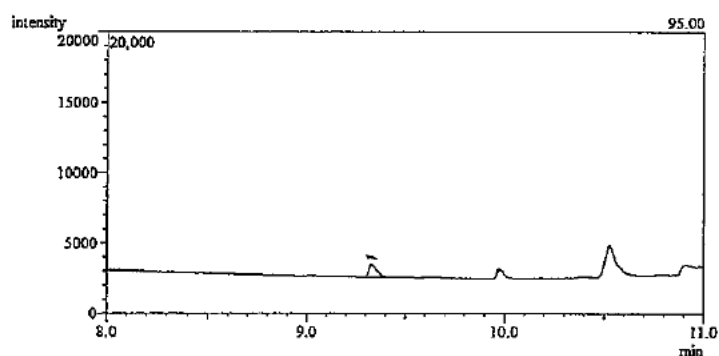


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	21444

Test fish after 10 days (Level 2-a)

Operating date : Feb. 23, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070223\1fla.qgd

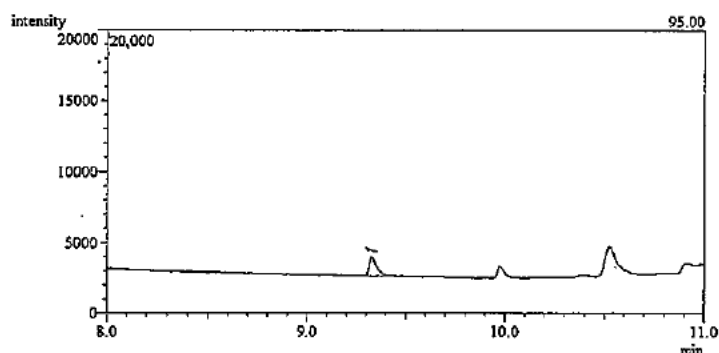


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	2260

Test fish after 10 days (Level 2-b)

Operating date : Feb. 23, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070223\1flb.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	3019

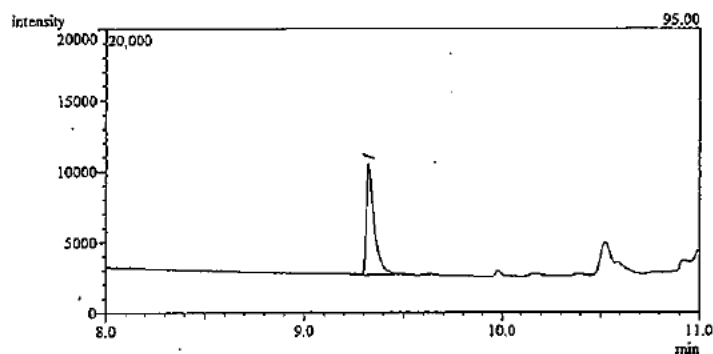
Fig. 10-1 Mass fragmentograms of GC-MS analysis for test fish (Level 2).

Date : Feb. 23, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Mar. 02, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070302\3fls.qgd

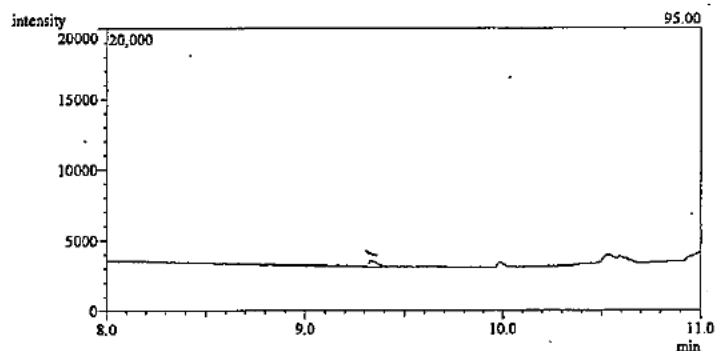


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	20849

Test fish after 17 days (Level 2-a)

Operating date : Mar. 02, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070302\3fla.qgd

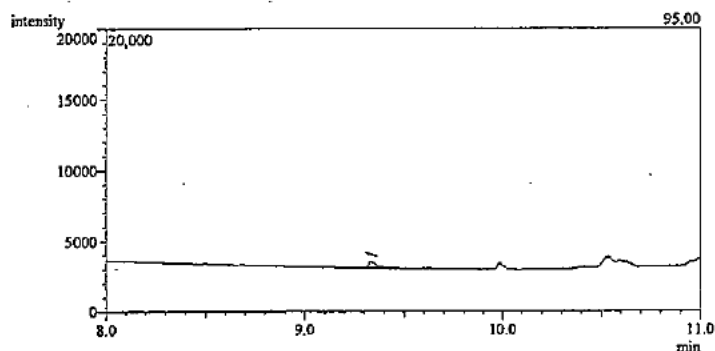


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	1222

Test fish after 17 days (Level 2-b)

Operating date : Mar. 02, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070302\3flb.qgd



Peak No.	Time(min)	m/z	Area
1	9.34	95.00	1066

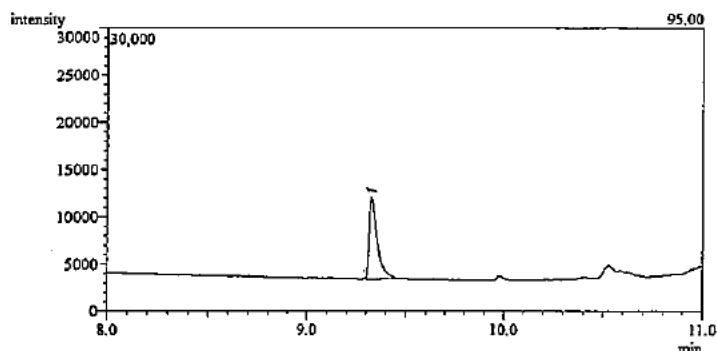
Fig. 10-2 Mass fragmentograms of GC-MS analysis for test fish (Level 2).

Date : Mar. 4, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Mar. 06, 2007

File name : C:\GCMSSolution\Data\Section2\44807\070305\2fls.qgd

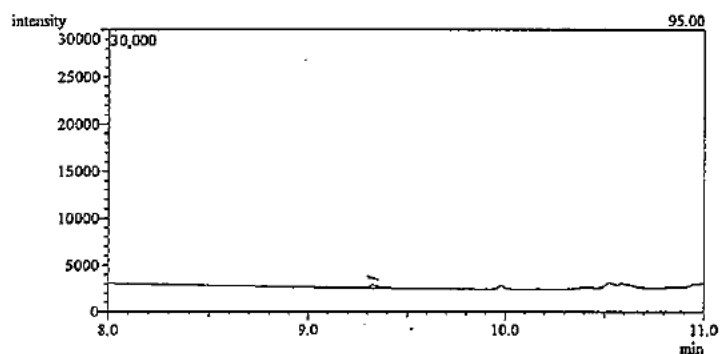


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	24513

Test fish after 20 days (Level 2-a)

Operating date : Mar. 06, 2007

File name : C:\GCMSSolution\Data\Section2\44807\070305\2fla.qgd

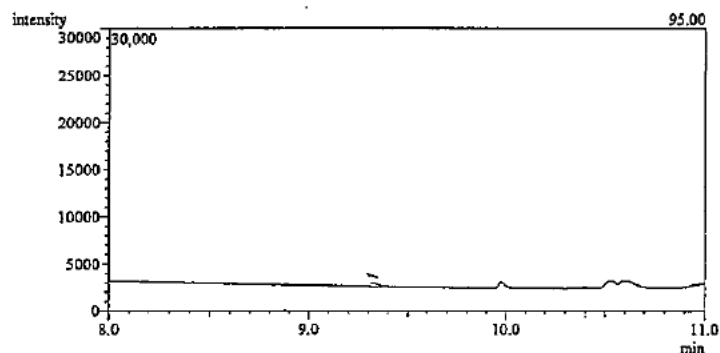


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	1004

Test fish after 20 days (Level 2-b)

Operating date : Mar. 06, 2007

File name : C:\GCMSSolution\Data\Section2\44807\070305\2flb.qgd



Peak No.	Time(min)	m/z	Area
1	9.32	95.00	944

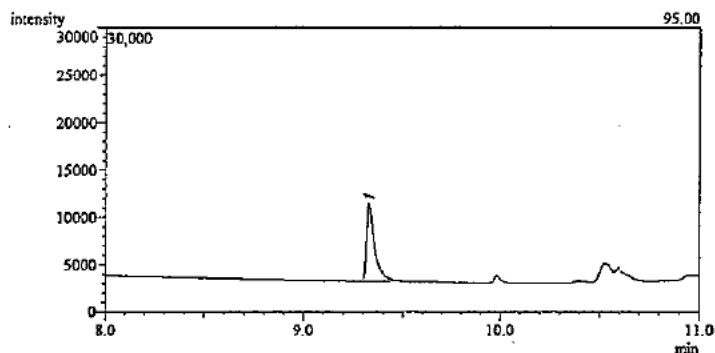
Fig. 10-3 Mass fragmentograms of GC-MS analysis for test fish (Level 2).

Date : Mar. 6, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Mar. 08, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070308\1fis.qgd

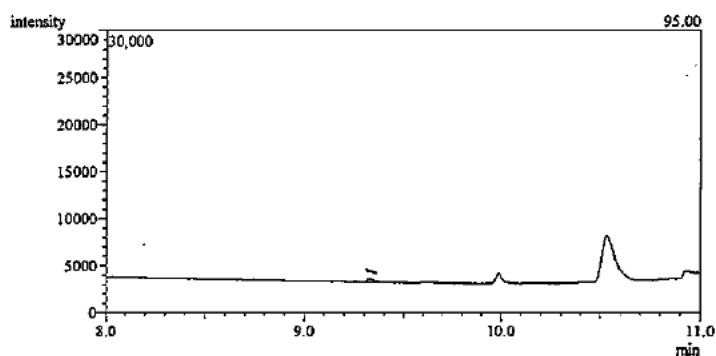


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	22890

Test fish after 23 days (Level 2-a)

Operating date : Mar. 08, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070308\1fla.qgd

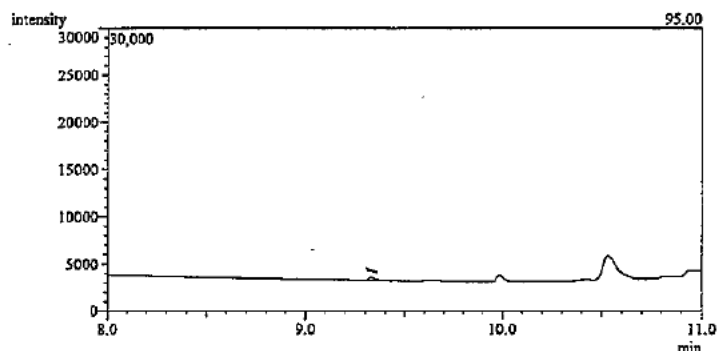


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	983

Test fish after 23 days (Level 2-b)

Operating date : Mar. 08, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070308\1flb.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	803

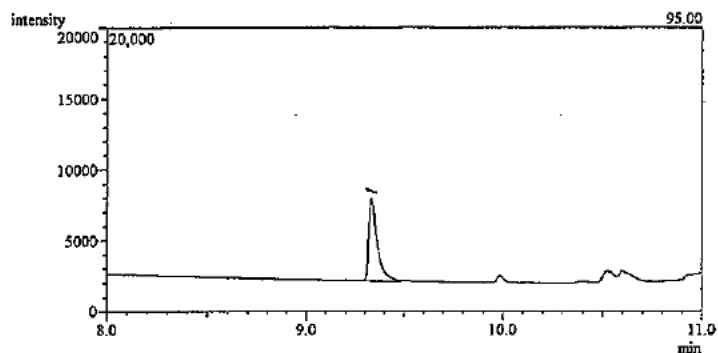
Fig. 10-4 Mass fragmentograms of GC-MS analysis for test fish (Level 2).

Date : Mar. 9, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Mar. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070313\1fls.qgd

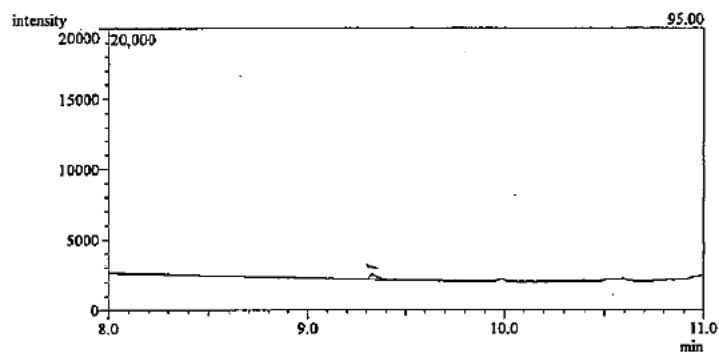


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	16813

Test fish after 28 days (Level 2-a)

Operating date : Mar. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070313\1fla.qgd

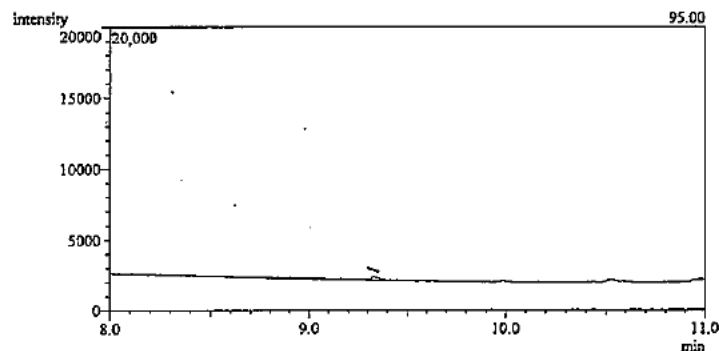


Peak No.	Time(min)	m/z	Area
1	9.33	95.00	1018

Test fish after 28 days (Level 2-b)

Operating date : Mar. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070313\1flb.qgd



Peak No.	Time(min)	m/z	Area
1	9.33	95.00	813

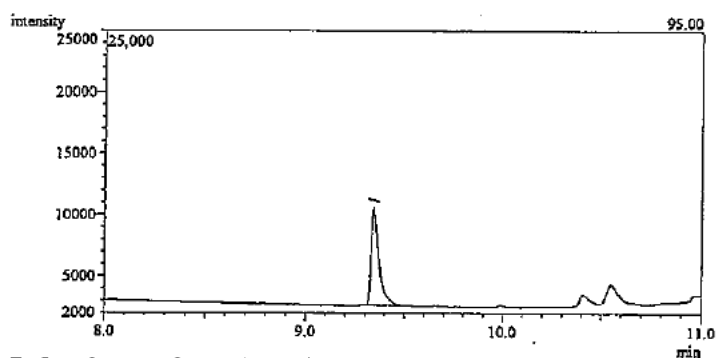
Fig. 10-5 Mass fragmentograms of GC-MS analysis for test fish (Level 2).

Date : Mar. 14, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Feb. 13, 2007

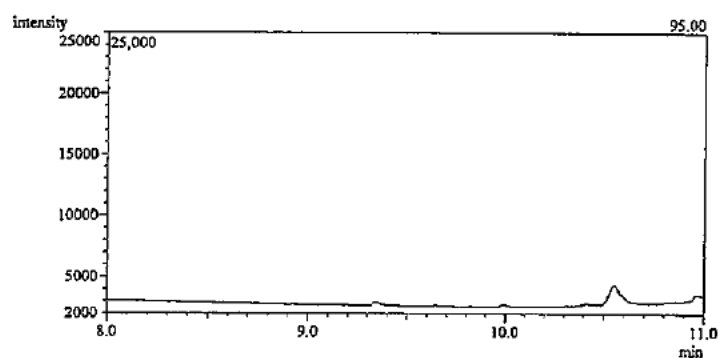
File name : C:\GCMSsolution\Data\Section2\44807\070213\fb.s.qgd



Before the experimental start (Control-a)

Operating date : Feb. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070213\ca.qgd



Before the experimental start (Control-b)

Operating date : Feb. 13, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070213\cb.qgd

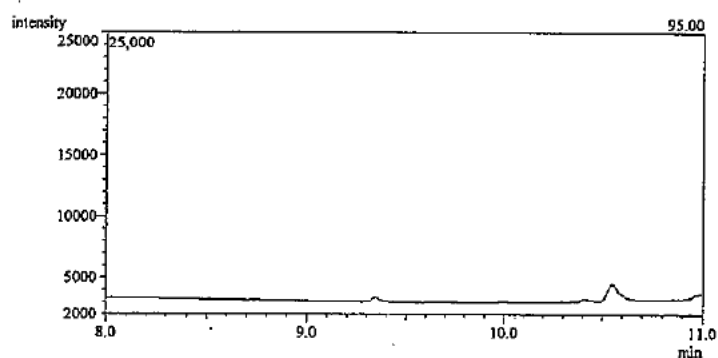


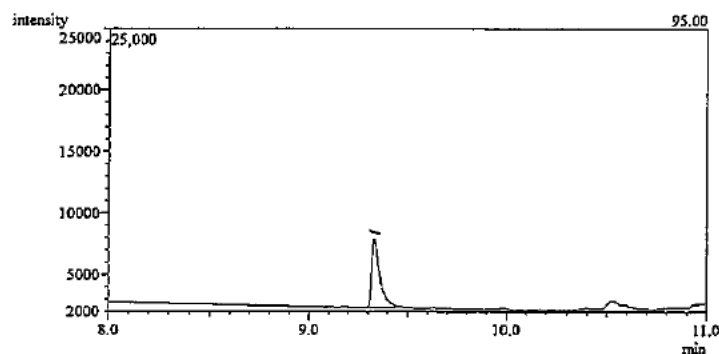
Fig. 11-1 Mass fragmentograms of GC-MS analysis for test fish (Control).

Date : Feb. 13, 2007 Name :

Standard solution 50.0 ug/L (F)

Operating date : Mar. 14, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070314\fb.s.qgd

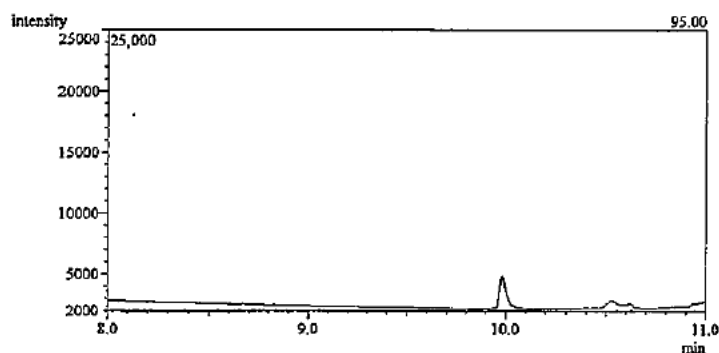


Peak No.	Time(min)	m/z	Area
1	9.34	95.00	16472

After the experimental completion (Control-a)

Operating date : Mar. 14, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070314\ca.qgd

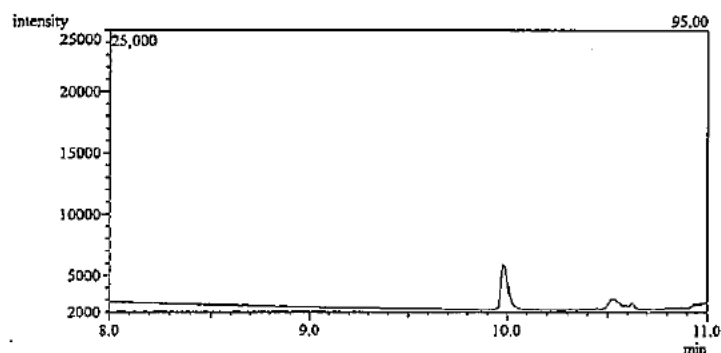


Peak No.	Time(min)	m/z	Area
1	-	95.00	---

After the experimental completion (Control-b)

Operating date : Mar. 14, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070314\cb.qgd



Peak No.	Time(min)	m/z	Area
1	-	95.00	---

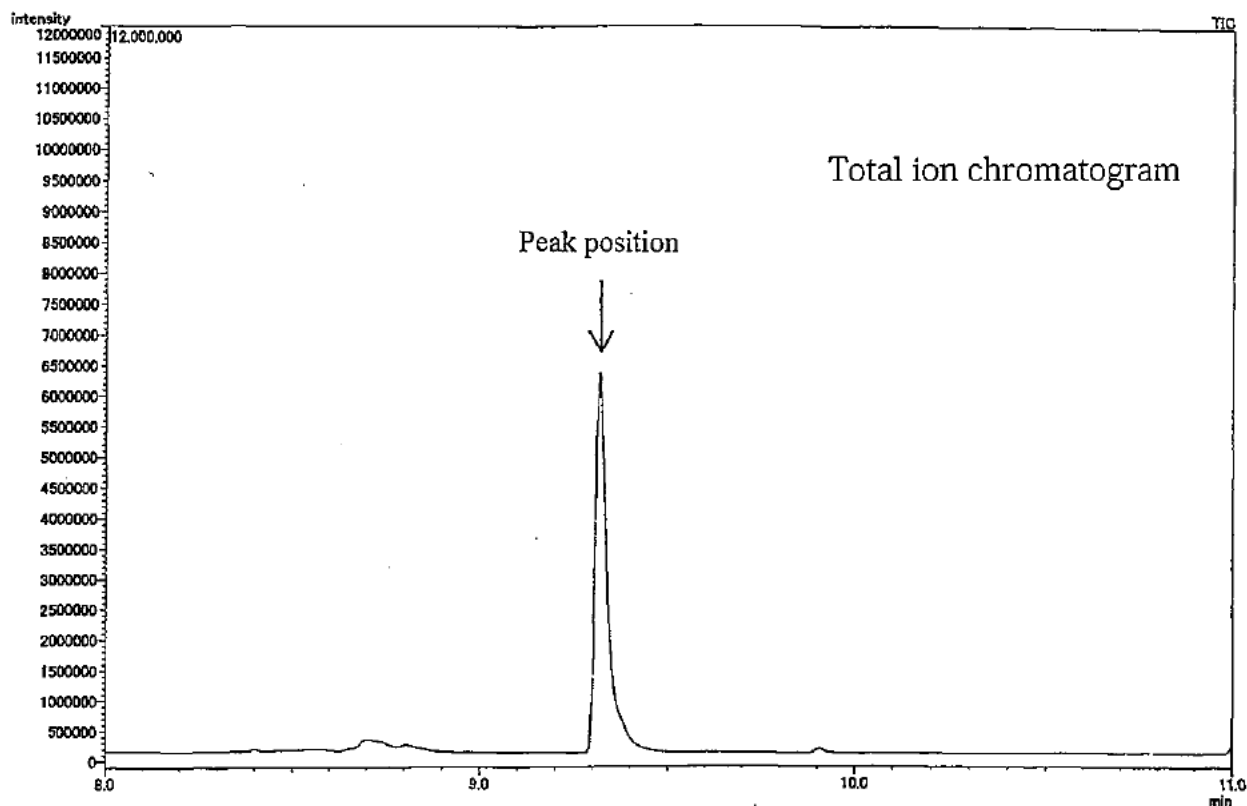
Fig. 11-2 Mass fragmentograms of GC-MS analysis for test fish (Control).

Date : Mar. 14, 2007 Name :

Instrument	<u>GCMS-QP-2010</u>
Sample	<u>Test item (Standard solution 20.0mg/L)</u>
GC Conditions	
Column	<u>HP-INNOWAX (Agilent Technologies)</u>
Size	<u>30m × 0.25mm I.D.</u>
Film thickness	<u>0.25μm (Fused silica)</u>
Temp.	<u>35°C (5min)—①→135°C (0min)—②→250°C (10min)</u>
Temp. rate	<u>① 20°C /min ② 50°C /min</u>
Sample size	<u>1μL (Solvent:ethyl acetate)</u>
Inlet mode	<u>Splitless</u>
Injection temp.	<u>200°C</u>
Total flow	<u>20.0mL/min</u>
Column flow	<u>1.1mL/min</u>
Sampling time	<u>2min</u>
MS Conditions	
Ionization mode	<u>EI</u>
Monitoring ion	<u>m/z 50-370</u>
Interface temp.	<u>250°C</u>
Ion source temp.	<u>230°C</u>
Ionization voltage	<u>70 V</u>

Fig. 12-1 Mass spectrum of test item (analytical conditions).

2007. 1.19



Peak No. 1 R.Time:9.32(Scan#:579)
MassPeaks:271 BasePeak:95(418929)
RawMode:Averaged 9.29-9.40(576-589)

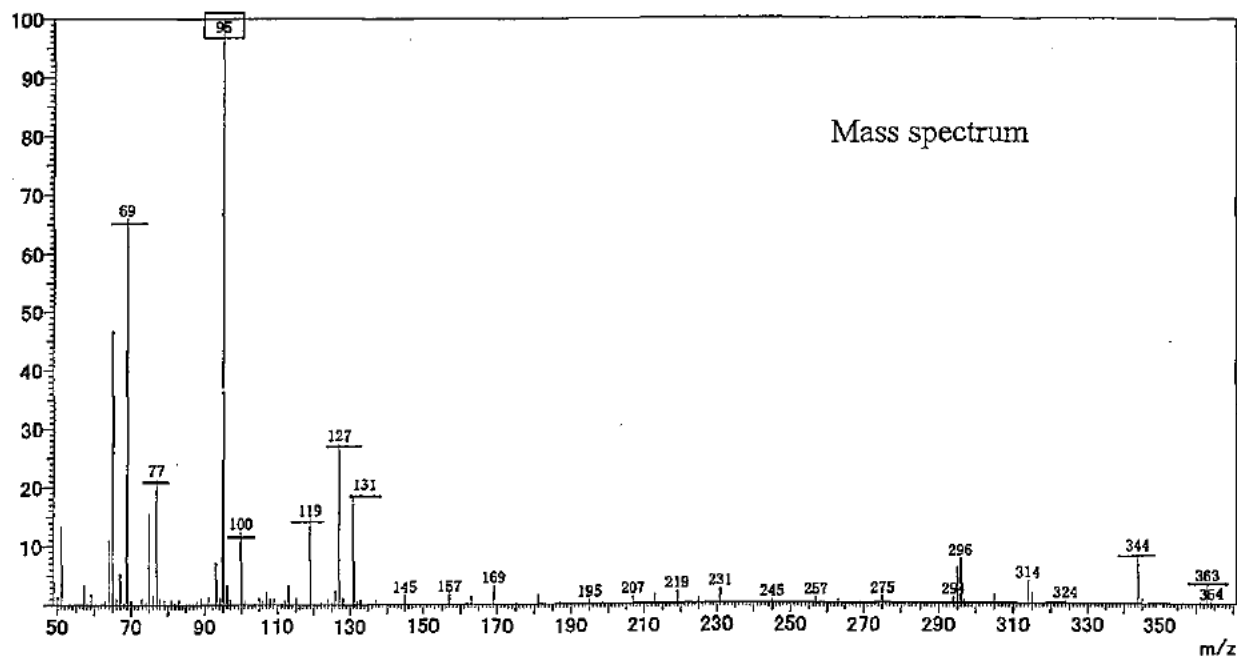


Fig. 12-2 Mass spectrum of test item.

Date : Jan. 19, 2007 Name :

Operating date : Jan. 19, 2007

File name : C:\GCMSsolution\Data\Section2\44807\070119\scan002.qgd

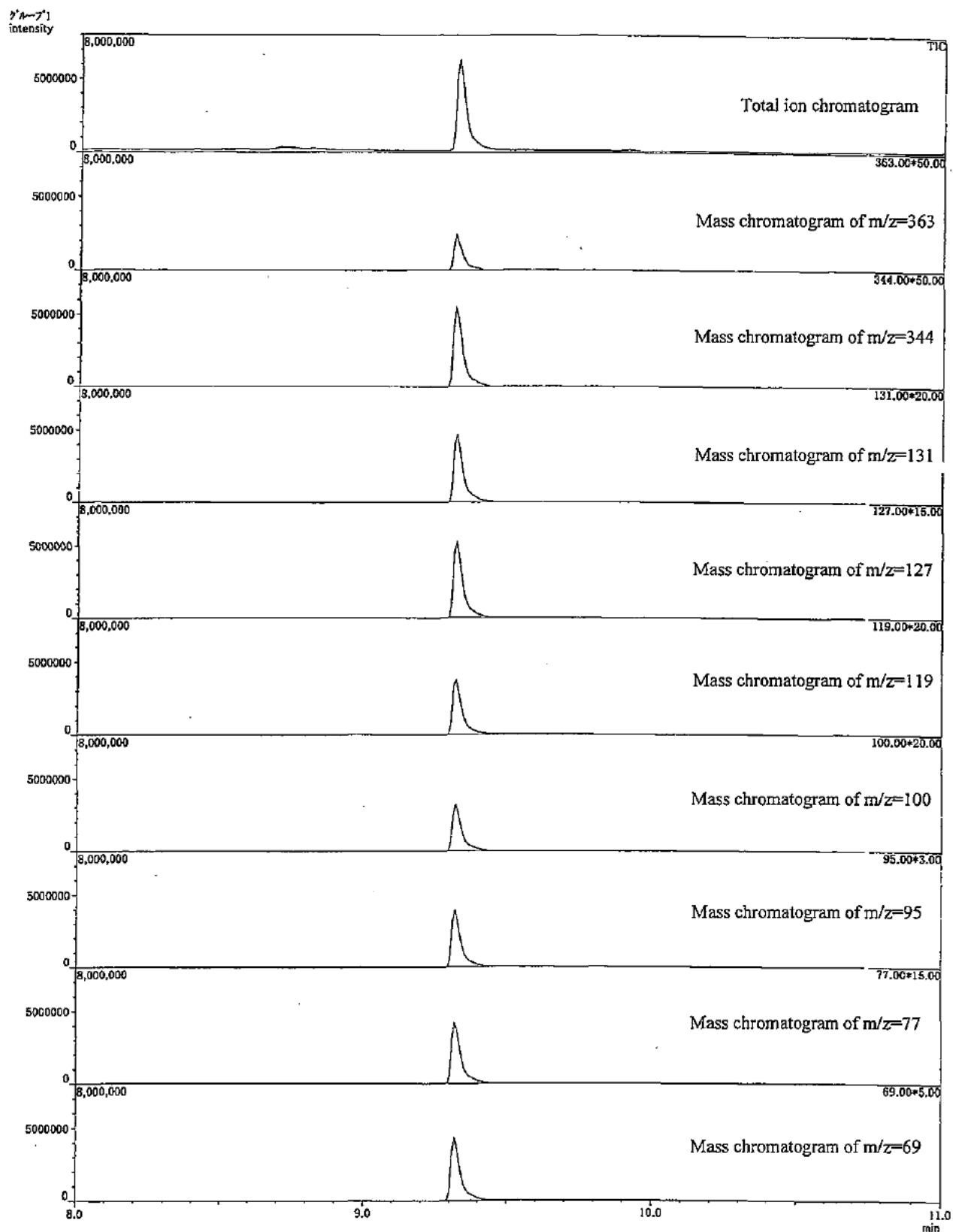


Fig. 12-3 Mass spectrum of test item.

Date : Jan. 19, 2007 Name :

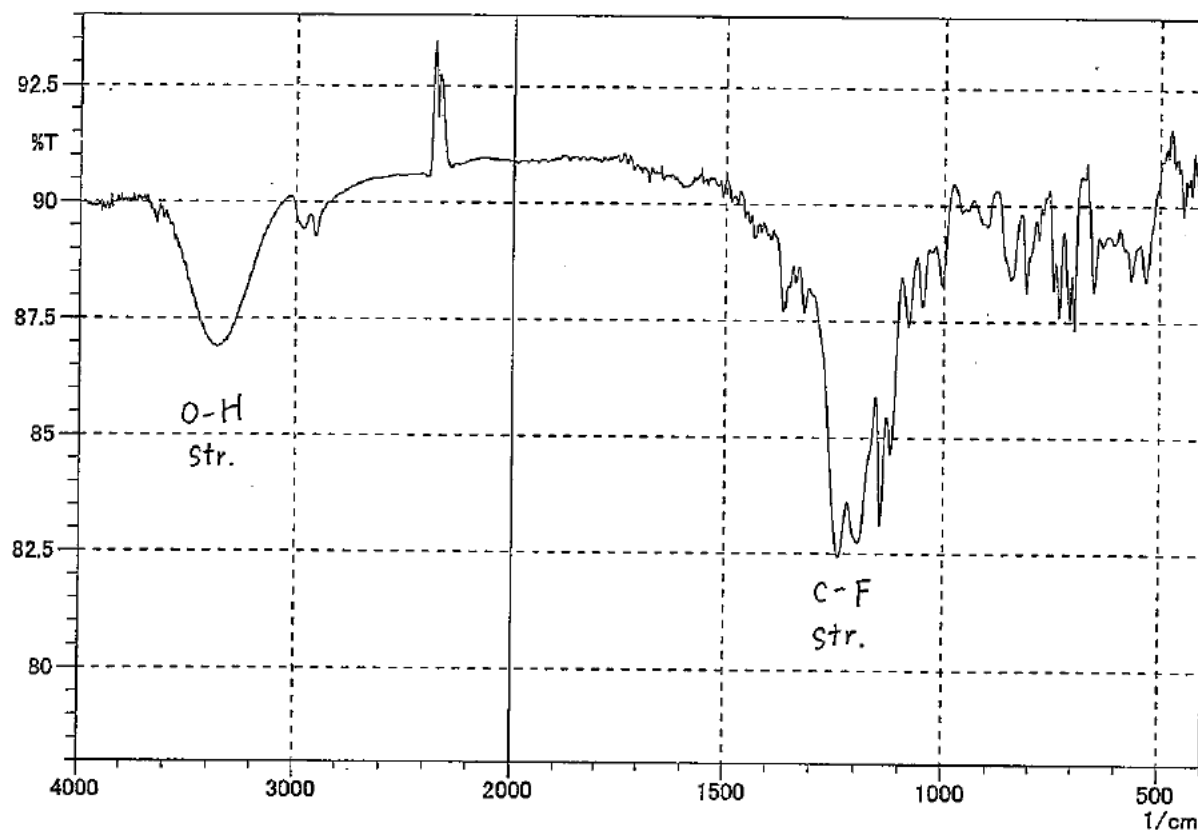


Mass number 364

m/z	Fragment ion
363	(M-H) ⁺
344	(M-HF) ⁺
131	(C ₃ F ₅) ⁺
127	(C ₂ F ₄ C ₂ H ₃) ⁺
119	(CF ₂ CF ₃) ⁺
100	(C ₂ F ₄) ⁺
95	(CF ₂ C ₂ H ₄ OH) ⁺
77	(CF ₂ C ₂ H ₃) ⁺
69	(CF ₃) ⁺

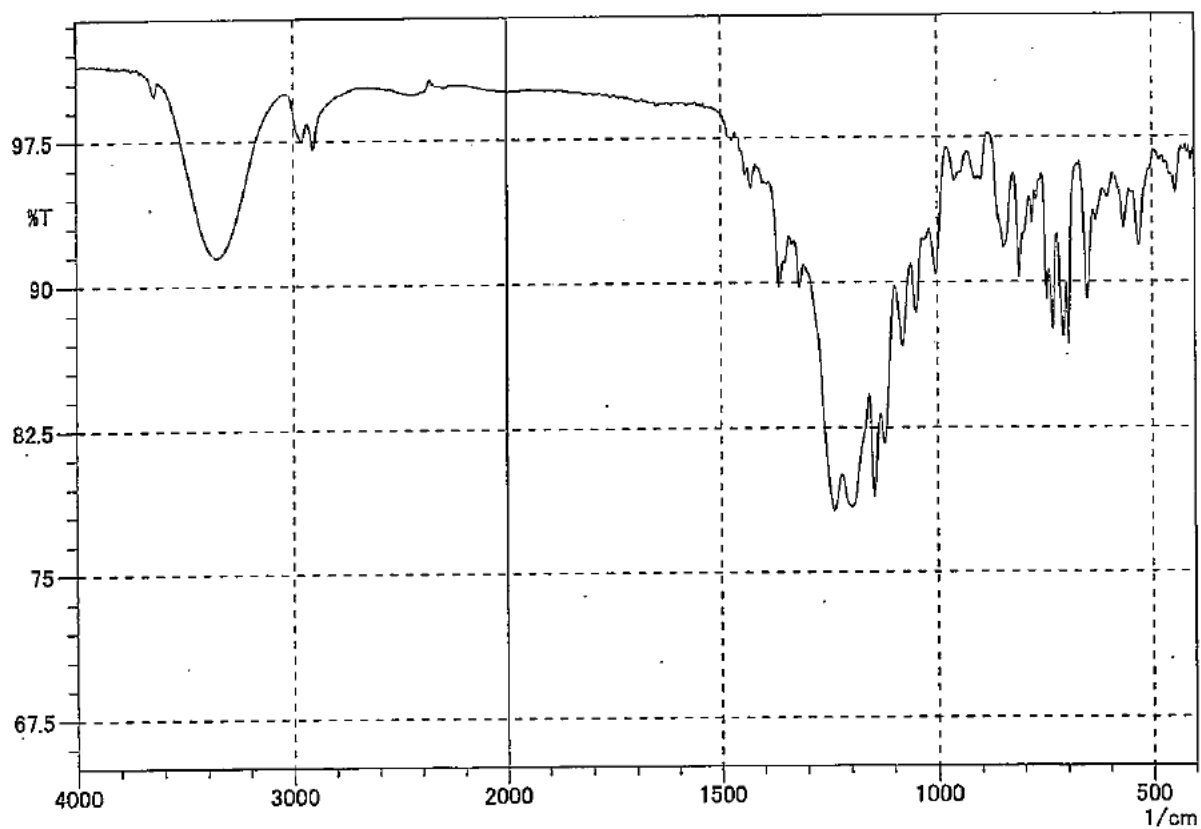
Fig. 12-4 Mass spectrum of test item (main presumed fragments).

2007.1.19



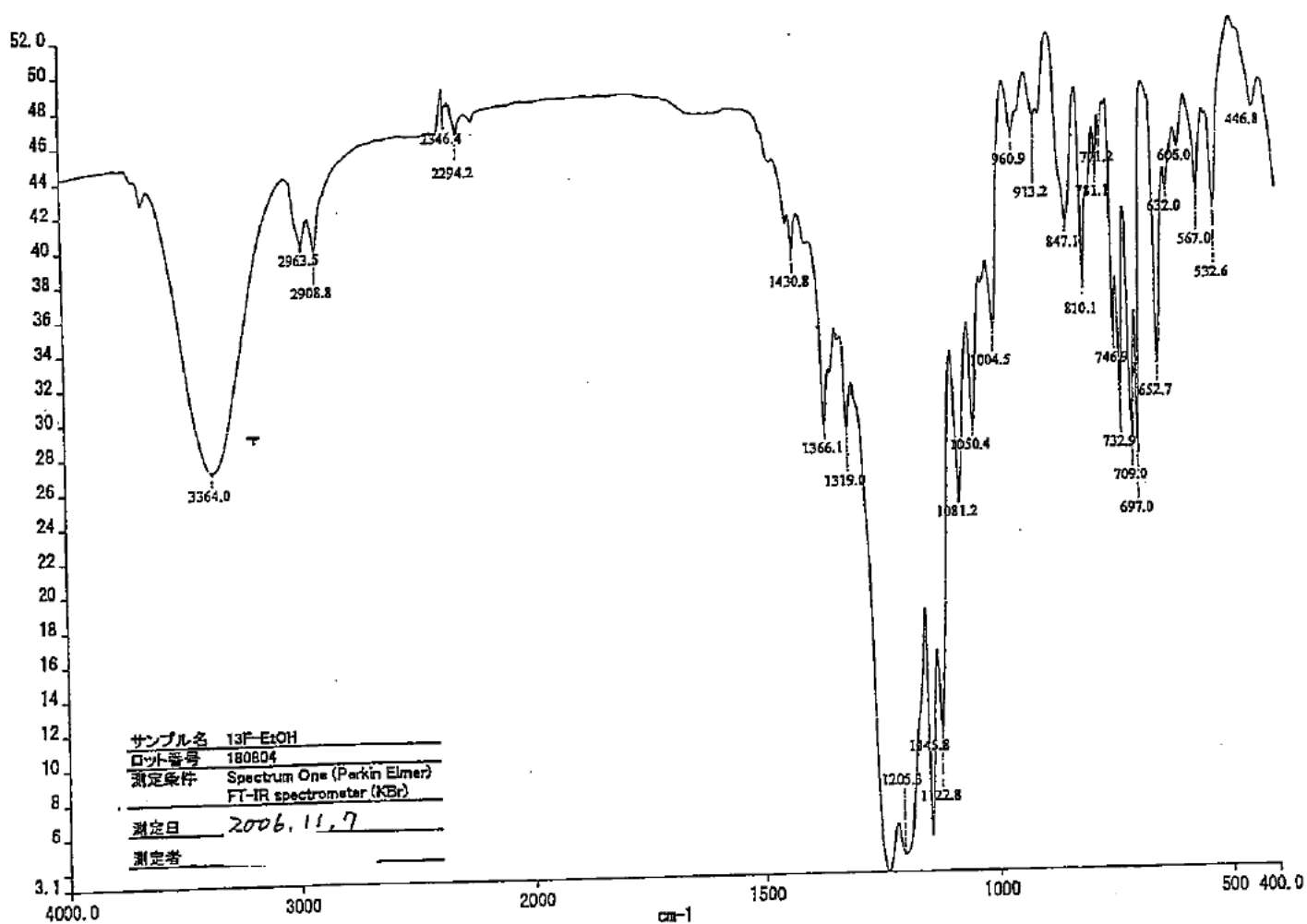
Instrument : Shimadzu IRPrestige-21
Study No. : 44807
Sample : Test item
Method : Neat
Date : Dec. 20, 2006
Name :

Fig. 13- 1 IR spectrum of test item measured before experimental start.



Instrument : Shimadzu IRPrestige-21
Study No. : 44807
Sample : Test item
Method : Neat
Date : Mar. 14, 2007
Name :

Fig. 13- 2 IR spectrum of test item measured after experimental completion.



c:\pel_data\spectra\dk5548.sp - 13F-EtOH (Lot. 180804)

Reference 2

IR spectrum supplied by sponsor

確認番号 001

基準適合試験施設確認書

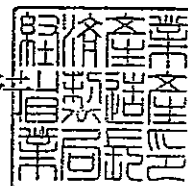
財団法人化学物質評価研究機構
理事長 近藤 雅臣 殿

化学物質の審査及び製造等の規制に関する法律に係る試験施設に関する基準適合確認実施要領に基づき、下記試験施設については、新規化学物質に係る試験並びに第一種監視化学物質及び第二種監視化学物質に係る有害性の調査の項目等を定める省令第4条に規定する試験施設に関する基準に適合していることを確認します。

なお、確認の有効期間は、本確認書の交付日から起算して3年間とします。

平成16年12月22日

経済産業省製造産業局長 石毛 博行



記

試験施設の名称	財団法人化学物質評価研究機構 久留米事業所
試験施設の所在地	福岡県久留米市宮ノ陣三丁目2番7号
試験項目	分解度試験、濃縮度試験及び分配係数試験