FINAL REPORT

Bioconcentration study of C6-2 alcohol in carp

January 31, 2002

Kurume Laboratory
Chemicals Evaluation and Research Institute, Japan

CONTAINS CONFIDENTIAL BUSINESS INFORMATION
STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor ASAHI GLASS CO., LTD.

Title Bioconcentration study of C₆₂ alcohol in carp

Study number 43771

I, the undersigned, hereby declare that this report provides a correct English translation of the Final Report (Study No. 43771, issued on January 31, 2002).

The Study Director was changed from Yurika Mouri to Yoshiyuki Inoue, because Yurika Mouri had been retired.

Date

Study Director

Yoshiyuki Inoue

CONTAINS CONFIDENTIAL BUSINESS INFORMATION
GLP STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor
ASAHI GLASS CO., LTD.

Title
Bioconcentration study of C62 alcohol in carp

Study number
43771

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
January 31, 2002

Study Director
Signed in original
Yurika Mouri
QUALITY ASSURANCE STATEMENT

Kurume Laboratory
Chemicals Evaluation and
Research Institute, Japan

Sponsor
ASAHI GLASS CO., LTD.

Title
Bioconcentration study of C₆₂ alcohol in carp

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43771

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

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<th>Item of audit or inspection</th>
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<th>Date of report to test facility management</th>
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It has been assured that the final report describes accurately the test method used, that details in the report are in compliance with the study plan and Standard Operating Procedures and that the final report reflects the raw data accurately.

Date
January 31, 2002

Quality Assurance Unit, Head
Signed in original

Kyoshiro Hori

CONTAINS CONFIDENTIAL BUSINESS INFORMATION
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Fig. 24 MS spectrum of carboxylic acid

Fig. 25 MS/MS spectrum of carboxylic acid

Reference 2 IR spectrum supplied by sponsor
Title
Bioconcentration study of C₆₂ alcohol in carp

Sponsor
ASAHI GLASS CO., LTD.
1-12-1, Yurakucho, Chiyoda-ku, Tokyo 100-8405 JAPAN

Test facility
Kurume Laboratory
Chemicals Evaluation and Research Institute, Japan
19-14 Chuo-machi, Kurume-shi, Fukuoka 830-0023, Japan

Objective
This study was performed to evaluate the bioconcentration potential of C₆₂ alcohol 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), and "Bioconcentration : Flow-through Fish Test (Guideline 305, June 14, 1996)" in the OECD Guidelines for Testing of Chemicals.
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, Yakuhatsu 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).

Dates

- Study initiation date: December 13, 2001
- Experimental starting date: December 27, 2001
- Experimental completion date: January 24, 2002
- Study completion date: January 31, 2002

Storage of test item, raw data, etc.

(1) Test item

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, 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 ten years after the receipt of the notice specified under Clause 1, Clause 2 or Clause 3 in Article 4 of "Law Concerning Examination and Regulation of Manufacture, etc. of Chemical Substances". Treatment of raw data and materials, etc. after the storage period is discussed with the sponsor.
Personnel

Study Director

Yurika Mouri
(2nd Chemical Safety Section)

Study personnel
(Operation of bioconcentration test)

Yoshiyuki Inoue
Yuka Kida
Yasuo Kawashima
Chitose Fukuda

Staff for fish care

Yasuo Kawashima

Person to conduct of fish acute toxicity test

Yasuo Kawashima
Tadayoshi Tonai

Approval of final report

Study Director

Date

January 31, 2002

Signature

Signed in original

Yurika Mouri
SUMMARY

Title
Bioconcentration study of C₆₂ alcohol in carp

Test conditions

Acute toxicity test
(1) Test fish: Orange-red killifish (Oryzias latipes)
(2) Duration of exposure: 96 hours
(3) Exposure method: Semi static system
(renewal of test water, at every 8 - 16 hours)

Bioconcentration test
(1) Test fish: Carp (Cyprinus carpio)
(2) Nominal concentrations of test item
   High exposure level (Level 1): 10 µg/L
   Low exposure level (Level 2): 1 µg/L
(3) Duration of exposure: 28 days
(4) Exposure method: Continuous flow system
(5) Analytical method
   Test item: Gas chromatography-mass spectrometry
   Carboxylic acid: Liquid chromatography-tandem mass spectrometry

Results

(1) 96-hour LC₅₀ value: 10.4 mg/L
(2) Bioconcentration factors at a steady state
   Test item: Level 1: 46
               Level 2: ≤ 36
   Carboxylic acid: Level 1: ≤ 1.1
                 Level 2: ≤ 12
1. Test item

In this report, C₅₂ alcohol has the following chemical name, etc.

1.1 Chemical name*¹  3,3,4,4,4,5,5,6,6,7,7,8,8,8-Tridecafluoro-1-octanol

1.2 Chemical structure, etc.

   Structural formula*¹

   \[
   \text{C}_{6}\text{F}_{13}\text{C}_{2}\text{H}_{4}\text{OH}
   \]

   Molecular formula  \( \text{C}_{6}\text{H}_{13}\text{F}_{13}\text{O} \)

   Molecular weight  364.10

*¹ Information supplied by the sponsor
2. Item supplied by sponsor

2.1 Supplier and lot number*¹
   (1) Supplier            ASAHI GLASS CO., LTD.
   (2) Lot number          Lot 2

2.2 Purity*¹
   Test item              99.8 wt%

   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. 22 and Reference 2).

2.4 Physicochemical properties*¹
   Appearance              Colorless and transparent liquid
   Vapor pressure          36 mmHg (91 - 93 °C)
   Density                 1.678 g/cm³ (20°C)

*¹ Information supplied by the sponsor

2.5 Storage and stability

   (1) Storage condition
      Room temperature

   (2) Stability
      The test item was stable under the storage condition as shown by the finding
      that IR spectra of the test item before and after the experiment were identical (see
      Fig. 22).

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 Ogawa shoten
(Address: 181 Ooishi-machi Kurume-shi, Fukuoka 830-0049, Japan)

(3) Conditions for fish care before acclimatization

Period
The fish were checked visually at receipt and those with any abnormalities were removed. The remainder was reared for 57 days in a flow-through system after the external disinfection for sick prevention and parasitic extermination.

External disinfection
The external disinfection for sick prevention was carried out in an aqueous solution containing 20 mg/L ELBAZIU and 7 g/L sodium chloride for 24 hours. The external disinfection for parasitic extermination was carried out two times in an aqueous solution containing 30 μL/L formalin for 24 hours.

(4) Conditions of acclimatization

Period
After rearing, the fish 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 26 days in a flow-through system at the temperature of 25 ± 2 °C. The fish were checked for health conditions and reared for 25 days after the external disinfection.

External disinfection
The first external disinfection was carried out in an aqueous solution containing 20 mg/L ELBAZIU and 7 g/L sodium chloride for 24 hours. The second external disinfection was carried out in an aqueous solution containing 20 mg/L ELBAZIU and 7 g/L sodium chloride for 24 hours.
(5) Weight average 0.35 g
(6) Length average 3.3 cm

(7) Certification
The 48-hour LC50 value of the reference substance*2 for the fish of the same lot (TFO-011022) was 0.704 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 September 3, 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 (Shadanhojin Nihon Suissansigen 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 Glass gallon vessel
(2) Volume of test water 3.85 L × 2 / level
(3) Temperature of test water
At initial exposure 24.0 °C
Before renewal of test water 24.0 °C
(4) Concentration of dissolved oxygen in test water
At initial exposure 8.1 mg/L
Before renewal of test water 7.0 - 7.7 mg/L
(5) pH of test water
   At initial exposure  8.5
   Before renewal of test water  8.2

(6) Number of fish  10 / level

(7) Duration of exposure  96 hours

(8) Exposure method  Semi static system
   (renewal of test water, at every 8 - 16 hours)

3.5 Preparation of stock solution

(1) Dispersant
   HCO-20 (Hydrogenated castor oil)

(2) Preparation
   The item supplied by the sponsor and HCO-20 (20 times amount of it) were
   mixed and kneaded. And 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   December 17, 2001 - December 21, 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  10.4 mg/L (see Fig. 3)
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
Sugishima fish farm
(Address: 123-2 Gunchiku Ichibancho, Yatsushiro-shi, Kumamoto 866-0024, Japan)

Date received: October 15, 2001

(3) Conditions for fish care before acclimatization

   Period
   The fish were checked visually at receipt and those showing any abnormalities were removed. The remainder was reared for 13 days in a flow-through system after the external disinfection for sick prevention and parasitic extermination.

   External disinfection
   The external disinfection for sick prevention was carried out in an aqueous solution containing 50 mg/L OTC (Oxytetracycline hydrochloride) for fisheries and 7 g/L sodium chloride for 24 hours. The external disinfection for parasitic extermination was carried out in an aqueous solution containing 30 μL/L formalin for 24 hours.

(4) Conditions for acclimatization

   Period
   After rearing, the fish 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 15 days in a flow-through system at the temperature of 25 ± 2 °C. The fish were checked for health conditions and transferred to test tanks. Thereafter the fish were reared at the same temperature in a flow-through system for 41 days, following an 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 20 mg/L ELBAZIU and 7 g/L sodium chloride for 24 hours.

(5) Length
6.6 - 8.6 cm
(6) Lot No. TFC-011015

(7) Age Yearling fish

(8) Feeding

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.
The fish were starved for 24 hours before sampling.

4.2 Dilution water for test

The same 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

Level 1 and 2 100-L glass tank for volatile item
Control 100-L glass tank

(3) Flow rate of test water

Level 1 and 2

0.02 mL/min for stock solution and 1600 mL/min for dilution water, 2304 L/day of test water, were supplied.

Control

2 mL/min for stock solution and 1600 mL/min for dilution water, 2307 L/day of test water, were supplied.

(4) Stock solution bottle

Level 1 and 2

500-mL glass bottle (Cooling in the refrigerator)
(Frequency of renewal 1 time/week)

Level 1 and 2

25-L glass bottle
(Frequency of renewal 1 time/week)

(5) Temperature of test water

Level 1 24.6 - 25.1 °C
Level 2 25.1 - 25.9 °C
Control 25.1 - 25.4 °C
(6) Concentrations of dissolved oxygen in test water
   Level 1       7.0 - 7.7 mg/L (see Fig. 19)
   Level 2       7.0 - 7.7 mg/L (see Fig. 20)
   Control       7.4 - 8.0 mg/L (see Fig. 21)

(7) pH of test water
   Level 1       7.6 - 7.8
   Level 2       7.6 - 7.8
   Control       7.7 - 7.9

(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  29
   Control        12

(10) Duration of exposure  28 days
    Reason: The time to reach a steady-state was estimated to be within 28 days from preliminary test results.

(11) Place  213 Aquatron room

4.4 Preparation of stock solutions

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

(2) Preparation
   · Level 1
     800 mg/L stock solution was prepared in the same way as described in Section 3.5 (2).
   · Level 2
     80 mg/L stock solution was prepared in the same way as described in Section 3.5 (2).
   · Control
     HCO-20 was dissolved in ion-exchanged water to prepare 160 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 item were decided as follows. The control was set as a blank test.

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration (µg/L)</th>
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<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
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</table>

4.6 Observation, measurement and cleaning of test tank

(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 test item in test water and test fish was performed with gas chromatography-mass spectrometry (GC-MS) analysis.

If the test item was bioaccumulated in the test fish, the test item had a possibility to be metabolized in the fish body. Therefore, C₆F₁₄COOH (hereafter mentioned as "carboxylic acid") which was an estimated metabolite of the test item in the test fish was analyzed at the same time. Analysis of the carboxylic acid in the test fish was performed with the liquid chromatography - tandem mass spectrometry (LC/MS/MS).

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.*3

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, two fish were taken out and three groups (two fish per group) were used for measurement of lipid contents.

*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,

<table>
<thead>
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<th>Level</th>
<th>mL</th>
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<tr>
<td>Level 1</td>
<td>25</td>
</tr>
<tr>
<td>Level 2</td>
<td>250</td>
</tr>
</tbody>
</table>

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

```
Test water
→ Water for recovery test 225 mL (graduated cylinder) (only Level 1)
   • Column chromatography*4 (see below)

Eluate

• Filling up to 5 mL (ethyl acetate, volumetric flask)

Sample for GC-MS analysis
```

*4 Conditions of column chromatograph

Mega Bond Elut C8

<table>
<thead>
<tr>
<th>Conditionings</th>
<th>Volume</th>
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<tr>
<td>Ethyl acetate</td>
<td>10 mL</td>
</tr>
<tr>
<td>Methanol</td>
<td>10 mL</td>
</tr>
<tr>
<td>Water*5</td>
<td>10 mL</td>
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</tbody>
</table>

Loading

Whole volume of the solution was loaded.

Eluent 1 Water*5 5 mL

Nitrogen purge was conducted after the first elution, then the cartridge for dehydration*6 was connected.

Eluent 2 Ethyl acetate 4.5 mL

Test item was eluted with eluent 2.

*5 City water was treated by Ultra pure water system.

*6 Conditions of the cartridge for dehydration

Sep-Pak Dry

<table>
<thead>
<tr>
<th>Conditionings</th>
<th>Volume</th>
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<tbody>
<tr>
<td>Ethyl acetate</td>
<td>5 mL</td>
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</table>
(2) Test fish (test item and carboxylic acid)

Test fish were taken from each test tank and pretreated for gas chromatography-mass spectrometry (GC-MS) analysis (test item) and liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis (carboxylic acid) as follows:

Test fish

- Measurement of weight and body length
- Chopping into pieces (scissors)
- Refinement (polytron, 2 min. or more, on ice water)

Fine sample

- Taking out 1 - 5 g (analytical balance)
- Taking out 3 - 5 g (analytical balance)
  - Acetonitrile / isopropyl alcohol (7/3 V/V) 15 mL
    (graduated cylinder)
- Homogenization (polytron, about 1 min., on ice water)
- Washing (acetonitrile / isopropyl alcohol (7/3 V/V) 3 mL)
- Centrifugation (7000 × g, 5 min.)

Sample for storage

Residue

- Filtration (absorbent cotton)
- Filling up to 25 mL (acetonitrile / isopropyl alcohol (7/3 V/V), volumetric flask)

Supernatant

- Taking out 2.5 mL (transfer pipet)
- Column chromatography *7
  (see next page)
- Taking out 1 mL (transfer pipet)
  - Filling up to 20 mL (methanol *8 / water *5
    (1/1 V/V), volumetric flask)

Eluate

- Filling up to 5 mL
  (acetonitrile / isopropyl alcohol
  (7/3 V/V), volumetric flask)

Sample for GC-MS analysis
(test item)

Sample for LC/MS/MS analysis
(carboxylic acid)
*7 Conditions of column chromatograph

Sep-Pak Silica

Conditionings Acetonitrile / isopropyl alcohol (7/3 V/V) 5 mL
Loading Whole volume of the solution was loaded.
Elution Eluent Acetonitrile / isopropyl alcohol (7/3 V/V) 2 mL

Test item was eluted with the loaded solution and eluent.

*8 Containing 10 mmol/L di-n-butylammonium acetate.
4.7.3 Quantitative analysis for test item and carboxylic acid

(1) 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-5, 6, Fig. 6 and Tables-8, 9, 10, Figs. 9, 10, 11).

(a) Analytical conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Gas chromatograph-mass spectrometer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shimadzu Corporation . type QP-5000</td>
</tr>
</tbody>
</table>

**Conditions of gas chromatograph**

<table>
<thead>
<tr>
<th>Column</th>
<th>INNOWAX 30 m × 0.25 mm I.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>film thickness</td>
<td>0.25μm</td>
</tr>
<tr>
<td>Column temp.</td>
<td>Test water</td>
</tr>
<tr>
<td></td>
<td>35 °C (2 min.) → 150 °C (2 min.) → 200 °C (2 min.)</td>
</tr>
<tr>
<td></td>
<td>Test fish</td>
</tr>
<tr>
<td></td>
<td>35 °C (2 min.) → 150 °C (2 min.) → 200 °C (3 min.)</td>
</tr>
<tr>
<td>Temp. rate</td>
<td>(1) 20 °C/min.</td>
</tr>
<tr>
<td></td>
<td>(2) 35 °C/min.</td>
</tr>
<tr>
<td>Injection temp.</td>
<td>200 °C</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>Helium</td>
</tr>
<tr>
<td>Pressure</td>
<td>50 kPa</td>
</tr>
<tr>
<td>Total flow</td>
<td>10 mL/min.</td>
</tr>
<tr>
<td>Injection method</td>
<td>Splitless</td>
</tr>
<tr>
<td>Sample size</td>
<td>3 μL</td>
</tr>
</tbody>
</table>

**Conditions of mass spectrometer**

| Interface temp.   | 250 °C                       |
| Ionization mode   | Electron ionization (EI)     |
| Ionization voltage| 70 eV                        |
| Monitoring m/z    | 94.95                        |

*CONTAINS CONFIDENTIAL BUSINESS INFORMATION*
(b) Preparation of standard solution

The standard solution to determine the concentration of the test item in samples was prepared as follows.

1. Test water

100 mg of the item supplied by the sponsor was dissolved in ethyl acetate to prepare 1000 mg/L solution of the test item. 2.50 mg/L solution of the test item was then prepared from this solution by dilution with ethyl acetate. This solution was pretreated as follows to prepare 50.0 μg/L standard solution.

```
Water for recovery test 250 mL

- Column chromatography (see page 15)

Eluate

← 2.50 mg/L solution of the test item 100 μL
  (microsyringe)
  - Filling up to 5 mL (ethyl acetate, volumetric flask)

50.0 μg/L standard solution
  (analysis for test water, test item)
```
© Test fish

100 mg of the item supplied by the sponsor was dissolved in acetonitrile to prepare 1000 mg/L solution of the test item. 2.50 mg/L solution of the test item was then prepared from this solution by dilution with acetonitrile/isopropyl alcohol (7/3 V/V). This solution was pretreated as follows to prepare 50.0 μg/L standard solution.

Fine sample of the same sample as recovery and blank test 5 g as described in Section 4.7.4(1)

- Acetonitrile/isopropanol (7/3 V/V) 15 mL (graduated cylinder)
  - Homogenization (polytron, about 1 min., on ice water)
  - Washing (acetonitrile/isopropyl alcohol (7/3 V/V) 3 mL)
  - Centrifugation (7000 x g, 5 min.)

Residue  Supernatant

- Filtration (absorbent cotton)
- Filling up to 25 mL (acetonitrile/isopropyl alcohol (7/3 V/V), volumetric flask)
- Taking out 2.5 mL (transfer pipet)
- Column chromatography* (see page 17)

Eluate

- 2.50 mg/L solution of the test item 100 μL (microsyringe)
- Filling up to 5 mL (acetonitrile/isopropyl alcohol (7/3 V/V), volumetric flask)

50.0 μg/L Standard solution (analysis for test fish, test item)
(c) Calibration curve

1) Test water

25.0, 50.0 and 100 μg/L standard solutions were prepared by the same method as described in (b)1). These standard solutions were analyzed according to the analytical conditions described in (a). A calibration curve was drawn on the basis of the relation between the peak area on the mass fragmentograms and the respective concentrations.

In consideration of the noise level, the lowest detectable peak area of the test item was regarded as 150, which corresponded to 2.5 μg/L of the test item concentration (see Fig. 4).

2) Test fish

25.0, 50.0 and 100 μg/L standard solutions were prepared by the same method as described in (b)2). These standard solutions were analyzed according to the analytical conditions described in (a). A calibration curve was drawn on the basis of the relation between the peak area on the mass fragmentograms and the respective concentrations.

In consideration of the noise level, the lowest detectable peak area of the test item was regarded as 150, which corresponded to 2.2 μg/L of the test item concentration (see Fig. 7).
(2) Analysis for carboxylic acid

The samples for LC/MS/MS analysis in pretreatment were analyzed by liquid chromatography-tandem mass spectrometry under the following analytical conditions. The concentration of the carboxylic acid 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-12, 13, 14, Figs. 16, 17, 18).

(a) Analytical conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Liquid chromatograph-mass spectrometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid chromatograph</td>
<td>Agilent type HP-1100</td>
</tr>
<tr>
<td>Mass spectrometer</td>
<td>Micromass type Quattro Ultima</td>
</tr>
</tbody>
</table>

Conditions of liquid chromatograph

<table>
<thead>
<tr>
<th>Column</th>
<th>L-column ODS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column temp.</td>
<td>15 cm x 2.1 mm I.D. stainless steel</td>
</tr>
<tr>
<td>Eluent</td>
<td>A (40 %): Water\textsuperscript{9}</td>
</tr>
<tr>
<td></td>
<td>B (60 %): Methanol\textsuperscript{9}</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.2 mL/min.</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

Conditions of mass spectrometer

<table>
<thead>
<tr>
<th>Ionization mode</th>
<th>Electrospray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection ion</td>
<td>Negative</td>
</tr>
<tr>
<td>Detection mode</td>
<td>Selected reaction monitoring</td>
</tr>
<tr>
<td>Precursor ion</td>
<td>m/z 313</td>
</tr>
<tr>
<td>Product ion</td>
<td>m/z 269</td>
</tr>
<tr>
<td>Ion source temp.</td>
<td>130 °C</td>
</tr>
<tr>
<td>Desolution temp.</td>
<td>400 °C</td>
</tr>
<tr>
<td>Cone voltage</td>
<td>20 V</td>
</tr>
<tr>
<td>Collision energy</td>
<td>10 eV</td>
</tr>
</tbody>
</table>

\textsuperscript{9} Containing 5 mmol/L di-n-butylammonium acetate.
(b) Preparation of standard solution

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

100 mg of the carboxylic acid sample was accurately weighed and dissolved in methanol to prepare 1000 mg/L solution of the carboxylic acid. 1.00 µg/L standard solution was then prepared from this solution by dilution with methanol* with water** (1/1 V/V).

(c) Calibration curve

0.50, 1.00 and 2.00 µg/L standard solutions were prepared by the same method as described in (b). These solutions were analyzed according to the analytical conditions described in (a). 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 carboxylic acid was regarded as 1000, which corresponded to 0.091 µg/L of the carboxylic acid concentration (see Fig. 14).

4.7.4 Recovery and blank test

(1) Test item

(a) Method

Water and fine sample of two fish (about 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.

(b) 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-4, 7 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)

81.0 %, 82.9 % average 82.0 %

For analysis of test fish (5000 ng test item added)

74.2 %, 74.5 % average 74.3 %
(2) Carboxylic acid

(a) Method
Fine sample of two fish (about 10 g) spiked a specified amount of the carboxylic acid for the recovery test was prepared in the same way as described in Section 4.7.2(2). The blank test was also performed in the same manner without the carboxylic acid. All the recovery and blank tests were performed in duplicate.

(b) Results of recovery test
In the blank tests, the chromatogram of LC/MS/MS had no peaks interfering with determination of the carboxylic acid concentration. The duplicate recovery rates and the average of them in the pretreatment are shown below (see Table-11 and Fig. 15). The average recovery rate was used as correction factors for the determination of the carboxylic acid concentrations in the analytical samples.

For analysis of test fish (1000 ng carboxylic acid added)
94.4 %, 93.2 % average 93.8 %

4.7.5 Lipid content in test fish
Lipid contents in the sample for storage of the control test fish were determined with gravimetric analysis after chloroform-methanol extraction.
4.7.6 Calculation of the test item and carboxylic acid concentration in sample and minimum limit of determination

(1) Calculation of the test item concentration in test water
The equations in Tables-5 and 6 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*10 of the test item in test water was calculated on the basis of that obtained from the calibration curve in Section 4.7.3(1)(c) as follows.

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.61</td>
</tr>
<tr>
<td>2</td>
<td>0.061</td>
</tr>
</tbody>
</table>

(3) Calculation of the test item and carboxylic acid concentration in test fish
(a) Test item
The equations in Tables-8, 9 and 10 were used to obtain the concentrations, and they were rounded to 3 figures.

(b) Carboxylic acid
If the carboxylic acid was concentrated into the test fish, the equations in Tables-12, 13 and 14 were used to obtain the concentrations. However, the measured concentrations of carboxylic acid were not more than the determination limit.

(4) Determination limit of the test item and carboxylic acid in test fish
Assuming the fine sample of fish to be 5 g, the determination limit*10 of the test item and carboxylic acid in test fish were calculated on the basis of that obtained from the calibration curve in Section 4.7.3(1)(c) and 4.7.3(2)(c).

(a) Test item 30 ng/g
(b) Carboxylic acid 9.7 ng/g

*10 Minimum determination limit of the test item and carboxylic acid (μg/L or ng/g)

\[
\frac{A}{100} \times \frac{C \times E}{D}
\]

where

A : Minimum determination limit of the test item and carboxylic acid 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)

\[
\overline{C_{wt}} = \{ \overline{C_{w(1)}} + \cdots + \overline{C_{w(n)}} \} / n
\]

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

4.7.8 Calculation of bioconcentration factor (BCF)

Bioconcentration factor (BCF) of the test item and carboxylic acid were calculated as follows.

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

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

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

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

(2) Calculation of bioconcentration factor of the test item and carboxylic acid

\[
\text{BCF} = \frac{C_{f}}{C_{w}}
\]

where
- \(\text{BCF}\) : Bioconcentration factor of the test item or carboxylic acid
- \(C_{f}\) : Concentration of the test item or carboxylic acid in test fish (ng/g)
- \(C_{w}\) : The average concentration of the test item in test water for calculating BCF (\(\mu g/L\))
4.7.9 Calculable BCF

On the basis of the minimum determination limit of the test item and carboxylic acid 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.

<table>
<thead>
<tr>
<th>Test item</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Test item</td>
<td>3.3</td>
<td>36</td>
</tr>
<tr>
<td>(2) Carboxylic acid</td>
<td>1.1</td>
<td>12</td>
</tr>
</tbody>
</table>

4.7.10 Calculation of lipid content

Lipid contents were calculated with the following equation.

Lipid content (%) = \( \frac{T - T_0}{S} \times 100 \)

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, the concentration values of the test item and carboxylic acid in test fish were rounded to 3 figures. BCFs values were rounded 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 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 81% of each nominated concentration. The variation of the concentrations of the test item was within ± 20% of the average of the measured concentrations.

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

(Unit: μg/L)

<table>
<thead>
<tr>
<th>Level</th>
<th>After 1 day</th>
<th>After 11 days</th>
<th>After 15 days</th>
<th>After 19 days</th>
<th>After 22 days</th>
<th>After 28 days</th>
<th>Average (Standard deviation)</th>
<th>Table</th>
<th>Fig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.94</td>
<td>9.30</td>
<td>9.10</td>
<td>9.43</td>
<td>9.24</td>
<td>8.65</td>
<td>9.11 (0.283)</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>0.823</td>
<td>0.846</td>
<td>0.842</td>
<td>0.821</td>
<td>0.811</td>
<td>0.870</td>
<td>0.835 (0.0214)</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

6.2 Bioconcentration factors

BCFs are shown in Table-2 and Table-3. BCFs in Table-2 plotted against the duration of exposure are shown in Figs. 1, 2 and Figs. 12, 13.

BCFs of the test item were following.

(1) Test item

<table>
<thead>
<tr>
<th>Level</th>
<th>15 – 49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td></td>
</tr>
<tr>
<td>Level 2</td>
<td>≤36</td>
</tr>
</tbody>
</table>

(2) Carboxylic acid

<table>
<thead>
<tr>
<th>Level</th>
<th>≤1.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td></td>
</tr>
<tr>
<td>Level 2</td>
<td>≤12</td>
</tr>
</tbody>
</table>

Table-2  BCFs (test item)

( ) : average value

<table>
<thead>
<tr>
<th>Level</th>
<th>After 11 days</th>
<th>After 15 days</th>
<th>After 19 days</th>
<th>After 22 days</th>
<th>After 28 days</th>
<th>Table</th>
<th>Fig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>33</td>
<td>27</td>
<td>17</td>
<td>44</td>
<td>49</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(48)</td>
<td>(32)</td>
<td>(29)</td>
<td>(15)</td>
<td>(46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(45)</td>
<td>(32)</td>
<td>(28)</td>
<td>(16)</td>
<td>(46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>≤36</td>
<td>≤36</td>
<td>≤36</td>
<td>≤36</td>
<td>≤36</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>≤36</td>
<td>≤36</td>
<td>≤36</td>
<td>≤36</td>
<td>≤36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3  BCFs (carboxylic acid)

<table>
<thead>
<tr>
<th>Level</th>
<th>After 11 days</th>
<th>After 15 days</th>
<th>After 19 days</th>
<th>After 22 days</th>
<th>After 28 days</th>
<th>Table</th>
<th>Fig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>≤1.1</td>
<td>≤1.1</td>
<td>≤1.1</td>
<td>≤1.1</td>
<td>≤1.1</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>≤12</td>
<td>≤12</td>
<td>≤12</td>
<td>≤12</td>
<td>≤12</td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

( ) : average value

6.3 BCFs at a steady-state (BCFss)

(1) Test item
Because all BCFs were less than 100 (see 6.2), it was evaluated that a steady-state was reached within 28th day. Therefore BCFss was the average of BCFs after 28 days (Level 1 ≤ 1.1, Level 2 ≤ 36).

(2) Carboxylic acid
Because all BCFs were less than 100 (see 6.2), it was evaluated that a steady-state was reached within 28th day. Therefore BCFss was the average of BCFs after 28 days (Level 1 ≤ 1.1, Level 2 ≤ 12).

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

Before initiation of experiment  2.95 %
After termination of experiment  2.26 %

6.5 Results of test fish observation
No abnormality in behavior or appearance was noted.
7. Remarks

Instruments, apparatus, special apparatus 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 Electronics Co., Ltd. type F-102

pH meter
Toa Electronics Ltd. type HM-14P

(2) Instruments, apparatus, special apparatus and reagents

Instruments and apparatus
Gas chromatograph-mass spectrometer:
see page 18

Liquid chromatograph-mass spectrometer:
see page 22

Electronic analytical balance:
Sartorius AG type LP4200S
Shimadzu Corporation type AEX-200B
Sartorius AG type BP301S

Metler Toledo type PB602

Infrared spectrophotometer:
Shimadzu Corporation type FTIR-8200PC

Homogenizer (polytron):
Kinematica type PT3000

Kinematica type PT3100

Centrifuge:
Hitachi Koki Co., Ltd. type CR21G

Special apparatus
Sep-Pak Silica:
Nihon Waters K. K.

Sep-Pak Dry:
Nihon Waters K. K.

Mega Bond Elut C8
Varian

Reagents
Acetonitrile (HPLC grade):
Wako Pure Chemical Industries, Ltd.

Methanol (HPLC grade):
Wako Pure Chemical Industries, Ltd.

Ethyl acetate(extra pure):
Kanto Chemical Co., Inc.

Isopropyl alcohol (extra pure):
NACALAI TESQUE, INC.

Di-n-butylammonium acetate (IPC grade):
Tokyo Kasei Kogyo Co., Ltd.

HCO-20:
Nikko Chemicals Co., Ltd.
(3) Instruments, apparatus and reagents for gravimetric analysis of lipid content in test fish

Instruments and apparatus
Electronic analytical balance : Sartorius AG type BP301S
Rotary evaporator : Tokyo Rika Kikai Co., Ltd. type N-1
Homogenizer (polytron) :
Kinematica type PT3000
Kinematica type PT3100
Homogenizer (auto cell master) : Iuchi Seiido Co., Ltd. type CM-200
Vacuum pump :
Sinku Kiko Co., Ltd. type DA-20D
Sinku Kiko Co., Ltd. type DAH-20C
Vacuum desiccator : Iuchi Seiido Co., Ltd. type VL

Reagents
Purified water : Takasugi Pharmaceutical Co., Ltd.
Methanol (extra pure) : Wako Pure Chemical Industries, Ltd.
Chloroform (guaranteed reagent) : Wako Pure Chemical Industries, Ltd.
Anhydrous sodium sulfate (extra pure) : Katayama Chemical Industries Co., Ltd.
### Table-4 Calculation table for recovery and blank test
(Analysis of test water, test item)

<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 50.0μg/L</td>
<td>2909</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery a</td>
<td>2356</td>
<td>1</td>
<td>5</td>
<td></td>
<td>202</td>
<td>81.0%</td>
</tr>
<tr>
<td>Recovery b</td>
<td>2412</td>
<td>1</td>
<td>5</td>
<td></td>
<td>207</td>
<td>82.9%</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>82.0%</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>3061</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank a</td>
<td>n.d.</td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank b</td>
<td>n.d.</td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a, b: individual sample)

A: Peak area

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 item recovered (ng)

\[ E = P \times \left( \frac{A(t)}{A(std)} \right) / B \times C - D \]

F: Recovery rate (%)

\[ F = E / Q \times 100 \]

P: Concentration of test item in standard solution 50.0μg/L

Q: Amount of test item added (250ng)

See Fig. 5

January 29, 2002

Name L. [Signature]
Table-5  Calculation table for analysis of test water  
(Level 1, test item) 

<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 50.0μg/L</td>
<td>2845</td>
<td></td>
</tr>
<tr>
<td>Test water after 1 day</td>
<td>2085</td>
<td>8.94</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>3723</td>
<td></td>
</tr>
<tr>
<td>Test water after 11 days</td>
<td>2838</td>
<td>9.30</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2811</td>
<td></td>
</tr>
<tr>
<td>Test water after 15 days</td>
<td>2096</td>
<td>9.10</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>3078</td>
<td></td>
</tr>
<tr>
<td>Test water after 19 days</td>
<td>2379</td>
<td>9.43</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2886</td>
<td></td>
</tr>
<tr>
<td>Test water after 22 days</td>
<td>2185</td>
<td>9.24</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2927</td>
<td></td>
</tr>
<tr>
<td>Test water after 28 days</td>
<td>2074</td>
<td>8.65</td>
</tr>
</tbody>
</table>

Average concentration of test item in test water 9.11 (S.D. 0.283)

A: Peak area  
A(std): Standard solution  
A(t): Sample  
B: Ratio of portion used for analysis  
C: Final volume  
SmL  
F: Recovery rate 82.0%  
H: Volume of test water taken out 25mL  
I: Concentration of test item in test water (μg/L)  
I = P × (A(t)/A(std)) / B × C × F × 100 / H  
J: Average concentration of test item 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 item in standard solution 50.0μg/L  
See Fig. 6

January 29, 2002  
Name [Signature]
Table-6  Calculation table for analysis of test water  
(Level 2, test item)  

<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 50.0μg/L</td>
<td>2845</td>
<td>0.823</td>
</tr>
<tr>
<td>Test water after 1 day</td>
<td>1918</td>
<td></td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>3723</td>
<td>0.846</td>
</tr>
<tr>
<td>Test water after 11 days</td>
<td>2580</td>
<td></td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2811</td>
<td>0.842</td>
</tr>
<tr>
<td>Test water after 15 days</td>
<td>1940</td>
<td></td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>3078</td>
<td>0.821</td>
</tr>
<tr>
<td>Test water after 19 days</td>
<td>2071</td>
<td></td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2886</td>
<td>0.811</td>
</tr>
<tr>
<td>Test water after 22 days</td>
<td>1918</td>
<td></td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2927</td>
<td>0.870</td>
</tr>
<tr>
<td>Test water after 28 days</td>
<td>2086</td>
<td></td>
</tr>
</tbody>
</table>

Average concentration of test item in test water 0.835  (S.D. 0.0214)

A : Peak area  
A( std) : Standard solution  A(i) : Sample  
B : Ratio of portion used for analysis 1  
C : Final volume 5mL  
F : Recovery rate 82.0%  
H : Volume of test water taken out 250mL  
I : Concentration of test item in test water (μg/L)  
I = P × ( A(i) / A( std) ) / B × C / F × 100 / H  
J : Average concentration of test item 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 item in standard solution 50.0μg/L  

See Fig. 6  

January 29, 2002  
Name  

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### Table 7: Calculation table for recovery and blank test
(Analysis of test fish, test item)

<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>C</th>
<th>D</th>
<th>B</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 50.0μg/L</td>
<td>2895</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery a</td>
<td>2148</td>
<td>2.5/25</td>
<td>5</td>
<td>-</td>
<td>3710</td>
<td>74.2%</td>
</tr>
<tr>
<td>Recovery b</td>
<td>2156</td>
<td>2.5/25</td>
<td>5</td>
<td>-</td>
<td>3720</td>
<td>74.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Average</td>
<td>74.3%</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2853</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank a</td>
<td>n.d.</td>
<td>2.5/25</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Blank b</td>
<td>n.d.</td>
<td>2.5/25</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

( a, b : individual sample )

A : Peak area

A(\text{std}) : Standard solution   \hspace{1cm} A(t) : Sample

B : Ratio of portion used for analysis (fish homogenate)   \hspace{1cm} 5/10

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 item recovered (ng)

\[ F = F \times (A(t) / A(\text{std})) / B \times C \times D \times E. \]

G : Recovery rate (%)

\[ G = F / Q \times 100 \]

P : Concentration of test item in standard solution \hspace{1cm} 50.0μg/L

Q : Amount of test item added (5000ng)

See Fig. 8

January 29, 2002

Name: [Signature]

CONTAINS CONFIDENTIAL BUSINESS INFORMATION
<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>G</th>
<th>K</th>
<th>H</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 50.0μg/L</td>
<td>2922</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test fish after 11 days a</td>
<td>1678</td>
<td>5.00</td>
<td>386</td>
<td>9.12</td>
<td>42</td>
</tr>
<tr>
<td>Test fish after 11 days b</td>
<td>1884</td>
<td>5.00</td>
<td>434</td>
<td>9.12</td>
<td>48</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2844</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test fish after 15 days a</td>
<td>760</td>
<td>3.00</td>
<td>299</td>
<td>9.11</td>
<td>33</td>
</tr>
<tr>
<td>Test fish after 15 days b</td>
<td>733</td>
<td>3.00</td>
<td>289</td>
<td>9.11</td>
<td>32</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2898</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test fish after 19 days a</td>
<td>1079</td>
<td>5.00</td>
<td>250</td>
<td>9.28</td>
<td>27</td>
</tr>
<tr>
<td>Test fish after 19 days b</td>
<td>1168</td>
<td>5.00</td>
<td>271</td>
<td>9.28</td>
<td>29</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2986</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test fish after 22 days a</td>
<td>685</td>
<td>5.00</td>
<td>154</td>
<td>9.26</td>
<td>17</td>
</tr>
<tr>
<td>Test fish after 22 days b</td>
<td>607</td>
<td>5.00</td>
<td>137</td>
<td>9.26</td>
<td>15</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>3119</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test fish after 28 days a</td>
<td>1860</td>
<td>5.00</td>
<td>401</td>
<td>9.11</td>
<td>44</td>
</tr>
<tr>
<td>Test fish after 28 days b</td>
<td>2049</td>
<td>5.00</td>
<td>442</td>
<td>9.11</td>
<td>49</td>
</tr>
</tbody>
</table>

(a, b: individual sample)

A: Peak area
A(Std): Standard solution  A(t): Sample
B: Ratio of portion used for analysis  2.5/25
C: Final volume  5mL
D: Dilution factor  1
E: Average concentration of blank in analysis of control  0μg/g
F: Recovery rate  74.3%
G: Weight of fine sample (g)
K: Concentration of test item in test fish (μg/g)
\[ K = \left( P \times \frac{(A(t)/A(Std)) \times B \times D \times C - E}{G - E} \right) / F \times 100 \]
H: Average concentration of test item in test water (μg/L)
\[ H = \left( \frac{\sum_{n=2}^{n} (1(n-2) + 1(n-1) + 1(n))}{m} \right) / \text{n: Number of test water analyses ; m = 2 when n = 2, m = 3 when n \geq 3} \]
I: Concentration of test item in test water (μg/L)
J: BCF
\[ J = \frac{K}{H} \]
P: Concentration of test item in standard solution  50.0μg/L

See Fig. 9

January 31, 2002  Name: [Signature]
### Table 9: Calculation table for analysis of test fish

_(Level 2, test item)_

<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>G</th>
<th>K</th>
<th>H</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 50.0µg/L</td>
<td>3025</td>
<td>n.d.</td>
<td>5.00</td>
<td>0.834</td>
<td>n.d.</td>
</tr>
<tr>
<td>Test fish after 11 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td>n.d.</td>
<td>0.834</td>
<td>n.d.</td>
</tr>
<tr>
<td>Test fish after 11 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td>n.d.</td>
<td>0.834</td>
<td>n.d.</td>
</tr>
<tr>
<td>Standard 50.0µg/L</td>
<td>3005</td>
<td>n.d.</td>
<td>3.00</td>
<td>0.837</td>
<td>n.d.</td>
</tr>
<tr>
<td>Test fish after 15 days a</td>
<td>n.d.</td>
<td>3.00</td>
<td>n.d.</td>
<td>0.837</td>
<td>n.d.</td>
</tr>
<tr>
<td>Test fish after 15 days b</td>
<td>n.d.</td>
<td>3.00</td>
<td>n.d.</td>
<td>0.837</td>
<td>n.d.</td>
</tr>
<tr>
<td>Standard 50.0µg/L</td>
<td>2808</td>
<td>n.d.</td>
<td>5.00</td>
<td>0.836</td>
<td>n.d.</td>
</tr>
<tr>
<td>Test fish after 19 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td>n.d.</td>
<td>0.836</td>
<td>n.d.</td>
</tr>
<tr>
<td>Test fish after 19 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td>n.d.</td>
<td>0.836</td>
<td>n.d.</td>
</tr>
<tr>
<td>Standard 50.0µg/L</td>
<td>3065</td>
<td>n.d.</td>
<td>5.00</td>
<td>0.825</td>
<td>n.d.</td>
</tr>
<tr>
<td>Test fish after 22 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td>n.d.</td>
<td>0.825</td>
<td>n.d.</td>
</tr>
<tr>
<td>Test fish after 22 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td>n.d.</td>
<td>0.825</td>
<td>n.d.</td>
</tr>
<tr>
<td>Standard 50.0µg/L</td>
<td>2927</td>
<td>n.d.</td>
<td>5.00</td>
<td>0.834</td>
<td>n.d.</td>
</tr>
<tr>
<td>Test fish after 28 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td>n.d.</td>
<td>0.834</td>
<td>n.d.</td>
</tr>
<tr>
<td>Test fish after 28 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td>n.d.</td>
<td>0.834</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

_(a, b : individual sample)_

A : Peak area
A(std) : Standard solution   A(t) : Sample
B : Ratio of portion used for analysis   2.5/2.5
C : Final volume  5mL
D : Dilution factor  1
E : Average concentration of blank in analysis of control  0µg/g
F : Recovery rate  74.3%
G : Weight of fine sample (g)
K : Concentration of test item in test fish (µg/g)
    \[ K = \frac{P \times (A(t) / A(std))}{B \times D \times C / G - B} \times 100 \]
H : Average concentration of test item in test water (µg/L)
    \[ H = \frac{I(n-2) + I(n-1) + I(n)}{m} ; m : \text{Number of test water analyses} ; m = 2 \text{ when } n = 2, m = 3 \text{ when } n \geq 3 \]
I : Concentration of test item in test water (µg/L)
J : BCF
    \[ J = \frac{K}{H} \]
P : Concentration of test item in standard solution  50.0µg/L

See Fig. 10

January 29, 2002  Name: [Signature]
### Table-10  Calculation table for analysis of test fish
(Control, test item)

<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>E</th>
<th>G</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 50.0μg/L</td>
<td>2598</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before the experimental start a</td>
<td>n.d.</td>
<td>-</td>
<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>Before the experimental start b</td>
<td>n.d.</td>
<td>-</td>
<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>Standard 50.0μg/L</td>
<td>2867</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After the experimental completion a</td>
<td>n.d.</td>
<td>-</td>
<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>After the experimental completion b</td>
<td>n.d.</td>
<td>-</td>
<td>5.00</td>
<td>-</td>
</tr>
</tbody>
</table>

( a, b : individual sample )

A : Peak area

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

B : Ratio of portion used for analysis  2.5/25

C : Final volume  5mL

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

\[ E = P \times \left( \frac{A(t)}{A(std)} \right) \times B \times C \]

G : Weight of fine sample (g)

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

\[ I = \frac{E}{G} \]

P : Concentration of test item in standard solution  50.0μg/L

See Fig. 11

January 29, 2002  Name ____________________
Table-11 Calculation table for recovery and blank test
(Analysis of test fish, carboxylic acid)

<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1.00µg/L</td>
<td>10021</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery a</td>
<td>9455</td>
<td>1/25</td>
<td>20</td>
<td>-</td>
<td>944</td>
<td>94.4 %</td>
</tr>
<tr>
<td>Recovery b</td>
<td>9341</td>
<td>1/25</td>
<td>20</td>
<td>-</td>
<td>932</td>
<td>93.2 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Average 93.8 %</td>
</tr>
<tr>
<td>Standard: 1.00µg/L</td>
<td>10006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank a</td>
<td>n.d.</td>
<td>1/25</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Blank b</td>
<td>n.d.</td>
<td>1/25</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a, b: individual sample)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A : Peak area
A(std) : Standard solution  A(t) : Sample
B : Ratio of portion used for analysis (fish homogenate)  S/10
C : Ratio of portion used for analysis (extracted solution)
D : Final volume (mL)
E : Amount of blank in test fish (ng)
F : Amount of carboxylic acid recovered (ng)
   \[ F = P \times \left( \frac{A(t)}{A(std)} \right) \times \frac{1}{B/C \times D - E} \]
G : Recovery rate (%)
   \[ G = \frac{F}{Q} \times 100 \]
P : Concentration of carboxylic acid in standard solution  1.00µg/L
Q : Amount of carboxylic acid added (1000ng)
See Fig. 15

January 29, 2002

Name [Signature]
## Table-12 Calculation table for analysis of test fish

(Level 1, carboxylic acid)

<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>G</th>
<th>K</th>
<th>H</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1.00μg/L</td>
<td>10090</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test fish after 11 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>9.12</td>
<td></td>
</tr>
<tr>
<td>Test fish after 11 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>9.12</td>
<td></td>
</tr>
<tr>
<td>Standard 1.00μg/L</td>
<td>10304</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test fish after 15 days a</td>
<td>n.d.</td>
<td>3.00</td>
<td></td>
<td>9.11</td>
<td></td>
</tr>
<tr>
<td>Test fish after 15 days b</td>
<td>n.d.</td>
<td>3.00</td>
<td></td>
<td>9.11</td>
<td></td>
</tr>
<tr>
<td>Standard 1.00μg/L</td>
<td>10859</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test fish after 19 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>9.28</td>
<td></td>
</tr>
<tr>
<td>Test fish after 19 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>9.28</td>
<td></td>
</tr>
<tr>
<td>Standard 1.00μg/L</td>
<td>10429</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test fish after 22 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>9.26</td>
<td></td>
</tr>
<tr>
<td>Test fish after 22 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>9.26</td>
<td></td>
</tr>
<tr>
<td>Standard 1.00μg/L</td>
<td>10143</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test fish after 28 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>9.11</td>
<td></td>
</tr>
<tr>
<td>Test fish after 28 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>9.11</td>
<td></td>
</tr>
</tbody>
</table>

(α, β: individual sample)

A: Peak area
A(Std): Standard solution
A(t): Sample
B: Ratio of portion used for analysis
C: Final volume
D: Dilution factor
E: Average concentration of blank in analysis of control
F: Recovery rate
G: Weight of fine sample (g)
H: Average concentration of test item in test water (μg/L)

K: Concentration of carboxylic acid in test fish (μg/g)

\[
K = \{ P \times (A(t) / A(Std)) / B \times D \times C / G \times E \} / F \times 100
\]

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

\[
I = \{ I(n-2) + I(n-1) + I(n) \} / m
\]

J: BCF

\[
J = K / H
\]

P: Concentration of carboxylic acid in standard solution

\[
P = 1.00μg/L
\]

See Fig. 16

January 29, 2002
Name: [Signature]

**CONTAINS CONFIDENTIAL BUSINESS INFORMATION**
### Table-13  Calculation table for analysis of test fish  
*(Level 2, carboxylic acid)*

<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>G</th>
<th>K</th>
<th>H</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1.00µg/L</td>
<td>10196</td>
<td></td>
<td></td>
<td>0.834</td>
<td></td>
</tr>
<tr>
<td>Test fish after 11 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>0.834</td>
<td></td>
</tr>
<tr>
<td>Test fish after 11 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>0.834</td>
<td></td>
</tr>
<tr>
<td>Standard 1.00µg/L</td>
<td>10317</td>
<td></td>
<td></td>
<td>0.837</td>
<td></td>
</tr>
<tr>
<td>Test fish after 15 days a</td>
<td>n.d.</td>
<td>3.00</td>
<td></td>
<td>0.837</td>
<td></td>
</tr>
<tr>
<td>Test fish after 15 days b</td>
<td>n.d.</td>
<td>3.00</td>
<td></td>
<td>0.837</td>
<td></td>
</tr>
<tr>
<td>Standard 1.00µg/L</td>
<td>10630</td>
<td></td>
<td></td>
<td>0.836</td>
<td></td>
</tr>
<tr>
<td>Test fish after 19 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>0.836</td>
<td></td>
</tr>
<tr>
<td>Test fish after 19 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>0.836</td>
<td></td>
</tr>
<tr>
<td>Standard 1.00µg/L</td>
<td>10488</td>
<td></td>
<td></td>
<td>0.825</td>
<td></td>
</tr>
<tr>
<td>Test fish after 22 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>0.825</td>
<td></td>
</tr>
<tr>
<td>Test fish after 22 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>0.825</td>
<td></td>
</tr>
<tr>
<td>Standard 1.00µg/L</td>
<td>10611</td>
<td></td>
<td></td>
<td>0.834</td>
<td></td>
</tr>
<tr>
<td>Test fish after 28 days a</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>0.834</td>
<td></td>
</tr>
<tr>
<td>Test fish after 28 days b</td>
<td>n.d.</td>
<td>5.00</td>
<td></td>
<td>0.834</td>
<td></td>
</tr>
</tbody>
</table>

(a, b : individual sample)

A : Peak area
A(std) : Standard solution  A(i) : Sample
B : Ratio of portion used for analysis  1/25
C : Final volume  20mL
D : Dilution factor  1
E : Average concentration of blank in analysis of control  0µg/g
F : Recovery rate  93.8%
G : Weight of fine sample (g)
K : Concentration of carboxylic acid in test fish (µg/g)
   \[ K = \left\{ \frac{P \times (A(i) / A(std))}{B \times D \times C / G - E} \right\} / F \times 100 \]
H : Average concentration of test item in test water (µg/L)
   \[ H = \left\{ \frac{I(n-2) + I(n-1) + I(n)}{m} \right\} ; \quad n : Number of test water analyses ; \quad m = 2 \text{ when } n = 2, \quad m = 3 \text{ when } n \geq 3 \]
I : Concentration of test item in test water (µg/L)
J : BCF
   \[ J = K / H \]
P : Concentration of carboxylic acid in standard solution  1.00µg/L

See Fig. 17

January 29, 2002

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BUSINESS INFORMATION
<table>
<thead>
<tr>
<th>Sample description</th>
<th>A</th>
<th>E</th>
<th>G</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1.00μg/L</td>
<td>11762</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before the experimental start a</td>
<td>n.d.</td>
<td>-</td>
<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>Before the experimental start b</td>
<td>n.d.</td>
<td>-</td>
<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>Standard 1.00μg/L</td>
<td>10113</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After the experimental completion a</td>
<td>n.d.</td>
<td>-</td>
<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>After the experimental completion b</td>
<td>n.d.</td>
<td>-</td>
<td>5.00</td>
<td>-</td>
</tr>
</tbody>
</table>

( a, b : individual sample )

A : Peak area

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

B : Ratio of portion used for analysis  
1/25

C : Final volume  
20mL

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

\[ E = \frac{P \times \left( \frac{A(t)}{A(std)} \right)}{B \times C} \]

G : Weight of fine sample (g)

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

\[ I = \frac{E}{G} \]

P : Concentration of carboxylic acid in standard solution  
1.00μg/L

See Fig. 18

January 29, 2002  
Name  

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BUSINESS INFORMATION
### Analytical results of dilution water

**Sampling date**: September 3, 2001

<table>
<thead>
<tr>
<th>Item</th>
<th>Unit</th>
<th>Measured value</th>
<th>Standard value</th>
<th>Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hardness (Ca, Mg)</td>
<td>mg/L</td>
<td>58.6</td>
<td>&lt; 300 *1</td>
<td></td>
</tr>
<tr>
<td>Suspended solid</td>
<td>mg/L</td>
<td>&lt; 1</td>
<td>&lt; 20 *2</td>
<td>1</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>8.3</td>
<td>6.5 ~ 8.5 *3</td>
<td></td>
</tr>
<tr>
<td>Total organic carbon</td>
<td>mg/L</td>
<td>1.6</td>
<td>&lt; 2 *2</td>
<td>0.1</td>
</tr>
<tr>
<td>Chemical oxygen demand</td>
<td>mg/L</td>
<td>0.1</td>
<td>&lt; 5 *3</td>
<td>0.1</td>
</tr>
<tr>
<td>Residual chlorine</td>
<td>mg/L</td>
<td>&lt; 0.01</td>
<td>&lt; 0.02 *3</td>
<td>0.01</td>
</tr>
<tr>
<td>Ammonia nitrogen</td>
<td>mg/L</td>
<td>0.25</td>
<td>&lt; 1 *3</td>
<td>0.01</td>
</tr>
<tr>
<td>Total cyan</td>
<td>mg/L</td>
<td>&lt; 0.01</td>
<td>n.d. *3</td>
<td>0.1</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>mg/L</td>
<td>198</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric conductivity</td>
<td>µS/cm</td>
<td>521</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic phosphorus</td>
<td>mg/L</td>
<td>&lt; 0.1</td>
<td>n.d. *3</td>
<td>0.1</td>
</tr>
<tr>
<td>Alkylmercury</td>
<td>mg/L</td>
<td>&lt; 0.0005</td>
<td>n.d. *3</td>
<td>0.0005</td>
</tr>
<tr>
<td>Mercury</td>
<td>mg/L</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005 *2</td>
<td>0.0005</td>
</tr>
<tr>
<td>Cadmium</td>
<td>mg/L</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01 *3</td>
<td>0.001</td>
</tr>
<tr>
<td>Cr⁶⁺</td>
<td>mg/L</td>
<td>&lt; 0.02</td>
<td>&lt; 0.05 *3</td>
<td>0.02</td>
</tr>
<tr>
<td>Lead</td>
<td>mg/L</td>
<td>&lt; 0.005</td>
<td>&lt; 0.1 *3</td>
<td>0.005</td>
</tr>
<tr>
<td>Arsenic</td>
<td>mg/L</td>
<td>&lt; 0.002</td>
<td>&lt; 0.05 *3</td>
<td>0.002</td>
</tr>
<tr>
<td>Iron</td>
<td>mg/L</td>
<td>0.02</td>
<td>&lt; 1.0 *3</td>
<td>0.01</td>
</tr>
<tr>
<td>Copper</td>
<td>mg/L</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005 *3</td>
<td>0.005</td>
</tr>
<tr>
<td>Cobalt</td>
<td>mg/L</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001 *5</td>
<td>0.001</td>
</tr>
<tr>
<td>Manganese</td>
<td>mg/L</td>
<td>0.01</td>
<td>&lt; 0.05 *1</td>
<td>0.01</td>
</tr>
<tr>
<td>Zinc</td>
<td>mg/L</td>
<td>0.01</td>
<td>&lt; 1.0 *1</td>
<td>0.01</td>
</tr>
<tr>
<td>Aluminium</td>
<td>mg/L</td>
<td>0.014</td>
<td>&lt; 0.2 *1</td>
<td>0.001</td>
</tr>
<tr>
<td>Nickel</td>
<td>mg/L</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01 *1</td>
<td>0.001</td>
</tr>
<tr>
<td>Silver</td>
<td>mg/L</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001 *5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Organochlorine pesticides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2-Dichloropropane</td>
<td>mg/L</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.06 *4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chlorelathion</td>
<td>mg/L</td>
<td>&lt; 0.0002</td>
<td>&lt; 0.04 *4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Propyzamide</td>
<td>mg/L</td>
<td>&lt; 0.0002</td>
<td>&lt; 0.008 *4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Chlornitrofen</td>
<td>mg/L</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001 *1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Simazine</td>
<td>mg/L</td>
<td>&lt; 0.0003</td>
<td>&lt; 0.003 *4</td>
<td>0.0003</td>
</tr>
<tr>
<td>Thioencarb</td>
<td>mg/L</td>
<td>&lt; 0.001</td>
<td>&lt; 0.02 *4</td>
<td>0.001</td>
</tr>
<tr>
<td>Organophosphorous pesticides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diazinon</td>
<td>mg/L</td>
<td>&lt; 0.0002</td>
<td>&lt; 0.005 *4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Isoxathion</td>
<td>mg/L</td>
<td>&lt; 0.0002</td>
<td>&lt; 0.008 *4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>mg/L</td>
<td>&lt; 0.0002</td>
<td>&lt; 0.003 *4</td>
<td>0.0002</td>
</tr>
<tr>
<td>EPN</td>
<td>mg/L</td>
<td>&lt; 0.0002</td>
<td>&lt; 0.006 *4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>mg/L</td>
<td>&lt; 0.0002</td>
<td>&lt; 0.01 *4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Iprobenfos</td>
<td>mg/L</td>
<td>&lt; 0.0002</td>
<td>&lt; 0.008 *4</td>
<td>0.0002</td>
</tr>
<tr>
<td>PCB</td>
<td>mg/L</td>
<td>&lt; 0.0005</td>
<td>n.d. *3</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

*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).

The minimum calculation limit of bioconcentration factor.

Fig. 2  Correlation between exposure period and bioconcentration factor (Level 2).
Ten data after 11, 15, 19, 22 and 28 days were lower than detection limit.

January 31, 2002  Name [Signature]

CONTAINS CONFIDENTIAL BUSINESS INFORMATION
96-hour LC50 = 10.4 mg/L

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Cumulative Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hours</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>0.854</td>
<td>0</td>
</tr>
<tr>
<td>1.88</td>
<td>0</td>
</tr>
<tr>
<td>4.13</td>
<td>0</td>
</tr>
<tr>
<td>9.09</td>
<td>0</td>
</tr>
<tr>
<td>20.0</td>
<td>20</td>
</tr>
</tbody>
</table>

Fig. 3 Concentration - Mortality Curve.

Date: December 21, 2001  Name: [Redacted]
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Fig. 4-1 (1/2) Mass fragmentogram of GC/MS analysis for calibration curve (test water, test item)
Fig. 4-1 (2/2) Mass fragmentogram of GC/MS analysis for calibration curve
(test water, test item)
Conditions of GC-MS analysis

Instrument : Shimadzu QP5000
Sample : C₆₂アルコール
Solvent : Ethyl acetate

GC Conditions
Injection vol. : 3μL
Column (Size) : INNOWAX (30m x 0.25mm I.D.) Film thickness 0.25μm
Col. temp. : 35°C(2min.) → 150°C(2min.) → 200°C(2min.)
Rate : 0°C/2min., 25°C/2min.
Injection Temp. : 200°C
Carrier Gas : He (Pressure 50kPa, Total flow rate 10mL/min.)
Inlet mode : Splitless

MS Conditions
Ionization mode : EI
Interface temp. : 250°C
Ionization vol. : 70eV
Monitoring Ion : m/z = 94, 95

Fig. 4 - 2 Calibration curve and conditions of GC/MS analysis for C₆₂アルコール (test water).

Name : [Signature]
January 29, 2002

CONTAINS CONFIDENTIAL BUSINESS INFORMATION
Fig. 5-1 Mass fragmentogram of GC/MS analysis for recovery and blank test (analysis of test water, test item)
Fig. 5-2  Mass fragmentogram of GC/MS analysis for recovery and blank test
(analysis of test water, test item)
Fig. 6-1  Mass fragmentogram of GC/MS analysis for test water (test item)
Fig. 6–2  Mass fragmentogram of GC/MS analysis for test water (test item)
Fig. 6–3 Mass fragmentogram of GC/MS analysis for test water (test item)
Fig. 6-4  Mass fragmentogram of GC/MS analysis for test water (test item)
Fig. 6-5  Mass fragmentogram of GC/MS analysis for test water (test item)
Fig. 6-6  Mass fragmentogram of GC/MS analysis for test water (test item)
Fig. 7-1 (1/2) Mass fragmentogram of GC/MS analysis for calibration curve
(test fish, test item)
Fig. 7-1 (2/2) Mass fragmentogram of GC/MS analysis for calibration curve
(test fish, test item)
Conditions of GC-MS analysis

Instrument: Shimadzu QP5000
Sample: CsAlcohol
Solvent: Acetonitrile/Isopropyl alcohol (7/3 V/V)

GC Conditions
Injection vol.: 3µL
Column (Size): INNOWAX (30m × 0.25mm I.D.) Film thickness 0.25µm
Col. temp.: 35°C (2min.) → 150°C (2min.) → 200°C (3min.)
Rate: 10°C/min., 25°C/min.
Injection Temp.: 200°C
Carrier Gas: He (Pressure 50kPa, Total flow rate 10mL/min.)
Inlet mode: Splitless

MS Conditions
Ionization mode: EI
Interface temp.: 250°C
Ionization vol.: 70eV
Monitoring ion: m/z = 94.95

Fig. 7-2 Calibration curve and conditions of GC/MS analysis for CsAlcohol (test fish).

January 30, 2002

Name
Fig. 8-1 Mass fragmentogram of GC/MS analysis for recovery and blank test
(analysis of fish, test item)
Fig. 8-2 Mass fragmentogram of GC/MS analysis for recovery and blank test (analysis of fish, test item)
Fig. 9-1 Mass fragmentogram of GC/MS analysis for test fish (Level 1, test item)
Fig. 9-2 Mass fragmentogram of GC/MS analysis for test fish (Level 1, test item)
Fig. 9-3 Mass fragmentogram of GC/MS analysis for test fish (Level 1, test item)
Fig. 9-4 Mass fragmentogram of GC/MS analysis for test fish (Level 1, test item)
Fig. 9–5 Mass fragmentogram of GC/MS analysis for test fish (Level 1, test item)
Fig. 10-1 Mass fragmentogram of GC/MS analysis for test fish (Level 2, test item)
Fig. 10-2 Mass fragmentogram of GC/MS analysis for test fish (Level 2, test item)
Fig. 10-3 Mass fragmentogram of GC/MS analysis for test fish (Level 2, test item)
Fig. 10-4 Mass fragmentogram of GC/MS analysis for test fish (Level 2, test item)
Fig. 10-5 Mass fragmentogram of GC/MS analysis for test fish (Level 2, test item)
Fig. 11-1 Mass fragmentogram of GC/MS analysis for test fish (Control, test item)
Standard solution 50 μg/L (F)

Study No. 43771

Data: 02/01/23
File name: C:\GCMSolution\Data\試験第2課V43771V43771z42.qgd

Intensity

7500
7000
6500
6000
5500
5000
4500
4000
3500

Peak No. Time(min) m/z Area
1 6.71 94.95 2887

Jan. 25, 2002 Name: [Signature]

After the experimental completion (Control-a)

Study No. 43771

Data: 02/01/23
File name: C:\GCMSolution\Data\試験第2課V43771V43771z43.qgd

Intensity

7500
7000
6500
6000
5500
5000
4500
4000
3500

Peak position

n.d.

Jan. 25, 2002 Name: [Signature]

After the experimental completion (Control-b)

Study No. 43771

Data: 02/01/23
File name: C:\GCMSolution\Data\試験第2課V43771V43771z44.qgd

Intensity

7500
7000
6500
6000
5500
5000
4500
4000
3500

Peak position

n.d.

Jan. 25, 2002 Name: [Signature]

Fig. 11-2 Mass fragmentogram of GC/MS analysis for test fish (Control, test item)
Fig. 12  Correlation between exposure period and bioconcentration factor (Level 1, carboxylic acid).
Ten data after 11, 15, 19, 22 and 28 days were lower than detection limit.

Fig. 13  Correlation between exposure period and bioconcentration factor (Level 2, carboxylic acid).
Ten data after 11, 15, 19, 22 and 28 days were lower than detection limit.

January 31, 2002  Name: Yosh
Fig. 14-1 Chromatogram of LC/MS/MS analysis for calibration curve (carboxylic acid)
Conditions of LC-MS analysis

**Instrument** : Agilent HP-1100, Micromass Quattro Ultima

**Sample** : Carboxylic acid

**Solvent** : Methanol*/water*(1:1 V/V)

**LC Conditions**

- **Column (Size)** : L-column ODS (15 cm × 2.1 mm I.D.)
- **Col. temp.** : 25°C
- **Eluent** : A (40%)=Water* * B (60%)=Methanol*
- **Flow rate** : 0.2 mL/min
- **Injection vol.** : 20 μL

**MS/MS Conditions**

- **Ionization mode** : ESI
- **Detection ion** : Negative
- **Detection mode** : Selected reaction monitoring
- **Precursor ion** : m/z = 313
- **Product ion** : m/z = 269
- **Ion source temp.** : 130°C
- **Cone voltage** : 20V
- **Cone voltage** : 10 eV

---

**Fig. 14 - 2** Calibration curve and conditions of LC/MS/MS analysis for Carboxylic acid (test fish).

January 29, 2002

[Signature]
Fig. 15-1 Chromatogram of LC/MS/MS analysis for recovery and blank test (analysis of test fish, carboxylic acid)
Fig. 15-2 Chromatogram of LC/MS/MS analysis for recovery and blank test
(analysis of test fish, carboxylic acid)
Fig. 16-1 Chromatogram of LC/MS/MS analysis for test fish (Level 1, carboxylic acid)
Fig. 16-2 Chromatogram of LC/MS/MS analysis for test fish (Level 1, carboxylic acid)
Fig. 16-3 Chromatogram of LC/MS/MS analysis for test fish (Level 1, carboxylic acid)
Fig. 16-4 Chromatogram of LC/MS/MS analysis for test fish (Level 1, carboxylic acid)
Fig. 16-5 Chromatogram of LC/MS/MS analysis for test fish (Level 1, carboxylic acid)
Fig. 17-1 Chromatogram of LC/MS/MS analysis for test fish (Level 2, carboxylic acid)
Fig. 17-2 Chromatogram of LC/MS/MS analysis for test fish (Level 2, carboxylic acid)
Fig. 17-3 Chromatogram of LC/MS/MS analysis for test fish (Level 2, carboxylic acid)
Fig. 17-4 Chromatogram of LC/MS/MS analysis for test fish (Level 2, carboxylic acid)
Fig. 17-5 Chromatogram of LC/MS/MS analysis for test fish (Level 2, carboxylic acid)
Fig. 18-1 Chromatogram of LC/MS/MS analysis for test fish (Control carboxylic acid)
Fig. 18-2 Chromatogram of LC/MS/MS analysis for test fish (Control, carboxylic acid)
Study No. 43771

Fig. 19  Concentration of dissolved oxygen (Level 1).

Fig. 20  Concentration of dissolved oxygen (Level 2).

Fig. 21  Concentration of dissolved oxygen (Control).

January 31, 2002

Name: [Signature]
Fig. 22-1 IR spectrum of test item measured before experimental start.

Study No.: 43771
Sample: C6-e 7142-16
Method: Neat
Date: Dec. 13, 2001
Name: L. Gasul

CONTAINS CONFIDENTIAL
Fig. 22-2 IR spectrum of test item measured after the experimental completion.

Study No.: 43771
Sample: C6-H23-O
Method: Neat
Date: Jan. 29, 2002
Name: I. Yadi

CONTAINS CONFIDENTIAL BUSINESS INFORMATION
Instrument  Shimadzu QP-5000

Sample  Test item

GC Conditions

Column  INNOWAX

Size 30 m x 0.25 mm i.D., Film thickness 0.25 μm
Temp. 35 °C (2 min) → 150 °C (2 min)
Temp. rate 20 °C/min
Sample size 1 μL (Solvent Ethyl acetate)
Inlet mode (Splitless), Injection temp. 200 °C
Column head pressure 50 kPa, Carrier gas flow (He) 1.0 mL/min
Total flow 10 mL/min, Sampling time 1.0 min

MS Conditions

Ionization Mode  EI, Detection Mode  Positive
Monitoring ion m/z 50 – 500
Interface temp. 250 °C
Ionization voltage 70 eV

Note

Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan

Fig. 23-1 MS spectrum of test item (analytical conditions)

Date 2001/11/1  Operator  

CONTAINS CONFIDENTIAL BUSINESS INFORMATION
Fig. 23-2 MS spectrum of test item (main presumed fragments)
$C_6F_{13}C_2H_4OH$

M.W. 364

<table>
<thead>
<tr>
<th>m/z</th>
<th>Fragment ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>363</td>
<td>(M - H)$^+$</td>
</tr>
<tr>
<td>344</td>
<td>(M - HF)$^{++}$</td>
</tr>
<tr>
<td>131</td>
<td>(C$_3$F$_5$)$^+$</td>
</tr>
<tr>
<td>119</td>
<td>(CF$_2$CF$_3$)$^+$</td>
</tr>
<tr>
<td>100</td>
<td>(C$_2$F$_4$)$^{++}$</td>
</tr>
<tr>
<td>95</td>
<td>(CF$_2$C$_2$H$_4$OH)$^+$</td>
</tr>
<tr>
<td>69</td>
<td>(CF$_3$)$^+$</td>
</tr>
</tbody>
</table>

Fig. 23-3 MS spectrum of test item
(main presumed fragments)

Date 23/11/1 Name [Signature]

CONTAINS CONFIDENTIAL
BUSINESS INFORMATION
Instrument: MS: Micromass Quattro Ultima
HPLC: Agilent HP-1100
Sample: Carboxylic acid

HPLC Conditions
Inlet system: Column
Sample size: 5 µL
(Solvent: Methanol/water (1/1 V/V))
Column: L-column ODS (SUS)
Column size: 15 cm x 2.1 mm ID; Column temp. 25°C
Eluent: A (40%) : Water (containing 5mmol/L di-n-butylamine acetate)
B (60%) : Methanol (containing 5mmol/L di-n-butylamine acetate)
Flow rate: 0.2 mL/min

MS Conditions
Ionization mode: ESI, Detection mode: Negative
Probe ESI: Capillary: 3.5 kV, Desolvation temp. 400 °C, Desolvation gas: 650 L/hr
Source Cone: 20 V
Source block temp.: 120 °C
MS: Ion energy: -0.2 V, Multiplier: 600 V
Monitoring ion m/z: 50 - 800

Note:

Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan

Fig. 24-1 MS spectrum of carboxylic acid (analytical conditions)

Date: 2001/01/01  Operator: [Signature]
Fig. 24-2 MS spectrum of carboxylic acid
Fig. 24-3 MS spectrum of carboxylic acid
**F(CF₂)₅COOH**

M.W. 314

<table>
<thead>
<tr>
<th>m/z</th>
<th>Molecular-related ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>756</td>
<td>(2M + (C₄H₉)₂NH - H)⁻</td>
</tr>
<tr>
<td>627</td>
<td>(2M - H)⁻</td>
</tr>
<tr>
<td>313</td>
<td>(M - H)⁻</td>
</tr>
</tbody>
</table>

Fig. 24-4  MS spectrum of carboxylic acid

Date 2011/9/1  Name D. Yoshida
Instrument: MS: Micromass Quattro Ultima
         HPLC: Agilent HP-1100
Sample: Carboxylic acid

HPLC Conditions
Inlet system: Column
Sample size: 5 μL
(Solvent: Methanol/water (1/1 V/V))
Column: L-column ODS (SUS)
Column size: 15 cm x 2.1 mm I.D., Column temp. 25 ºC
Eluent A (40%): Water (containing 5 mmol/L di-n-butylamine acetate)
         B (60%): Methanol (containing 5 mmol/L di-n-butylamine acetate)
Flow rate: 0.2 mL/min

MS Conditions
Ionization mode: ESI, Detection mode: Negative
Probe ESI: Capillary 3.5 kV, Desolvation temp. 400 ºC, Desolvation gas 650 L/hr
Source Cone: 20 V
Source block temp: 120 ºC
MS Ion energy: -0.2 V, Multiplier: 600 V
Monitoring ion m/z: 100 - 600

Note: 

Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan

Fig. 25-1 MS/MS spectrum of carboxylic acid (analytical conditions)

Date: 2011-8-11
Operator: [Signature]

CONTAINS CONFIDENTIAL BUSINESS INFORMATION
Fig. 25-2 MS/MS spectrum of carboxylic acid
Standard solution 100mg/L

01-Aug-2001
4: Daughters of 313ES-
TIC
1.30e8

Total ion chromatogram

10.12

Mass chromatogram of 269

10.23

Mass chromatogram of 313

10.12

2001. P. 1
L. Knoll

Fig. 25-3 MS/MS spectrum of carboxylic acid

CONTAINS CONFIDENTIAL BUSINESS INFORMATION
F\((CF_2)_5\)COOH

<table>
<thead>
<tr>
<th>m/z</th>
<th>Product ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>313</td>
<td>(M - H)(^-)</td>
</tr>
<tr>
<td>269</td>
<td>(M - COOH)(^-)</td>
</tr>
</tbody>
</table>

Fig. 25-4 MS/MS spectrum of carboxylic acid

Date 2001/8/1 Name Yoski
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 平石 次郎 殿

化学物質の審査及び製造等の規制に関する法律に基づく試験施設に関する基準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及び指定化学物質に係る有害性の調査の項目等を定める命令第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、確認の有効期限は、本確認書の交付日から起算して3年間とします。

平成12年11月17日

通商産業省基礎産業局長 岡本 善

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 福岡県久留米市中央町19番14号

試験項目 分解度試験、濃縮度試験、分配係数試験