Translation Statement

Institute of Ecotoxicology Co., Ltd.

Sponsor:

Title: Bioconcentration Test of in Carp, *Cyprinus carpio*

Test No.: C-186

I, the undersigned, hereby declare that this report provides a correct English translation of the Final Report (Study No. C-186, issued on March 1, 2011).

Date May 15, 2012

Study Director K. Kaneko

Kenji Kaneko

Final Report

Bioconcentration Test of in Carp, *Cyprinus carpio*

Report No. E4 -11006·C186·NS

March 1, 2011

Institute of Ecotoxicology Co., Ltd.

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This report includes 90 pages

GLP Statement

Institute of Ecotoxicology Co., Ltd.

Sponsor:

Title of the test: Bioconcentration Test of in Carp, *Cyprinus carpio*

Test No.: C－186

The aforementioned test was conducted in compliance with following GLP principles:

"Standards Concerning Test Facilities Performing Tests Concerning New Chemical Substances" on Japanese GLP (Yakushokuhatsu No. 1121003, Heisei15・11・17 Seikyoku No. 3 and Kanpokihatsu No. 031121004, Joint notification of Director General of Pharmaceutical and Food Safety Bureau in Ministry of Health, Labor and Welfare, Director General of Manufacturing Industries Bureau in Ministry of Economy, Trade and Industry and Director General of Environmental Policy Bureau in Ministry of the Environment) (dated November 21, 2003)

"OECD Principles of Good Laboratory Practice" (as revised in 1997).

March 1, 2011

Study Director: Signed in original

Kenji Kaneko

Report No. E4 -11006· C186 · NS Test No. C－186

Quality Assurance Certificate

Title:	Bioconcentration Test of	in Carp, *Cyprinus carpio*
Sponsor:		
Study Director:	Kenji Kaneko	
Study Personnel:	Kenji Kaneko	
Test Facility:	Institute of Ecotoxicology Co., Ltd.	

We certify that the present final report describes the test methods accurately and the contents were performed according to the test protocol and standard operation procedures, and the original data are accurately presented. The inspection and audit were performed as follows.

		Dates performed	Dates reported to Test Facility Management and Study Director
Status of performance of test inspected	Water/fish analysis on Day 7 from the start of exposure	February 2, 2011	February 2, 2011
	Water/fish analysis on Day 14 from the start of exposure	February 9, 2011	February 9, 2011
	Water/fish analysis on Day 21 from the start of exposure	February 16, 2011	February 16, 2011
Test protocol audited	Protocol audited Protocol (revision) audited	January 12, 2011 January 18, 2011	January 12, 2011 January 18, 2011
Final report audited	Report audited	March 1, 2011	March 1, 2011

Institute of Ecotoxicology Co., Ltd., Department of Quality Assurance Unit

Quality Assurance Director : Signed in original
Mikiyasu Goto
Signature date: March 1, 2011
(Inspections of study conduct, protocol and final report)

Quality Assurance : Signed in original
Mitsuko Takamatsu
Signature date: March 1, 2011
(Inspections of protocol and final report)

Quality Assurance : Signed in original
Miyuki Kuribara
Signature date: March 1, 2011
(Inspection of final report)

Final Report

– Preface –

1. **Title** Bioconcentration Test of in Carp, *Cyprinus carpio*

2. **Sponsor**

3. **Test facility** Institute of Ecotoxicology Co., Ltd.
   2-28-1 Yoshino-cho, Kita-ku, Saitama City, 331-0811 Japan
   Representative Mikiyasu Goto
   Test Facility Management Tadayoshi Kan

4. **Objective of the test**
   The objective of this test is to determine the degree of bioconcentration of the test substance in fish in order to file for a Chemical Substance Control Law in Japan (No. 117 of October 16, 1973). (abbreviated to 'CSCL' below)

5. **Test methods**
   The methods used in this test were based on "Test Methods Concerning New Chemical Substances < Bioconcentration Test of Chemical Substances in the Bodies of Fish and Shellfish>" (Yakushokuhatsu No.1121002, Heisei 15・11・13 Seikyoku No.2 and Kanpokihatsu No. 031121002, Joint notification of Director General of Pharmaceutical and Food Safety Bureau at the Ministry of Health, Labor and Welfare, Director General of Manufacturing Industries Bureau in Ministry of Economy, Trade and Industry and Director General of Environmental Policy Bureau in Ministry of the Environment, Japan) (dated November 21, 2003) and OECD Guidelines for the Testing of Chemicals, "No. 305 Bioconcentration: Flow-through Fish Test" (adopted on June 14, 1996).

6. GLP applied

Japanese Chemical Substance GLP: "Standards Concerning Test Facilities Performing the Tests Concerning New Chemical Substances" (Yakushokuhatsu No.1121003, Heisei 15・11・17 Seikyoku No.3 and Kanpokihatsu No. 031121004, Joint notification of Director General of Pharmaceutical and Food Safety Bureau at the Ministry of Health, Labor and Welfare, Director General of Manufacturing Industries Bureau in Ministry of Economy, Trade and Industry and Director General of Environmental Policy Bureau in Ministry of the Environment)(dated November 21, 2003)

OECD GLP: "the OECD Principles of Good Laboratory Practice" (as revised in 1997).

7. Name of the test substance

Chemical name :

Abbreviation :

CAS No. :

8. Test periods

(1) Study initiation date	:	January	12, 2011
(2) Duration of the test			
Date of arrival of fish	:	August	23, 2010
Acclimation completion date	:	January	26, 2011
Experiment starting date	:	January	26, 2011
Experiment completion date	:	February	23, 2011
(3) Study completion date	:	March	1, 2011

9. Test results

The bioconcentration test of the test substance in carp, *Cyprinus carpio* was conducted. The test substance is a salt consisting of , and dissociates completely in water. In this test, the bioconcentration of only was confirmed. The bioconcentration factors of during the 28-day exposure period are summarized in the next page as a table.

Bioconcentration factor of	High exposure level (Test substance: 0.2 mgL^{-1}) (: 0.193 mgL^{-1})	Low exposure level (Test substance: 0.02 mgL^{-1}) (: 0.0193 mgL^{-1})
Bioconcentration factors (BCF)	under a detectable limit (less than 1-fold)	under a detectable limit (less than 11-fold)

10. Evaluation, discussion of the results and conclusion

The test substance is a salt consisting of . In this test, the bioconcentration of only was confirmed. Since listed as an existing chemical substance, is not high bioconcentration, the bioconcentration of is considered to be confirmed by analogy.

The 96h LC_{50} value of the test substance in Zebrafish (*Danio rerio*) was greater than 101 mgL^{-1}. Since the solubility in water was $>1.0 \times 10^5$ mgL^{-1}, two concentrations of the test substance in water were set up to be 0.2 mgL^{-1} (0.193 mgL^{-1}) for the high exposure level (Concentration Level 1) and 0.02 mgL^{-1} (0.0193 mgL^{-1}) for the low exposure level (Concentration Level 2). According to the results of the bioconcentration test, the bioconcentration factors (BCFs) in both Concentration Levels 1 and 2 were less than 50-fold. Thus, a steady state was assumed to be achieved within 28 days of exposure. It was confirmed that the bioconcentration was under a detectable limit and the bioconcentration factors were less than 1-fold in Concentration Level 1 and less than 11-fold in Concentration Level 2. Therefore, bioconcentration of anion of the test substance was confirmed to be low.

11. Storage of the test source materials

The following records and samples concerning the tests will be stored for 10 years after receiving notification based on the provisions of Chemical Substances Control Law (CSCL) (No. 117, 1973): Article 4, Paragraphs 1 or 2, Article 4-2, Paragraphs 2, 3 or 8, Article 5-4, Paragraph 2, Article 24, Paragraph 2 or Article 25-3, Paragraph 2, and the test substance, reference substance and other items will be stored for 10 years after receiving notification based on the provisions of the CSCL: Article 4, Paragraphs 1 or 2, Article 4-2, Paragraphs 2, 3 or 8, Article 5-4, Paragraph 2, Article 24, Paragraph 2 or

Article 25-3, Paragraph 2 or the period wherein they can be stored stably without causing any interference with quality, which is at least one of the periods that is shorter, at the Institute of Ecotoxicology Co., Ltd.. Regarding the storage after these periods, it will be determined separately after discussion.

(1) Protocols, records of modifications and changes

(2) Records concerning the test substance, etc.

(3) Experimental notes, raw data, charts

(4) Test substance, reference substance, other items (stored at about 20°C in a dark place)

(5) Inspection and audit records recorded by the personnel in charge of quality assurance regarding the present test

(6) Final report

(7) Other items that are required

12. Environmental factors assumed to have an effect on reliability of the data

No particular abnormalities were found.

13. Personnel related to the test and their assigned positions

Study Director: Kenji Kaneko

Study Personnel: Kenji Kaneko

- 96h LC_{50} test
- Establishment of analytical methods for test water and fish
- Preparation of calibration curve for analysis of test water and fish
- Recovery tests of test water and fish
- Preliminary test to arrange the study period and the sampling schedule
- Analyses of test water and fish during the experimental period
- Preparation and maintenance of test solution during the experimental period
- Measurement of lipid contents in fish body
- Measurement of IR
- Observation of the conditions of fish
- Measurement of water environment

Final report prepared by Kenji Kaneko

14. Name and address of scientists having contributed reports to the final report

Not applicable

15. Approval of the final report

Study Director: Signed in original

Kenji Kaneko

Date signed: March 1, 2011

Main Subject

1. Test substance

In the present report, the test substance has the following name and structural formula (based on the material provided by the sponsor).

1.1 Name

Chemical name:

Abbreviation:

CAS No.:

1.2 Structural formula

The abbreviation words were used in this test as shown below.

Molecular formula:

1.3 Supplier and Lot No.

(1) Supplier

(2) Lot No.

1.4 Identification

It was identified by the agreement of the IR spectrum provided by the sponsor with the IR spectrum measured at our institution at the time of the start of the test. Further, the stability of the test substance during the test period was confirmed by the agreement of the IR spectrum at the end of the test with the IR spectrum measured at the start of the test (see, Ref. 12).

1.5 Physico-chemical properties (*based on the data provided by the sponsor)

Appearance*:

Purity*:

Solubility (25°C) measured at Institute of Ecotoxicology:

water	> 1.0×10^5	mgL^{-1}
dimethyl sulfoxide	> 1.0×10^5	mgL^{-1}
acetone	> 1.0×10^5	mgL^{-1}
acetonitrile	> 1.0×10^5	mgL^{-1}
methanol	> 1.0×10^5	mgL^{-1}
dichloromethane	5.1×10^1	mgL^{-1}
n-hexane	6.0×10^{-2}	mgL^{-1}

2. Test methods and analytical methods

The methods used in this test were based on the Japanese Guideline, "Test Methods Concerning New Chemical Substances < Bioconcentration Test of Chemical Substances in the Bodies of Fish and Shellfish>" (Yakushokuhatsu No. 1121002, Heisei 15・11・13 Seikyoku No. 2 and Kanpokihatsu No. 031121002) (dated November 21, 2003)

and

the OECD Guidelines for Testing of Chemicals, "No. 305 Bioconcentration: Flow-through Fish Test" (adopted on June 14, 1996)

2.1 Acute toxicity test (96h LC_{50}) in Zebrafish (*Danio rerio*) for selecting the substance concentration (SOP 2.6)

Based on the results of a preliminary test, the concentrations of the test substance in test water of a 96h LC_{50} test were selected. The 96h LC_{50} value was determined from the test result (the test was performed with reference to the method described in OECD Test Guidelines 203).

(1) Test fish	Zebrafish (*Danio rerio*)	Lot No. 101202
	Supplier	Kamihata Fish Ind., Ltd. 292-1 Chigusa-cho, Hanamigawa-ku, Chiba City, 262-0012 Japan
	Date received	December 2, 2010
	Mean total length	3.2 ± 0.1 cm
	Mean bodyweight	0.24 ± 0.03 g
	Acclimation period	December 2, 2010 — December 20, 2010

Receiving and acclimatization procedures were based on SOP 2.1 and SOP 2.5.

(2) Test solution	Test water was prepared by dissolving the test substance in water for test.
(3) Test temperature	24.9 ± 0.2°C
(4) Test aquarium	Made of glass, amount of test water; 4 L
(5) Exposure method	Semi-static method (replacing test water after 48 hours)
(6) Water for test	Dechlorinated city water of Saitama Analysis of water quality: it was confirmed that water quality was adopted to 'the standard of quality of fisheries water' (see, Ref.10).
(7) Number of fish	10 fish/4 L test aquarium
(8) Experimental period	December 20, 2010 — December 24, 2010
(9) Sensitivity test of *Danio rerio*	A sensitivity test was conducted using $CuSO_4 \cdot 5H_2O$ as a standard substance. The fish in the same lot which passed in the sensitivity test (96h LC_{50} value; 0.08 to 0.3 mgL^{-1}) were used as 96h LC_{50} test fish.
(10) Test results	Since the preliminary 96h LC_{50} value of the test substance in Zebrafish (*Danio rerio*) was greater than 100 mgL^{-1}, the test was done by "a limited test" in which the concentration of the test substance was 100 mgL^{-1}. As a result, it was confirmed that the 96h LC_{50} value was greater than 101 mgL^{-1}. The concentration was an initial experimental value calculated from LC/MS analysis (see, the following table).

Test water		Mortality (%)			
Compound	Concentration (mgL^{-1})	24 hours	48 hours	72 hours	96 hours
Copper (II) sulfate pentahydrate (sensitivity test)	0.5	0	20	70	100
	0.05	0	0	0	0
Test substance	101	0	0	0	0
Control (water for test)	–	0	0	0	0

2.2 Performing a bioconcentration test

(1) Test fish

To confirm the bioconcentration in fish, the carp (*Cyprinus carpio*), representative fish species in Japan, was used (SOP 2.1.2) as the test fish for the bioconcentration test.

Common carp	*Cyprinus carpio* (Lot No. 100823)
Supplier:	Kitamura Carp Breeding Farm (12-388 Gunchiku, Yatsushiro-shi, Kumamoto Prefecture, Japan)

Date purchased: August 23, 2010

Feeding and acclimatization:

After receiving the fish at the Institute of Ecotoxicology Co., Ltd., those with a total length of approx. 8.0 ± 4.0 cm were selected (SOP 2.4). After the fish were treated with Elevage® (4-[2-(5-nitro-2-furanyl)ethenyl]benzoic acid, sodium salt) and rock salt, the fish were raised. Fish were selected again on January 5, 2011 and 150 fish were transferred to an acclimation water-tank. After they were treated again with Elevage®, the process of acclimatization was started (SOP 2.3, SOP 2.5). Finally, the fish were acclimatized at water temperature 25 ± 2.0°C for 7 days and it was confirmed that the mortality was 5% or less before use in the test.

Mean body weight	3.36 ± 0.21 g	(SOP 2.8)
Mean total length	6.9 ± 0.2 cm	(SOP 2.8)
Mortality of fish during the experiment		(SOP 2.7)
	High exposure level 0%	
	Low exposure level 0%	
	Control 0%	
Lipid contents (by chloroform/methanol extraction method)		
Before experiment	5.0% (mean value, n=2)	(SOP 2.9)
After experiment	4.8% (mean value, n=2)	(SOP 2.9)

(The conditions of the test fish were shown in Ref. 11)

(2) Feeding

Kind: Mixed feed for carp in pellet form (Swimmy)

Crude proteins	37.0%
Crude lipids	2.5%

Manufacturer: Nippon Pet Food Company

Feeding method: Fish were fed daily in an amount of approximately 1.5% of the bodyweight of the fish. However, no feed was given on the day before sampling of the test fish.

(3) Performing the test

Water for test: Same as in Section 2.1 (6)

Preparation of a stock solution:

A test substance was added to water for test to prepare 10^5 mgL^{-1} stock solution. The stock solution was diluted with water for test to obtain test water.

High exposure level:

The stock solution was diluted with water for test to obtain a solution of the test substance (20 mgL^{-1}). This was further diluted with water for test and continuously flowed through the test aquarium.

Low exposure level:

The stock solution was diluted with water for test to obtain a solution of the test substance (2 mgL^{-1}). This was further diluted with water for test and continuously flowed through the test aquarium.

Control: Water for test was continuously flowed through the test aquarium.

Bioconcentration test equipment:

see, Ref. 1

Test aquarium: "Flow-through system" (made of glass)

Capacity 100 L (amount of test water; 50 L)

Flow rate 300 mL min^{-1} (432 L day^{-1})

No. 9 aquarium for high exposure level, No. 8 aquarium for low exposure level and No. 7 aquarium for the control were used.

(4) Test concentrations

The 96h LC_{50} of in *Danio rerio* was greater than 101 mgL^{-1} and the water solubility of was $>1.0 \times 10^5$ mgL^{-1}. The setup concentrations for the bioconcentration test were determined to be less than the solubility in water. Based on the 96h LC_{50} value, 1/505 and 1/5050 were used as safety factors and the test concentrations were set as follows.

	High exposure level	Low exposure level	Control
Concentration of the test substance (concentration of	0.200 mgL^{-1} (0.193 mgL^{-1})	0.0200 mgL^{-1} (0.0193 mgL^{-1})	– –

(5) Test conditions

Temperature of test water: 25.3 ± 0.2°C

pH of test water: 7.5 ± 0.1

DO of test water: 7.4 ± 0.1 mgL^{-1}

(Temperature, pH and DO of test water were measured on each analysis day, see, Ref. 2-2)

Measurement of total organic carbon concentrations in the test water (SOP 2.12):

Since solvents or dispersant were not used in this test, the total organic carbon concentrations in the test water was not measured.

Light type:	UV- cut fluorescent lamp
Photoperiod:	About 10 hours
Experimental period:	January 26, 2011 — February 23, 2011

The analysis schedule is presented in Section 2.8.

Number of test fish:	High exposure level	50 fish
	Low exposure level	50 fish
	Control	15 fish

Number of fish analyzed and number repeated:

2 fish were paired for a single analysis (n=2).
2 fish were paired for analysis of the control group (n=1).

2.3 Analyses of fish and test water

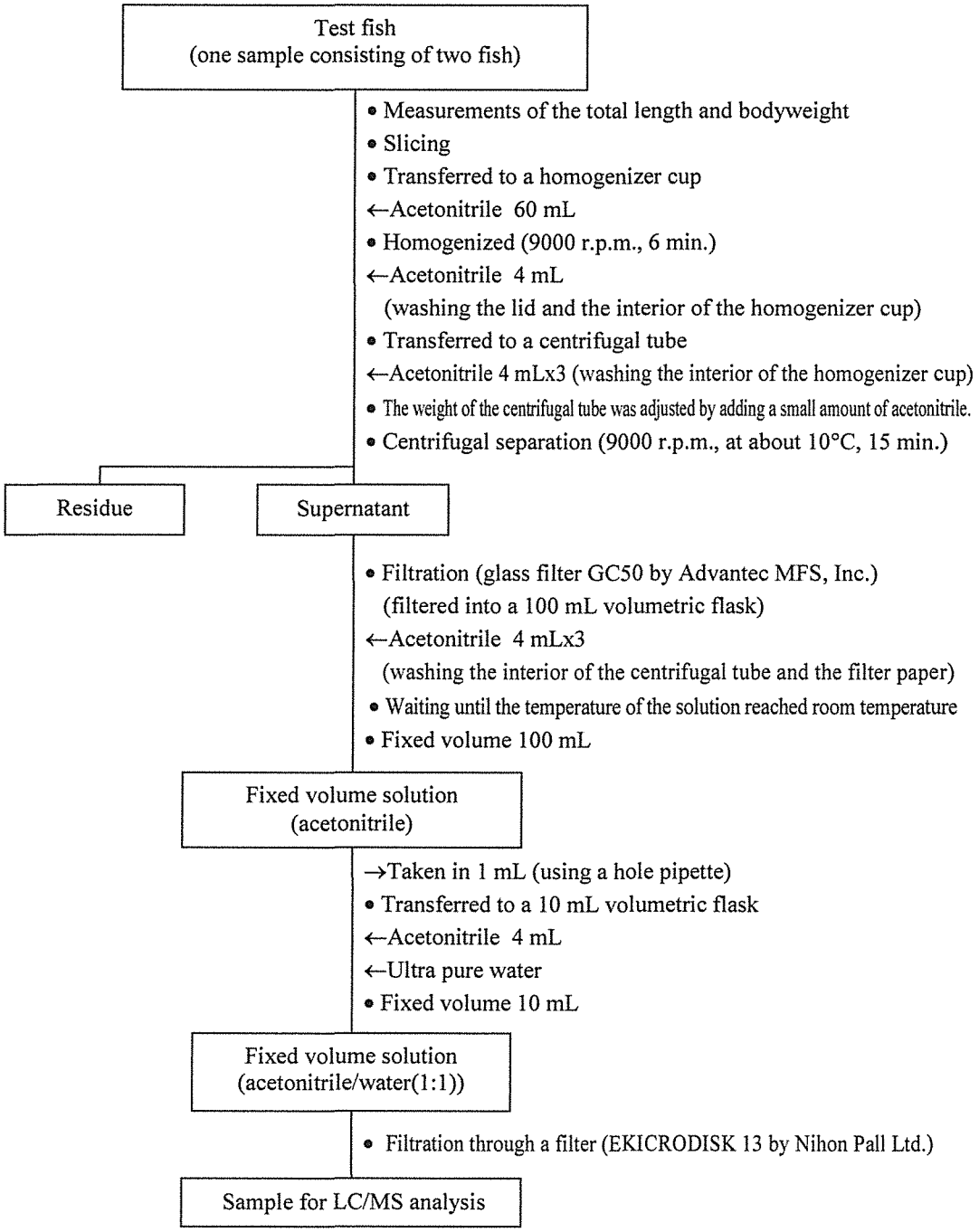
2.3.1 Analytical method for fish (SOP 2.11)

Pretreatment

Each six fish in the Concentration Level 1 and Level 2 and two fish in the control group were sampled using a landing net on the analysis day. The fish were temporarily paralyzed by placing them in an ice water. The body surface was washed with tap water and wiped lightly by gauze. Then, the body weight was measured in a unit of 0.01 g using a balance (SOP/INS/4.2.2), and the body length from the mouth to the tail fin was measured in a unit of 0.1 cm. In the test group, two fish were used as one sample and analyzed in duplicate, and other sample was put into a plastic bag and stored in a freezer after the fish were wrapped with aluminum-foil and packed in a plastic bag (SOP 2.15).

The fish were put into a homogenizer cup after slicing, and acetonitrile (60 mL) was added to the homogenizer cup. The mixture was homogenized at 9000 r.p.m. for 6 min. (SOP/INS/4.17.2). The lid and the interior of the homogenizer cup were rinsed with acetonitrile (4 mL) and the washings were transferred to a centrifugal tube. The interior of the said homogenizer cup was rinsed three times with 4 mL of acetonitrile and the washings were also transferred to the said centrifugal tube. The weight of the centrifugal tube was adjusted by adding a small amount of acetonitrile. After centrifugal separation for 15 min. (SOP/INS/4.16.3) (9000 r.p.m., at about 10°C), the supernatant was filtered into a 100 mL volumetric flask (a glass filter GC50 by Advantec MFS, Inc., Tokyo, Japan). The interior of the said centrifugal tube and the filter paper were rinsed three times with 4 mL of acetonitrile and the washings were also filtered into the said volumetric flask. After waiting until the temperature of the solution reached room temperature, the volume was adjusted to 100 mL by adding acetonitrile. A portion of the constant volume solution was taken with a hole pipette (1 mL) and was transferred to a 10 mL volumetric flask. After adding 4 mL of acetonitrile, the volume was adjusted to 10 mL by adding ultra pure water. The constant volume solution was filtered through a filter (EKICRODISK 13 by Nihon Pall Ltd., Tokyo, Japan). The filtrate was analyzed by liquid chromatography mass spectrometer (LC/MS).

The operations of the pretreatment are shown in the next page as a flow chart.

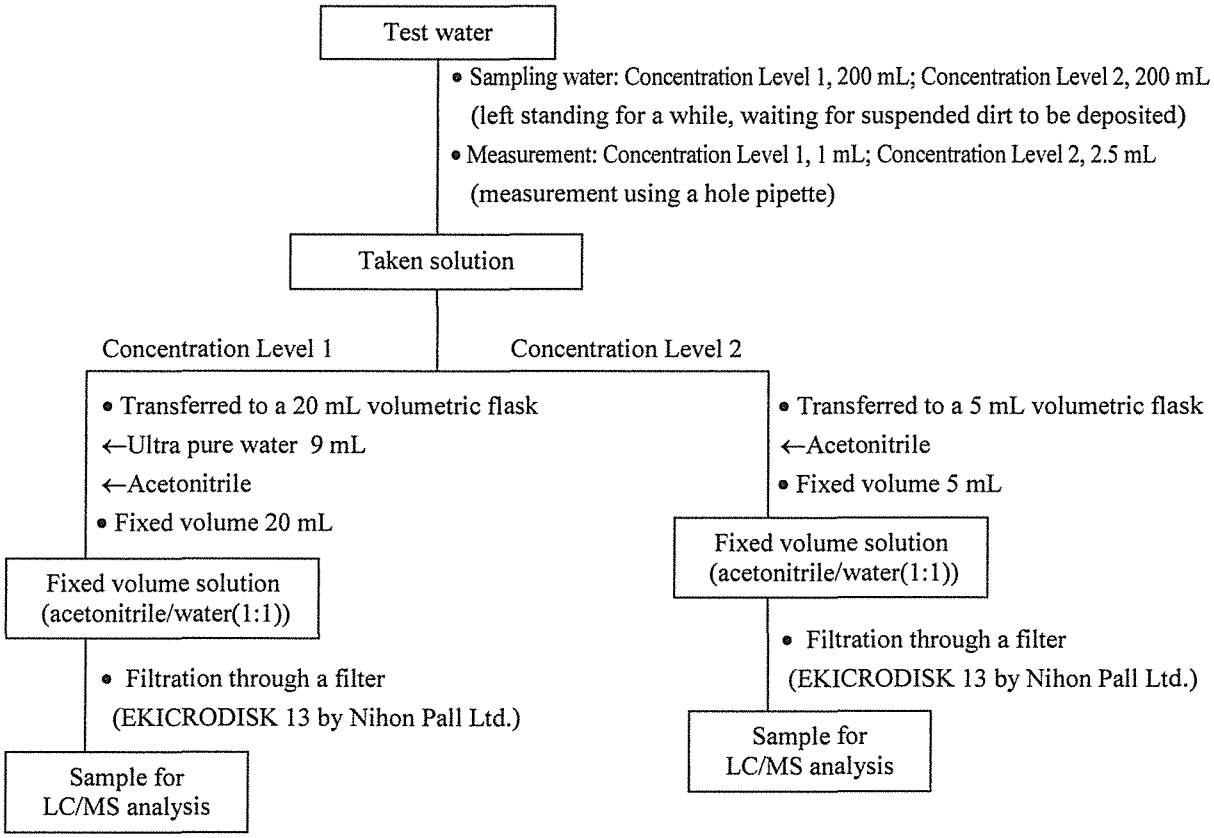

Test fish
(one sample consisting of two fish)
• Measurements of the total length and bodyweight
• Slicing
• Transferred to a homogenizer cup
←Acetonitrile 60 mL
• Homogenized (9000 r.p.m., 6 min.)
←Acetonitrile 4 mL
(washing the lid and the interior of the homogenizer cup)
• Transferred to a centrifugal tube
←Acetonitrile 4 mLx3 (washing the interior of the homogenizer cup)
• The weight of the centrifugal tube was adjusted by adding a small amount of acetonitrile.
• Centrifugal separation (9000 r.p.m., at about 10°C, 15 min.)
Residue
Supernatant
• Filtration (glass filter GC50 by Advantec MFS, Inc.)
(filtered into a 100 mL volumetric flask)
←Acetonitrile 4 mLx3
(washing the interior of the centrifugal tube and the filter paper)
• Waiting until the temperature of the solution reached room temperature
• Fixed volume 100 mL
Fixed volume solution
(acetonitrile)
→Taken in 1 mL (using a hole pipette)
• Transferred to a 10 mL volumetric flask
←Acetonitrile 4 mL
←Ultra pure water
• Fixed volume 10 mL
Fixed volume solution
(acetonitrile/water(1:1))
• Filtration through a filter (EKICRODISK 13 by Nihon Pall Ltd.)
Sample for LC/MS analysis

2.3.2 Analytical method for test water (SOP 2.10)

Pretreatment

Test water was sampled (approx. 200 mL from the Concentration Level 1 and approx. 200 mL from the Concentration Level 2) from the central area of each aquarium with an Erlenmeyer flask. After leaving it standing, the supernatant (1 mL in the Concentration Level 1 and 2.5 mL in the Concentration Level 2) was taken using a hole pipette and transferred to a volumetric flask (20 mL in Concentration Level 1 and 5 mL in Concentration Level 2). In Concentration Level 1, ultra pure water (9 mL) was added to the volumetric flask. The volume was adjusted with acetonitrile to 20 mL in Concentration Level 1 and 5 mL in Concentration Level 2. The constant volume solution was filtered through a filter (EKICRODISK 13 by Nihon Pall Ltd., Tokyo, Japan). The filtrate was analyzed by liquid chromatography mass spectrometer (LC/MS).

The operations of the pretreatment are shown below as a flow chart.



The operation of water analysis in the control group (when starting the test) was based on the method described in Concentration Level 2.

2.3.3 Measurement methods

<Analytical machine>

Liquid chromatography mass spectrometer (SOP/INS/4.33)

<Analytical apparatuses and conditions>

< Liquid chromatography (LC)>

Analytical apparatus	High performance liquid chromatography LC-20AD (Shimadzu Co., Ltd.)
Column	Hypersil GOLD 2.1 mm i.d. × 50 mm (5 μm) (Lot No. 9966) Thermo Scientific Inc.
Mobile phase	A: 20 mM ammonium formate/formic acid (1000:1), B: acetonitrile, A/B(1:1)
Flow rate	0.2 mL/min
Temperature	40°C
Injection volume	5 μL

< Mass spectrometer (MS)>

Analytical apparatus	TSQ Quantum Access MAX triple stage quadrupole mass spectrometer (Thermo Fisher Scientific Inc.)
Ionization method	Negative ESI
Vaporizer temperature	450°C
Spray voltage	2500 V
Sheath gas	50
AUX gas	15
Ion sweep gas	0
Capillary temperature	220°C
Scan type	Selected ion monitoring (SIM) m/z 180 $[\mathbf{M}\text{-Li}]^-$

<Quantitative calculation>

Instrument	Xcalibur by Thermo Fisher Scientific Inc.
Calculation method	Two-point calibration method (absolute calibration method)

The calibration using two standard solutions and the quantification of sample were performed on the analysis day.

2.4 Calibration curve by liquid chromatography mass spectrometry

2.4.1 Preparation of standard solutions

The test substances (5.03 mg and 3.15 mg) were weighed using Mettler Toledo XS105DU (SOP/INS/4.1.4) into 200 mL and 250 mL volumetric flasks and brought to the mark with acetonitrile/water(1:1), respectively. The concentrations of the solutions were 25.0242 mgL^{-1} and 12.5370 mgL^{-1} when considering the purity. The concentrations of the solutions were 24.0959 mgL^{-1} and 12.0719 mgL^{-1} when

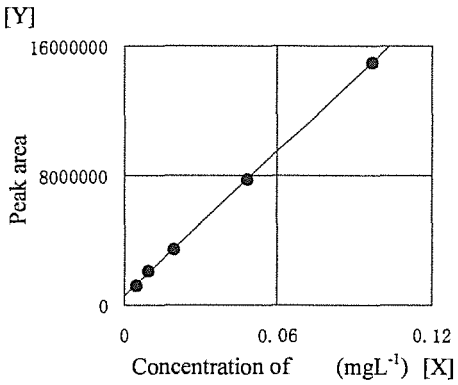
converting to the concentration of . The solutions were diluted with acetonitrile/water(1:1) to prepare standard solutions for calibration curve.

2.4.2 Preparation of calibration curve

The calibration curve was prepared using the standard solutions prepared in Section 2.4.1 and the linearity was checked.

Solution No.	Test substance concentration (mgL^{-1}) [X]	Anion concentration (mgL^{-1}) [X]	Peak area [Y]
1	0.0050	0.0048	1246726
2	0.0100	0.0096	2149557
3	0.0200	0.0193	3499884
4	0.0501	0.0483	7806108
5	0.1001	0.0964	15009834

Calibration curve Y = 149267349.5345 X +616562.7686
(by the least square method)
r = 1.000



■ The chromatograms used when preparing the calibration curve are shown in Ref. 6.

2.5 Recovery test and blank test for

2.5.1 Recovery test from fish for (SOP 2.11)

Fish bodies (two fish paired as one sample) were crashed with a blender after mincing, added an acetonitrile solution (1 ml) of the test substance , an analytical sample was prepared according to Section "2.3.1 Analytical method for fish" and was analyzed by liquid chromatography mass spectrometer. The recovery rates of were found to be as follows.

The amount of an addition of the test substance (the amount converted to : 21.9 μg) corresponded to 3.40 μgg^{-1} relative to the fish 3.22 g (6.44 g/2 fish) and the bioconcentration factor in Concentration Level 2 (the concentration converted to : 0.0193 mgL^{-1}) was approximately 176-fold.

Added group

Sample No.	Fish weight (g)	Fixed volume (mL)	Dilution factor	Quantity of addition		Recovery measured areas	Recovery rate (%)
				Measured areas	Calculated concentrated (mgL^{-1})		
1	3.12 3.21	100	10	4025794	0.0219	3951983	98.2
2	3.14 3.15	100	10	4025794	0.0219	3953287	98.2
3	3.38 3.22	100	10	4025794	0.0219	3849419	95.6

Mean recovery rate: 97.3 ± 1.2%

<Background of the fish>

After measuring bodyweight and total length of fish bodies (two fish were paired as one sample), the fish bodies were crashed with a blender after mincing. After adding 1 mL of acetonitrile, an analytical sample was prepared according to Section "2.3.1 Analytical method for fish" and was analyzed by liquid chromatography mass spectrometer. No background peaks were detected at the position of the peak of .

Control group

Sample No.	Fish bodyweight (g)	Fixed volume (mL)	Dilution factor	Measured areas
1	3.21 3.29	100	10	N.D.
2	3.25 3.22	100	10	N.D.
3	3.09 3.41	100	10	N.D.

■ The chromatograms used when calculating the recovery rates of from the fish bodies are shown in Ref. 7-1.

2.5.2 Recovery test from test water for (SOP 2.10)

Water used for raising fish was sampled and a solution containing the test substance was added. According to Section "2.3.2 Analytical method for test water", an analytical sample was prepared and analyzed by LC/MS. As shown in the following table, the mean recovery rates of were 96.2 ± 1.1% in Concentration Level 1 and 95.5 ± 1.2% in Concentration Level 2.

Recovery rates in Concentration Level 1 (Added group)

Sample No.	Amount of water measured (mL)	Fixed volume (mL)	Quantity of addition		Recovery measured areas	Recovery rate (%)
			Measured areas	Calculated concentrated (mgL^{-1})		
1	1	20	2377235	0.0114	2270545	95.5
2	1	20	2377235	0.0114	2266589	95.3
3	1	20	2377235	0.0114	2321802	97.7

Mean recovery rate: 96.2 ± 1.1%

Recovery rates in Concentration Level 2 (Added group)

Sample No.	Amount of water measured (mL)	Fixed volume (mL)	Quantity of addition		Recovery measured areas	Recovery rate (%)
			Measured areas	Calculated concentrated (mgL^{-1})		
1	2.5	5	2413659	0.0118	2334903	96.7
2	2.5	5	2413659	0.0118	2315855	95.9
3	2.5	5	2413659	0.0118	2264899	93.8

Mean recovery rate: 95.5 ± 1.2%

<Background of the test water>

Water used for raising fish was sampled and ultra pure water was added. According to Section "2.3.2 Analytical method for test water", an analytical sample was prepared and analyzed by LC/MS. No background peaks were detected at the position of the peak of either in Concentration Level 1 or in Concentration Level 2.

Control group

Concentration Level 1				Concentration Level 2			
Sample No.	Amount of water measured (mL)	Fixed volume (mL)	Measured areas	Sample No.	Amount of water measured (mL)	Fixed volume (mL)	Measured areas
1	1	20	N.D.	1	2.5	5	N.D.
2	1	20	N.D.	2	2.5	5	N.D.
3	1	20	N.D.	3	2.5	5	N.D.

■ The chromatograms used when calculating the recovery rates of from water are shown in Ref. 7-2.

2.6 Quantifiable limit

2.6.1 Quantifiable limit for fish

In the LC/MS chromatograms, there were no background peaks found due to fish body at the position of . If the peak area of 800000 counts is set as a detectable limit, the quantifiable bioconcentration factor is shown in the following table.

Setup concentration in each concentration level (concentration of)	Detectable concentration[1] (mgL^{-1})	Quantifiable limit[2] (μgg^{-1})	Bioconcentration factor[3]	
			Concentration Level 1 (0.193 mgL^{-1})	Concentration Level 2 (0.0193 mgL^{-1})
Concentraion Level 1 0.193 mgL^{-1}	0.0012	0.192	1-fold or greater	10-fold or greater
Concentraion Level 2 0.0193 mgL^{-1}				

(Note 1) The detectable concentration was calculated from the calibration curve in Section 2.4.2.

(Note 2) The quantifiable limit in the fish bodies was calculated by the calculation formula shown in the next page.

The quantifiable limit was calculated as the fish bodyweight; 6.44 g (3.22 g per fish, 2 fish were paired as one sample), recovery rate; 97.3%, fixed volume; 100 ml and dilution factor; 10.

$$\text{Quantifiable limit } (\mu gg^{-1}) = \frac{\text{Detectable concentration } (mgL^{-1}) \times \text{Fixed volume (mL)} \times \text{Dilution factor}}{\text{Recovery rate (\%)} \times \text{Fish bodyweight (g)}} \times 100$$

(Note 3)

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Quantifiable limit } (\mu gg^{-1})}{\text{Setup concentration in the test group (theoretical value) } (mgL^{-1})}$$

2.6.2 Quantifiable limit for test water

In the LC/MS chromatograms, there were no background peaks observed due to test water at the position of the peak of . If the peak area of 800000 counts is set as a detectable limit, the quantifiable limit for test water is shown in the following table.

Setup concentration in each concentration level (concentration of		Detectable concentration[1] (mgL^{-1})	Quantifiable limit[2] (mgL^{-1})	
Concentration Level 1	Concentration Level 2		Concentration Level 1 (0.193 mgL^{-1})	Concentration Level 2 (0.0193 mgL^{-1})
0.193 mgL^{-1}	0.0193 mgL^{-1}	0.0012	0.0249	0.00251

(Note 1) The detectable concentration was calculated from the calibration curve in Section 2.4.2.

(Note 2) The quantifiable limit for test water was calculated by the following calculation formula. The quantifiable limit was calculated as the fixed volume; 20 mL in Level 1 and 5 mL in Level 2, recovery rate; 96.2% in Level 1 and 95.5% in Level 2 and amount of water measured; 1 mL in Level 1 and 2.5 mL in Level 2.

$$\text{Quantifiable limit } (mgL^{-1}) = \frac{\text{Detectable concentration } (mgL^{-1}) \times \text{Fixed volume (mL)}}{\text{Recovery rate (\%)} \times \text{Amount of water measured (mL)}} \times 100$$

2.7 Preliminary test

Test fish (*Cyprinus carpio*) were exposed to test water (the concentration converted to 0.0193 mgL^{-1}) in Concentration Level 2 (the flow-through system). On the 7th day of exposure, the test fish (2 fish as a pair) and the test water were sampled and analyzed according to Sections "2.3.1 Analytical method for fish" and "2.3.2 Analytical method for test water". As a result, the BCF of was found to be under a detectable limit and less than 8-fold.

2.8 Schedule of analyses of fish and test water

Analyses of test water and fish were conducted according to the following schedule:

	Sampling date	Number of days after exposure	Test group	Control group
Exposure period (28 days)	January 26, 2011	Day 0	Analysis of water	Analysis of water Analysis of fish Lipid contents
	February 2, 2011	7th day	Analysis of water Analysis of fish	–
	February 9, 2011	14th day	Analysis of water Analysis of fish	–
	February 16, 2011	21st day	Analysis of water Analysis of fish	–
	February 18, 2011	23rd day	Analysis of water Analysis of fish	–
	February 23, 2011	28th day	Analysis of water Analysis of fish	Analysis of fish Lipid contents

On the day of analysis of water, pH, DO (adjusted to be approximately 7 mgL^{-1}) and water temperature were measured for each aquarium and the data were recorded (pH meter SOP/INS/4.3.3, DO meter SOP/INS/4.4.4).

In compliance with SOP 2.10 (measurement of concentrations of the test substance in the test water), analysis of test water was conducted according to Section "2.3.2 Analytical method for test water". The changes in the concentration of the test substance in water were confirmed to be ±20% or less relative to the mean of the measured values during the exposure period.

In compliance with SOP 2.11 (measurement of concentrations of the test substance in the fish), analysis of fish was conducted according to Section "2.3.1 Analytical method for fish". In compliance with SOP 2.9 (measurement of lipid contents in the fish), lipid contents were measured according to the "chloroform/methanol extraction method". The changes in the lipid contents from the beginning of the experiment to the end of the experiment were confirmed to be ± 25% or less.

Storage before analysis of fish and treatment of waste during analysis of fish were performed in compliance with SOP 2.15 (storage before analysis of fish) and SOP 2.16 (discharging waste during analysis of fish).

3. Test results

3.1 Concentrations of in test water

The concentrations of in the test water (mgL^{-1}) are shown in the following table with respect to the concentration levels.

Number of days after exposure / Test group	Day 0	7th day	14th day	21st day	23rd day	28th day
Concentration Level 1	0.177	0.181	0.177	0.181	0.187	0.185
Concentration Level 2	0.0186	0.0188	0.0184	0.0184	0.0193	0.0188

Unit: mgL^{-1}

- Analytical data during the analysis of test water are shown in Ref.5.

Based on the results shown above, the mean concentration during the test period and the standard deviation were as follows.

Concentration Level 1: 0.181 ± 0.00373 mgL^{-1}
Concentration Level 2: 0.0187 ± 0.000308 mgL^{-1}

The following calculation formula was used.

Mean

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} = \frac{\sum x}{n}$$

Standard deviation

$$\sigma_n = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n}} = \sqrt{\frac{\sum x^2 - (\sum x)^2 / n}{n}}$$

- Changes over time in the concentrations of the test substance in water, water temperature, pH and DO are shown in Ref. 2. The chromatograms at the time of analysis on Day 0, 7, 14, 21, 23 and 28 after exposure are shown in Ref. 9-1. Further, the chromatograms at the time of analysis of water in the control group are shown in Ref. 9-2.

3.2 Bioconcentration factors (BCFs)

According to the results of the bioconcentration test, the bioconcentration factors (BCFs) of were under a detectable limit in both Concentration Levels (less than 1-fold in Concentration Level 1 and less than 11-fold in Concentration Level 2). Since it was confirmed that the steady-state was achieved within 28 days in both Concentration Levels, exposure was finished. The bioconcentration factors (BCFs) of are shown below with reference to the day of analysis.

Number of days after exposure (Days)	Concentration Level 1		Concentration Level 2	
	Sample No. (n=2)	Bioconcentration factor (BCF)	Sample No. (n=2)	Bioconcentration factor (BCF)
7	No. 1 No. 2	< 1 < 1	No. 1 No. 2	< 10 < 10
14	No. 1 No. 2	< 1 < 1	No. 1 No. 2	< 11 < 10
21	No. 1 No. 2	< 1 < 1	No. 1 No. 2	< 10 < 10
23	No. 1 No. 2	< 1 < 1	No. 1 No. 2	< 10 < 10
28	No. 1 No. 2	< 1 < 1	No. 1 No. 2	< 10 < 10

(Note 1) The sample numbers in Concentration Levels 1 and 2 are symbols given to the fish taken from each concentration group when the amount of accumulation was measured.

(Note 2) The fish bodyweight during the analysis and other analytical data are shown in Ref. 4, and the chromatograms obtained at the time of analysis on the 7th, 14th, 21st, 23rd and 28th day are shown in Ref. 8-1. The chromatograms obtained at the time of analysis of fish in the control group are shown in Ref. 8-2.

(Note 3) The time-dependent changes in the bioconcentration factors in fish (BCF) are shown in Ref. 3.

(1) Mean of bioconcentration factors (BCFs) and standard deviation

The mean of the bioconcentration factors (BCFs) of during the experiment period and the standard deviation are shown below.

Concentration Level 1 BCF = The mean of the BCFs was not calculated because all the BCFs were less than the detectable limit.

Concentration Level 2 BCF = The mean of the BCFs was not calculated because all the BCFs were less than the detectable limit.

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Concentration of in fish } (\mu g g^{-1})}{\text{Concentration of in the aquarium } (mg L^{-1})}$$

Mean

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} = \frac{\sum x}{n}$$

Standard deviation

$$\sigma_n = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n}} = \sqrt{\frac{\sum x^2 - (\sum x)^2 / n}{n}}$$

(2) Confirmation of a steady-state

Since the bioconcentration factors (BCFs) of in both Concentration Levels were less than 50-fold during the 28-day exposure period, the steady state was assumed to be achieved on the 28th day of exposure and exposure was completed on the 28th day.

< Reference items>

Test fish in the control group were analyzed before exposure Day 0 (n=1) and at the end of exposure Day 28 (n=1). No background peaks due to fish were detected in the LC/MS chromatograms at the position of the peak of as in the control group in the added recovery test. These chromatograms are shown in Ref. 8-2.

3.3 Evaluation, discussion of the results and conclusion

The test substance is a salt consisting of . In this test, the bioconcentration of only was confirmed. Since lithium bromide [1-110], listed as an existing chemical substance, is not high bioconcentration, the bioconcentration of Li is considered to be confirmed by analogy.

The 96h LC_{50} value of the test substance in Zebrafish (*Danio rerio*) was greater than 101 mgL^{-1}. Since the solubility in water was >1.0 $\times 10^5$ mgL^{-1}, two concentrations of the test substance in water were set up to be 0.2 mgL^{-1} (: 0.193 mgL^{-1}) for the high exposure level (Concentration Level 1) and 0.02 mgL^{-1} (: 0.0193 mgL^{-1}) for the low exposure level (Concentration Level 2). According to the results of the bioconcentration test, the bioconcentration factors (BCFs) in both Concentration Levels 1 and 2 were less than 50-fold. Thus, a steady state was assumed to be achieved within 28 days of exposure. It was confirmed that the bioconcentration was under a detectable limit and the bioconcentration factors were less than 1-fold in Concentration Level 1 and less than 11-fold in Concentration Level 2. Therefore, bioconcentration of of the test substance was confirmed to be low.

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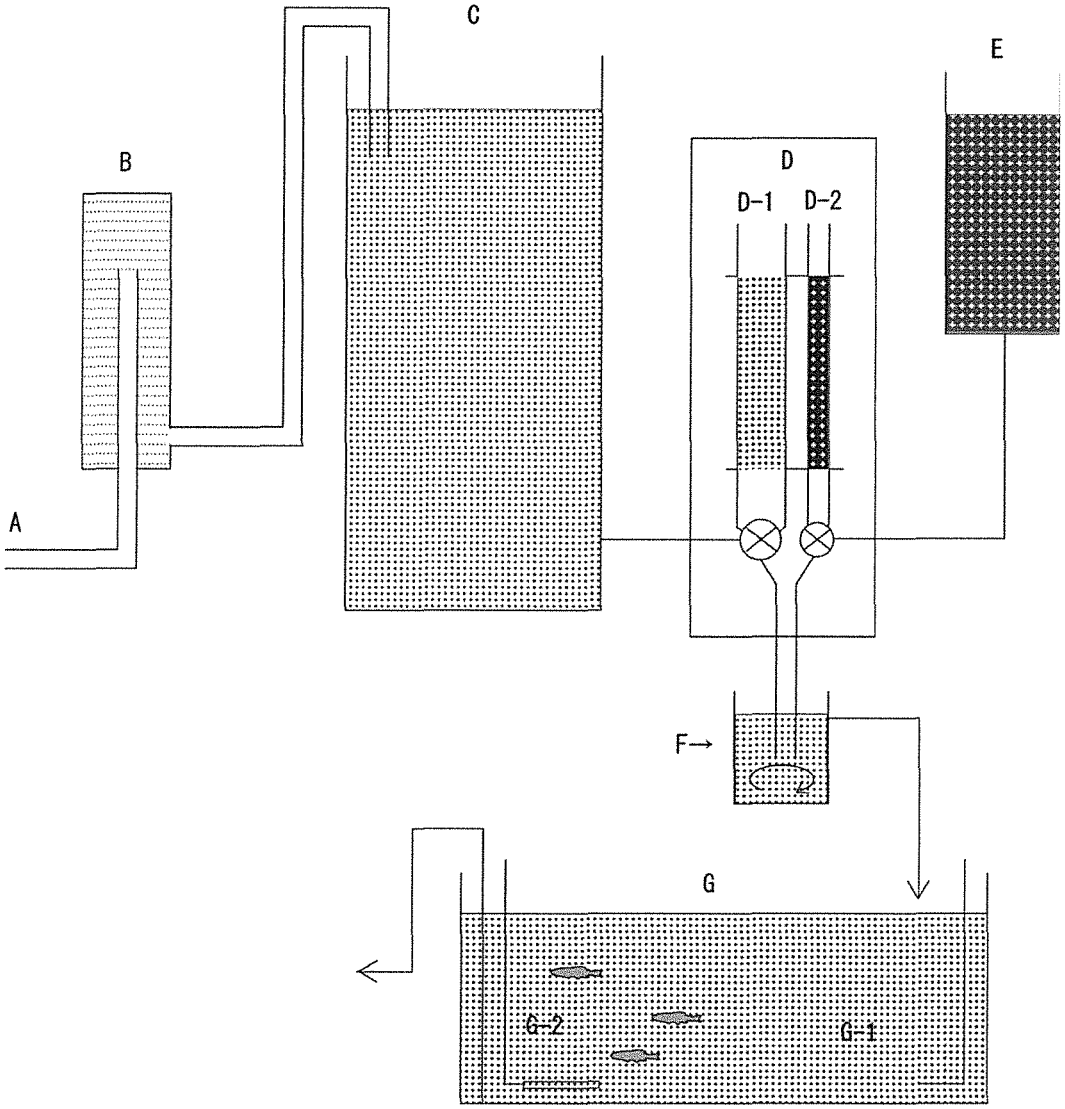
Reference 1 Bioconcentration test equipment outline

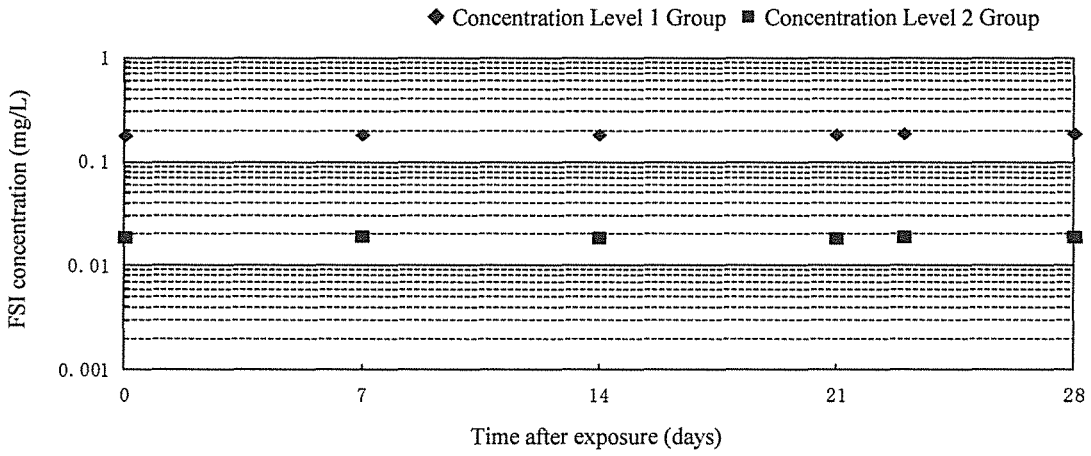


A: Tap water
B: Filter/activated charcoal tank (dechlorination equipment)
C: Tank for water for test (dechlorinated water)
D: Regulator for controlling the flow rate and mixing ratio
D-1: Water for test
D-2: Stock solution of test substance

E: Tank for stock solution of test substance (10L)
F: Mixing tank (2L) (mechanical stirrer)
G: Test aquarium (100L)
G-1: Aeration
G-2: Temperature regulator/heater

Reference 2 Aquatic environment (concentrations, water temperature, pH and DO)

2-1 Concentrations of in water

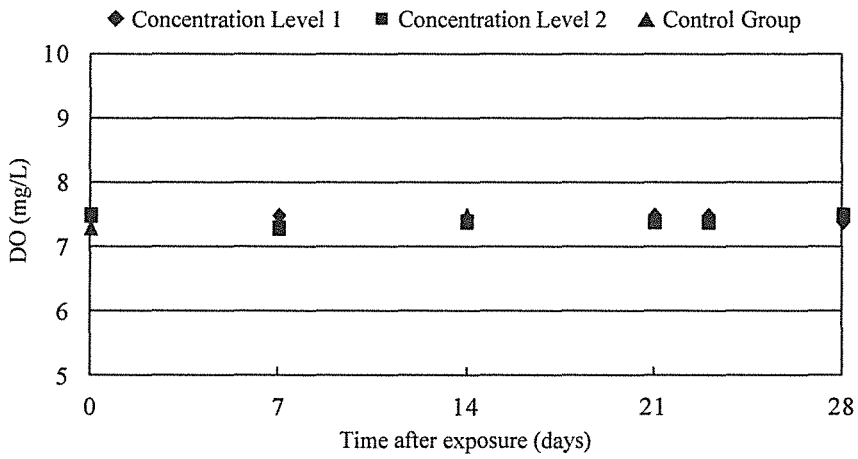


2-2 Measurement of temperature, pH and DO in water

The aquatic environment was measured at a fixed time (10 a.m.). For pH, water was sampled from the center of the aquarium and measured with a pH meter (SOP/INS/4.3.3). Further, DO in water was measured at the center of the aquarium using a DO meter (SOP/INS/4.4.4). The results are shown in the following table.

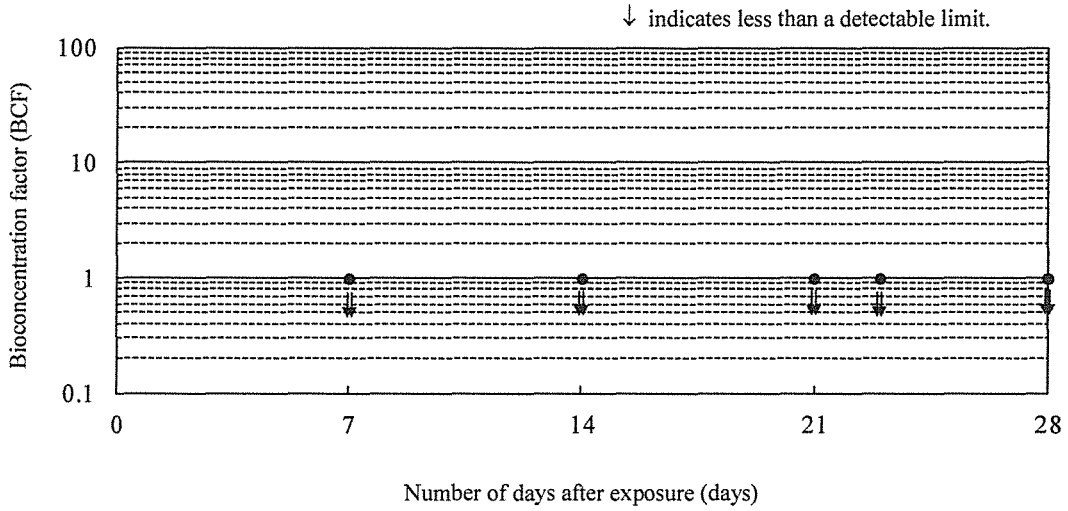
Number of days after exposure	Concentration Level 1			Concentration Level 2			Control group		
	Water temperature (°C)	pH	DO (mgL^{-1})	Water temperature (°C)	pH	DO (mgL^{-1})	Water temperature (°C)	pH	DO (mgL^{-1})
0	24.8	7.4	7.5	25.1	7.4	7.5	25.5	7.4	7.3
7	25.4	7.4	7.5	25.2	7.5	7.3	25.3	7.5	7.3
14	25.5	7.5	7.4	25.5	7.5	7.4	25.4	7.5	7.5
21	25.1	7.5	7.5	25.4	7.4	7.4	25.1	7.4	7.4
23	25.4	7.6	7.5	25.4	7.6	7.4	25.4	7.6	7.4
28	25.2	7.6	7.4	25.1	7.5	7.5	25.3	7.6	7.5

[DO in water]

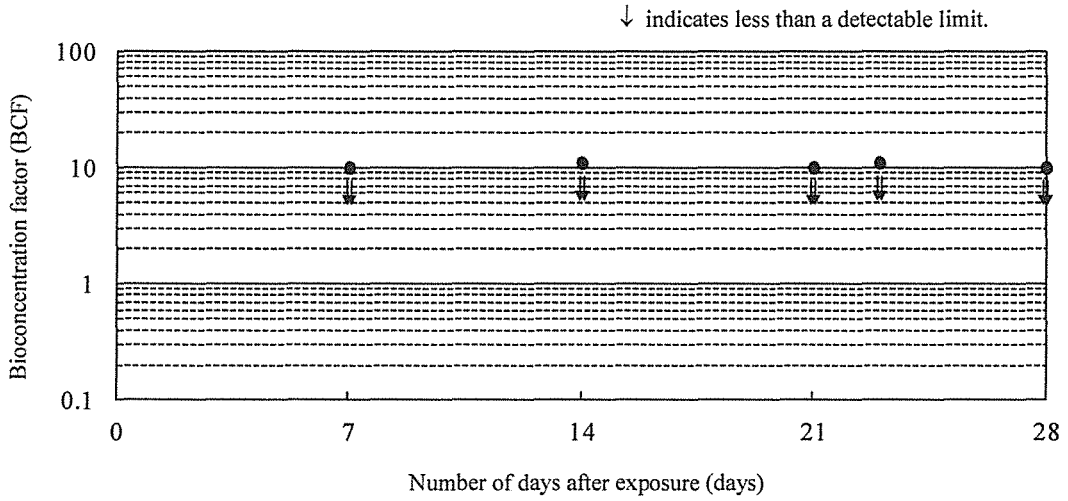


Reference 3 Time-dependent changes in bioconcentration factors

[Concentration Level 1]



[Concentration Level 2]



Reference 4 Fish analysis data

Fish analysis data (Concentration Level 1)

Items / Samples	A Fixed volume of the solution (mL)	B Dilution factor	C Peak area	D Concentration calculated by two-point calibration curve (mgL^{-1})	E Amount deposited in fish body (μg)	F Fish weight (g)	G Recovery rate (%)	H Concentration in fish body (μgg^{-1})	I Concentration in water tank (mgL^{-1})	J Bioconcentration factor (BCF)	K Date of measurement
Day 7 No.1	100	10	N.D.	<0.0012	<1.2	6.69 [3.24, 3.45]	97.3	<0.184	0.181	<1	2011. 2. 2
Day 7 No.2	100	10	N.D.	<0.0012	<1.2	6.85 [3.20, 3.65]	97.3	<0.180	0.181	<1	2011. 2. 2
Day 14 No.1	100	10	N.D.	<0.0012	<1.2	6.87 [3.58, 3.29]	97.3	<0.180	0.177	<1	2011. 2. 9
Day 14 No.2	100	10	N.D.	<0.0012	<1.2	6.75 [3.11, 3.64]	97.3	<0.183	0.177	<1	2011. 2. 9
Day 21 No.1	100	10	N.D.	<0.0012	<1.2	6.83 [3.41, 3.42]	97.3	<0.181	0.181	<1	2011. 2.16
Day 21 No.2	100	10	N.D.	<0.0012	<1.2	6.80 [3.66, 3.14]	97.3	<0.181	0.181	<1	2011. 2.16
Day 23 No.1	100	10	N.D.	<0.0012	<1.2	6.62 [3.14, 3.48]	97.3	<0.186	0.187	<1	2011. 2.18
Day 23 No.2	100	10	N.D.	<0.0012	<1.2	6.55 [3.07, 3.48]	97.3	<0.188	0.187	<1	2011. 2.18
Day 28 No.1	100	10	N.D.	<0.0012	<1.2	6.91 [3.11, 3.80]	97.3	<0.178	0.185	<1	2011. 2.23
Day 28 No.2	100	10	N.D.	<0.0012	<1.2	6.81 [3.53, 3.28]	97.3	<0.181	0.185	<1	2011. 2.23

Calculation methods:

Amount deposited in fish $E = A \times B \times D$

Concentration in fish $H = \frac{E \times 100}{F \times G}$ Total value of 2 fish was used for F.

Bioconcentration factor (BCF) $J = \frac{H}{I}$

(Note) Sample No. is a symbol given when two fish were paired when sampling fish and analyzed at n=2.
For the samples showing less than the detectable limit (peak area 800000), N.D. is recorded in the column C and the calculation was made by replacing the detectable limit of 0.0012 mgL^{-1} in the aforementioned D.

Fish analysis data (Concentration Level 2)

Items / Samples	A Fixed volume of the solution (mL)	B Dilution factor	C Peak area	D Concentration calculated by two-point calibration curve (mgL^{-1})	E Amount deposited in fish body (μg)	F Fish weight (g)	G Recovery rate (%)	H Concentration in fish body (μgg^{-1})	I Concentration in water tank (mgL^{-1})	J Bioconcentration factor (BCF)	K Date of measurement
Day 7 No.1	100	10	N.D.	<0.0012	<1.2	6.76 [3.58, 3.18]	97.3	<0.182	0.0188	<10	2011. 2. 2
Day 7 No.2	100	10	N.D.	<0.0012	<1.2	6.86 [3.36, 3.50]	97.3	<0.180	0.0188	<10	2011. 2. 2
Day 14 No.1	100	10	N.D.	<0.0012	<1.2	6.25 [2.89, 3.36]	97.3	<0.197	0.0184	<11	2011. 2. 9
Day 14 No.2	100	10	N.D.	<0.0012	<1.2	6.51 [3.36, 3.15]	97.3	<0.189	0.0184	<10	2011. 2. 9
Day 21 No.1	100	10	N.D.	<0.0012	<1.2	6.89 [3.24, 3.65]	97.3	<0.179	0.0184	<10	2011. 2.16
Day 21 No.2	100	10	N.D.	<0.0012	<1.2	6.78 [3.55, 3.23]	97.3	<0.182	0.0184	<10	2011. 2.16
Day 23 No.1	100	10	N.D.	<0.0012	<1.2	6.49 [3.29, 3.20]	97.3	<0.190	0.0193	<10	2011. 2.18
Day 23 No.2	100	10	N.D.	<0.0012	<1.2	6.55 [3.55, 3.00]	97.3	<0.188	0.0193	<10	2011. 2.18
Day 28 No.1	100	10	N.D.	<0.0012	<1.2	6.87 [3.33, 3.54]	97.3	<0.180	0.0188	<10	2011. 2.23
Day 28 No.2	100	10	N.D.	<0.0012	<1.2	6.76 [3.42, 3.34]	97.3	<0.182	0.0188	<10	2011. 2.23

Calculation methods:

Amount deposited in fish $E = A \times B \times D$

Concentration in fish $H = \frac{E \times 100}{F \times G}$ Total value of 2 fish was used for F.

Bioconcentration factor (BCF) $J = \frac{H}{I}$

(Note) Sample No. is a symbol given when two fish were paired when sampling fish and analyzed at n=2.
For the samples showing less than the detectable limit (peak area 800000), N.D. is recorded in the column C and the calculation was made by replacing the detectable limit of 0.0012 mgL^{-1} in the aforementioned D.

Fish analysis data (Control group)

Items / Samples	A Fixed volume of the solution (mL)	B Dilution factor	C Peak area	D Concentration calculated by two-point calibration curve (mgL^{-1})	E Amount deposited in fish body (μg)	F Fish weight (g)	G Recovery rate (%)	H Concentration in fish body (μgg^{-1})	I Concentration in water tank (mgL^{-1})	J Bioconcentration factor (BCF)	K Date of measurement
Day 0	100	10	N.D.	–	–	6.84 [3.53, 3.31]	–	–	–	–	2011. 1.26
Day 28	100	10	N.D.	–	–	6.62 [3.66, 2.96]	–	–	–	–	2011. 2.23

Reference 5 Water analysis data

Water analysis data (Concentration Level 1)

Items / Samples	A Fixed volume of the solution (mL)	B Peak area	C Concentration calculated by two-point calibration curve (mgL^{-1})	D Absolute amount in the fixed volume solution (μg)	E Amount of water measured (mL)	F Recovery rate (%)	G Concentration in water tank (mgL^{-1})	H Date of measurement
Day 0	20	1873661	0.0085	0.170	1	96.2	0.177	2011. 1.26
Day 7	20	1921305	0.0087	0.174	1	96.2	0.181	2011. 2. 2
Day 14	20	1895936	0.0085	0.170	1	96.2	0.177	2011. 2. 9
Day 21	20	1931160	0.0087	0.174	1	96.2	0.181	2011. 2.16
Day 23	20	1967483	0.0090	0.180	1	96.2	0.187	2011. 2.18
Day 28	20	1964054	0.0089	0.178	1	96.2	0.185	2011. 2.23

Calculation methods: Absolute amount in the fixed volume solution $D = A \times C$

Concentration in the aquarium $G = \frac{D \times 100}{E \times F}$

Water analysis data (Concentration Level 2)

Items / Samples	A Fixed volume of the solution (mL)	B Peak area	C Concentration calculated by two-point calibration curve (mgL^{-1})	D Absolute amount in the fixed volume solution (μg)	E Amount of water measured (mL)	F Recovery rate (%)	G Concentration in water tank (mgL^{-1})	H Date of measurement
Day 0	5	1937105	0.0089	0.0445	2.5	95.5	0.0186	2011. 1.26
Day 7	5	1970474	0.0090	0.0450	2.5	95.5	0.0188	2011. 2. 2
Day 14	5	1941077	0.0088	0.0440	2.5	95.5	0.0184	2011. 2. 9
Day 21	5	1943578	0.0088	0.0440	2.5	95.5	0.0184	2011. 2.16
Day 23	5	1988219	0.0092	0.0460	2.5	95.5	0.0193	2011. 2.18
Day 28	5	1985770	0.0090	0.0450	2.5	95.5	0.0188	2011. 2.23

Calculation methods: Absolute amount in the fixed volume solution $D = A \times C$

Concentration in the aquarium $G = \frac{D \times 100}{E \times F}$

Water analysis data (Control group)

Items / Samples	A Fixed volume of the solution (mL)	B Peak area	C Concentration calculated by two-point calibration curve (mgL^{-1})	D Absolute amount in the fixed volume solution (μg)	E Amount of water measured (mL)	F Recovery rate (%)	G Concentration in water tank (mgL^{-1})	H Date of measurement
Day 0	5	N.D.	–	–	2.5	–	–	2011. 1.26

Reference 6 Chromatograms for the preparation of calibration curve

Calibration curve

Standard solution
0.0048 mgL^{-1}

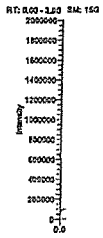
Data File:	C186CALS1-2	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\KANEKO\C 186\CAL	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Calibration curve

Standard solution
0.0096 mgL^{-1}

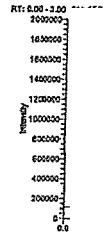
Data File:	C186CALS2-2	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\KANEKO\C 186\CAL	Sample ID:
Sample Name:	Std. 0.0096 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Calibration curve

Standard solution
0.0193 mgL^{-1}

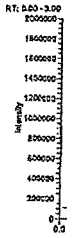
Data File:	C186CALS3-2	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\KANEKO\C 186\CAL	Sample ID:
Sample Name:	Std. 0.0193 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Calibration curve

Standard solution
0.0483 mgL^{-1}

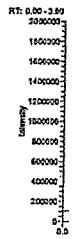
Data File:	C186CALS7-2	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\KANEKO\C 186\CAL	Sample ID:
Sample Name:	Std. 0.0483 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Calibration curve

Standard solution
0.0964 mgL^{-1}

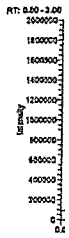
Data File:	C186CALS4-2	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\KANEKO\C 186\CAL	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Calibration curve

Chromatogram of solvent for adjustment

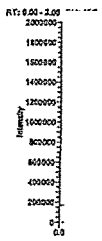
Data File:	C186CALS0-1	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\KANEKO\C 186\CAL	Sample ID:
Sample Name:	Solvent	Comments:
Sample Type:	Blank	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Reference 7 Chromatograms of for recovery rate

7-1 Chromatograms of for recovery rate from fish

Recovery test
(Fish analysis)

Standard solution
0.0048 mgL^{-1}

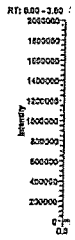
Data File:	C166FRS1	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\kaneko\C16 6\FR	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : ThermoFisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Fish analysis)

Standard solution
0.0483 mgL^{-1}

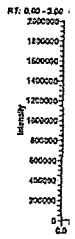
Data File:	C166FRS2-1	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\kaneko\C16 6\FR	Sample ID:
Sample Name:	Std. 0.0483 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Fish analysis)

Added Standard
solution

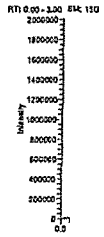
Data File:	C186FRAS	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\kaneko\C18 6\FR	Sample ID:
Sample Name:	Added Std.	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Fish analysis)

Added group
No.1

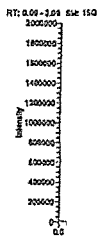
Data File:	C186FRA1-1	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\kaneko\C18 6\FR	Sample ID:
Sample Name:	Added No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Fish analysis)

Added group
No.2

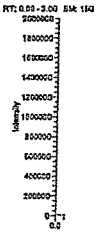
Data File:	C186FRA2	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\kaneko\C18 5\FR	Sample ID:
Sample Name:	Added No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Fish analysis)

Added group
No.3

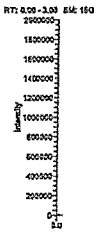
Data File:	C186FRA3	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\kaneko\C18 5\FR	Sample ID:
Sample Name:	Added No.3	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Fish analysis)

Control group
No.1

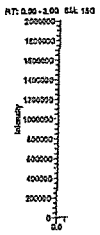
Data File:	C186FRC1-1	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\kaneko\C18 6\FR	Sample ID:
Sample Name:	Ctrl No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Fish analysis)

Control group
No.2

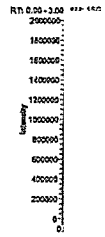
Data File:	C186FRC2	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\kaneko\C18 6\FR	Sample ID:
Sample Name:	Ctrl No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Fish analysis)

Control group
No.3

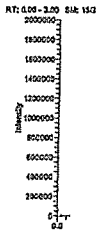
Data File:	C186FRC3	Acquisition Date:
Original Data Path:	C:\XCALIBUR\DATA\kanako\C18 6\FR	Sample ID:
Sample Name:	Ctrl No.3	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE	:	SHIMADZU LC-20AD
COLUMN	:	Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT	:	A; 20 mM Ammonium formate aq./formic acid (1000:1) B; Acetonitrile A/B(1:1)
FLOW RATE	:	0.2 mL/min
COLUMN TEMP	:	313 K
INJECT VOL	:	5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE	:	Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION	:	Negative ESI
VAPORIZER TEMP	:	723 K
SPRAY VOLTAGE	:	2500 V
SHEATH GAS	:	50
AUX GAS	:	15
ION SWEEP GAS	:	0
CAPILLARY TEMP	:	493 K
SCAN TYPE	:	SIM (m/z 180)



7-2 Chromatograms of for recovery rate from water

Recovery test
(Water analysis)

Standard solution
0.0048 mgL^{-1}

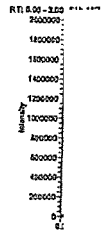
Data File:	C186WRHS1-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186WRH	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

Standard solution
0.0483 mgL^{-1}

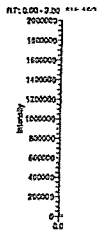
Data File:	C186WRHS2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186WRH	Sample ID:
Sample Name:	Std. 0.0483 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

High exposure level
Added Standard
solution

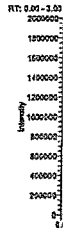
Data File:	C186WRHAS-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\WRH	Sample ID:
Sample Name:	Added Std.	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

High exposure level
Added group No.1

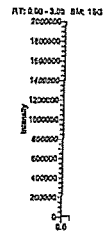
Data File:	C186WRHA1-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\WRH	Sample ID:
Sample Name:	Added No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

High exposure level
Added group No.2

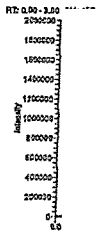
Data File:	C186WRHA2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\W RH	Sample ID:
Sample Name:	Added No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

High exposure level
Added group No.3

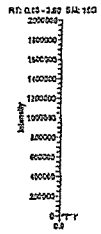
Data File:	C186WRHA3-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\W RH	Sample ID:
Sample Name:	Added No.3	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

High exposure level
Control group No.1

Data File:	C186WRHC1-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\WRH	Sample ID:
Sample Name:	Ctrl No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)

Recovery test
(Water analysis)

High exposure level
Control group No.2

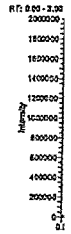
Data File:	C186WRHC2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\WRH	Sample ID:
Sample Name:	Ctrl No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

High exposure level
Control group No.3

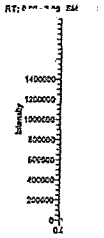
Data File:	C186WRHC3-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186WRH	Sample ID:
Sample Name:	Ctrl No.3	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

Standard solution
0.0048 mgL^{-1}

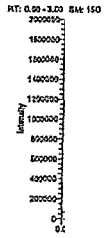
Data File:	C186WRLS1-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186WRL	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

Standard solution
0.0483 mgL^{-1}

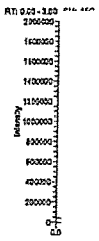
Data File:	C186WRLS2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\WRL	Sample ID:
Sample Name:	Std. 0.0483 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

Low exposure level
Added Standard
solution

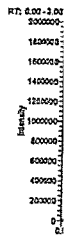
Data File:	C186WRLAS-3	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\WRL	Sample ID:
Sample Name:	Added Std.	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

Low exposure level
Added group No.1

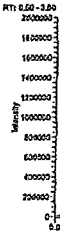
Data File:	C186WRLA1-4	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\WRL	Sample ID:
Sample Name:	Added No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

Low exposure level
Added group No.2

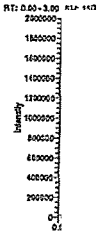
Data File:	C186WRLA2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\WRL	Sample ID:
Sample Name:	Added No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

Low exposure level
Added group No.3

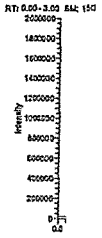
Data File:	C186WRLA3-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\WRL	Sample ID:
Sample Name:	Added No.3	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

Low exposure level
Control group No.1

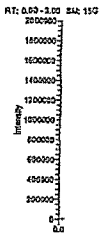
Data File:	C186WRLC1-3	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186\WRL	Sample ID:
Sample Name:	Ctrl No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

Low exposure level
Control group No.2

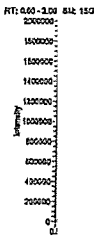
Data File:	C186WRLC2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186WRL	Sample ID:
Sample Name:	Ctrl No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Recovery test
(Water analysis)

Low exposure level
Control group No.3

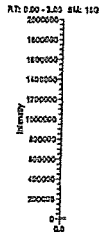
Data File:	C186WRLC3-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\data\kaneko\C186WRL	Sample ID:
Sample Name:	Ctrl No.3	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Reference 8 Chromatograms in fish

8-1 Test group

Fish analysis
Day 7

Standard solution
0.0048 mgL^{-1}

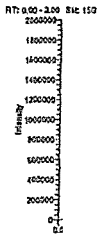
Data File:	C186WFA7S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 7	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 7

Standard solution
0.0964 mgL^{-1}

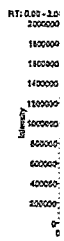
Data File:	C186WFA7S2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 7	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 7

High exposure level
No.1

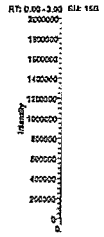
Data File:	C186FA7H1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 7	Sample ID:
Sample Name:	Fish analysis H Lv No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 160)



Fish analysis
Day 7

High exposure level
No.2

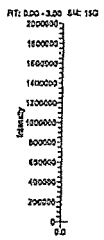
Data File:	C186FA7H2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 7	Sample ID:
Sample Name:	Fish analysis H Lv No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 160)



Fish analysis
Day 7

Low exposure level
No.1

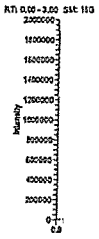
Data File:	C166FA7L1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C166\Day 7	Sample ID:
Sample Name:	Fish analysis L Lv No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 7

Low exposure level
No.2

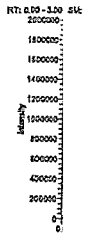
Data File:	C166FA7L2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C166\Day 7	Sample ID:
Sample Name:	Fish analysis L Lv No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 14

Standard solution
0.0048 mgL^{-1}

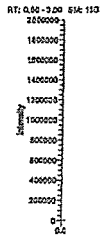
Data File:	C186WFA14S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 14	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 14

Standard solution
0.0964 mgL^{-1}

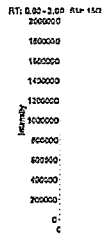
Data File:	C186WFA14S2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 14	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 14

High exposure level
No.1

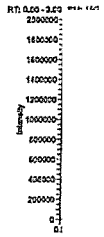
Data File:	C186FA14H1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 14	Sample ID:
Sample Name:	Fish analysis H Lv No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 14

High exposure level
No.2

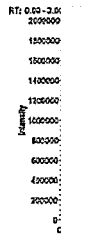
Data File:	C186FA14H2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 14	Sample ID:
Sample Name:	Fish analysis H Lv No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 14

Low exposure level
No.1

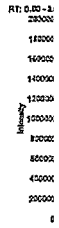
Data File:	C186FA14L1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 14	Sample ID:
Sample Name:	Fish analysis L Lv No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 160)



Fish analysis
Day 14

Low exposure level
No.2

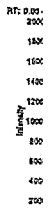
Data File:	C186FA14L2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 14	Sample ID:
Sample Name:	Fish analysis L Lv No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 160)



Fish analysis
Day 21

Standard solution
0.0048 mgL^{-1}

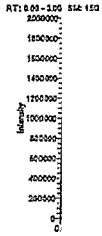
Data File:	C166WFA21S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C166\Day 21	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 21

Standard solution
0.0964 mgL^{-1}

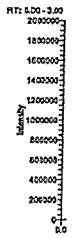
Data File:	C166WFA21S2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C166\Day 21	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 21

High exposure level
No.1

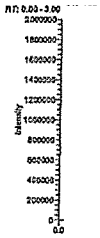
Data File:	C186FA21H1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 21	Sample ID:
Sample Name:	Fish analysis H Lv No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 21

High exposure level
No.2

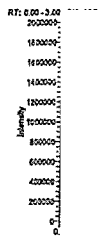
Data File:	C186FA21H2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 21	Sample ID:
Sample Name:	Fish analysis H Lv No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 21

Low exposure level
No.1

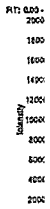
Data File:	C186FA21L1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 21	Sample ID:
Sample Name:	Fish analysis L Lv No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 21

Low exposure level
No.2

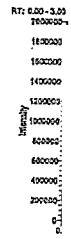
Data File:	C186FA21L2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 21	Sample ID:
Sample Name:	Fish analysis L Lv No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 23

Standard solution
0.0048 mgL^{-1}

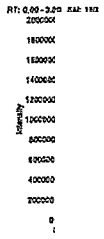
Data File:	C186WFA23S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 23	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 23

Standard solution
0.0964 mgL^{-1}

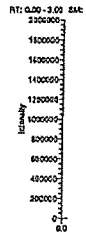
Data File:	C186WFA23S2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 23	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 23

High exposure level
No.1

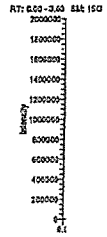
Data File:	C186FA23H1-1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 23	Sample ID:
Sample Name:	Fish analysis H Lv No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 23

High exposure level
No.2

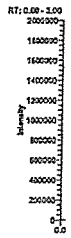
Data File:	C186FA23H2-1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 23	Sample ID:
Sample Name:	Fish analysis H Lv No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 23

Low exposure level
No.1

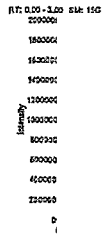
Data File:	C186FA23L1-1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 23	Sample ID:
Sample Name:	Fish analysis L Lv No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 160)



Fish analysis
Day 23

Low exposure level
No.2

Data File:	C186FA23L2-1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 23	Sample ID:
Sample Name:	Fish analysis L Lv No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 160)

Fish analysis
Day 28

Standard solution
0.0048 mgL^{-1}

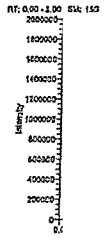
Data File:	C186WFA28S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 28

Standard solution
0.0964 mgL^{-1}

Data File:	C186WFA28S2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)

Fish analysis
Day 28

High exposure level
No.1

Data File:	C186FA28H1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Fish analysis H Lv No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)

Fish analysis
Day 28

High exposure level
No.2

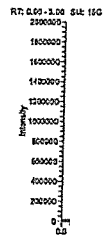
Data File:	C186FA28H2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Fish analysis H Lv No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 28

Low exposure level
No.1

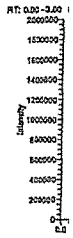
Data File:	C186FA28L1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Fish analysis L Lv No.1	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 28

Low exposure level
No.2

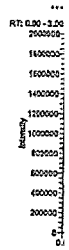
Data File:	C186FA28L2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Fish analysis L Lv No.2	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



8-2 Control group

Fish analysis
Day 0

Standard solution
0.0048 mgL^{-1}

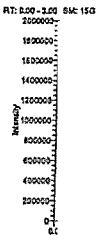
Data File:	C186WFA0S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 0	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A: 20 mM Ammonium formate aq./formic acid (1000:1)
B: Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 0

Standard solution
0.0964 mgL^{-1}

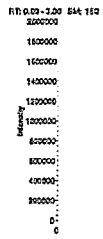
Data File:	C186WFA0S2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 0	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A: 20 mM Ammonium formate aq./formic acid (1000:1)
B: Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 0
(before starting exposure)

Control group

Data File:	C186FA0C	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 0	Sample ID:
Sample Name:	Fish analysis Ctrl	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)

Fish analysis
Day 28

Standard solution
0.0048 mgL^{-1}

Data File:	C186WFA28S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)

Fish analysis
Day 28

Standard solution
0.0964 mgL^{-1}

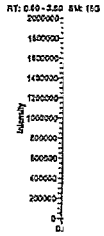
Data File:	C186WFA28S2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Fish analysis
Day 28
(at the termination
of exposure)
Control group

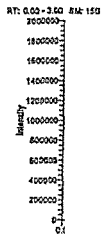
Data File:	C186FA28C	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Fish analysis Ctrl	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Reference 9 Chromatograms when measuring the concentrations in water

9-1 Test group

Water analysis
Day 0

Standard solution
0.0048 mgL^{-1}

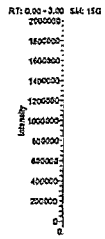
Data File:	C186WFA0S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 0	Sample ID:
Sample Name:	Std, 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 0

Standard solution
0.0964 mgL^{-1}

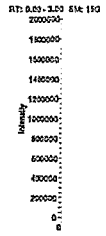
Data File:	C186WFA0S2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 0	Sample ID:
Sample Name:	Std, 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 0

High exposure level

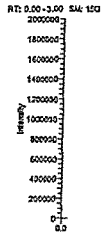
Data File:	C186WA0H3	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 0	Sample ID:
Sample Name:	Water analysis H Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 0

Low exposure level

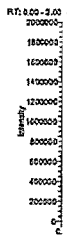
Data File:	C186WA0L	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 0	Sample ID:
Sample Name:	Water analysis L Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 7

Standard solution
0.0048 mgL^{-1}

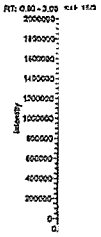
Data File:	C186WFA7S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 7	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 7

Standard solution
0.0964 mgL^{-1}

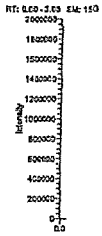
Data File:	C186WFA7S2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 7	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 7

High exposure level

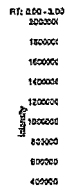
Data File:	C186WA7H	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 7	Sample ID:
Sample Name:	Water analysis H Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 7

Low exposure level

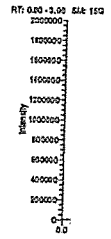
Data File:	C186WA7L	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 7	Sample ID:
Sample Name:	Water analysis L Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 14

Standard solution
0.0048 mgL^{-1}

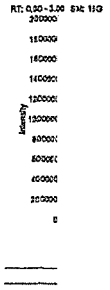
Data File:	C186WFA14S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 14	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 14

Standard solution
0.0964 mgL^{-1}

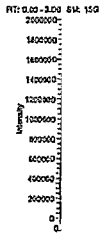
Data File:	C186WFA14S2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 14	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 14

High exposure level

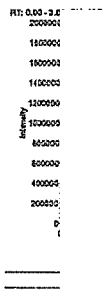
Data File:	C186WA14H	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 14	Sample ID:
Sample Name:	Water analysis H Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 14

Low exposure level

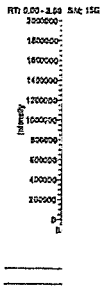
Data File:	C186WA14L	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 14	Sample ID:
Sample Name:	Water analysis L Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 21

Standard solution
0.0048 mgL^{-1}

Data File:	C186WFA21S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 21	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)

Water analysis
Day 21

Standard solution
0.0964 mgL^{-1}

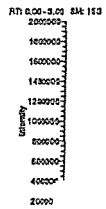
Data File:	C186WFA21S2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 21	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 21

High exposure level

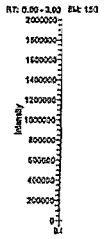
Data File:	C186WA21H	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 21	Sample ID:
Sample Name:	Water analysis H Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 21

Low exposure level

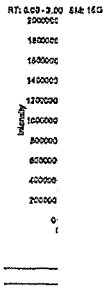
Data File:	C186WA21L	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 21	Sample ID:
Sample Name:	Water analysis L Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 23

Standard solution
0.0048 mgL^{-1}

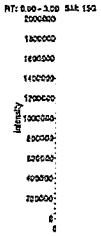
Data File:	C186WFA23S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 23	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 23

Standard solution
0.0964 mgL^{-1}

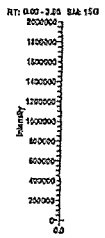
Data File:	C186WFA23S2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 23	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 23

High exposure level

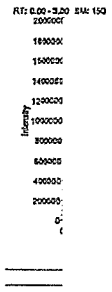
Data File:	C186WA23H	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 23	Sample ID:
Sample Name:	Water analysis H Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 23

Low exposure level

Data File:	C186WA23L	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 23	Sample ID:
Sample Name:	Water analysis L Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)

Water analysis
Day 28

Standard solution
0.0048 mgL^{-1}

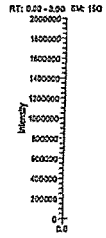
Data File:	C186WFA28S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 28

Standard solution
0.0964 mgL^{-1}

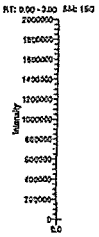
Data File:	C186WFA28S2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 28

High exposure level

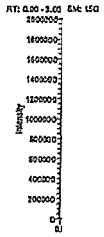
Data File:	C186WA28H	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Water analysis H Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 160)



Water analysis
Day 28

Low exposure level

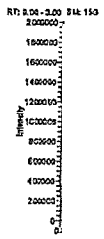
Data File:	C186WA28L	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 28	Sample ID:
Sample Name:	Water analysis L Lv	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 160)



9-2 Control group

Water analysis
Day 0

Standard solution
0.0048 mgL^{-1}

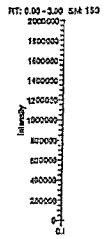
Data File:	C186WFA0S1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 0	Sample ID:
Sample Name:	Std. 0.0048 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 0

Standard solution
0.0964 mgL^{-1}

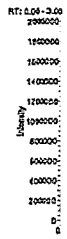
Data File:	C186WFA0S2-2	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C186\Day 0	Sample ID:
Sample Name:	Std. 0.0964 mg/L	Comments:
Sample Type:	Std Bracket	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Water analysis
Day 0

Control group

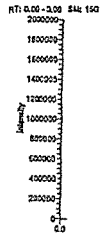
Data File:	C166WA0C1	Acquisition Date:
Original Data Path:	C:\Xcalibur\Data\kaneko\C166\Day 0	Sample ID:
Sample Name:	Water analysis Ctrl	Comments:
Sample Type:	Unknown	Operator:

*** ANALYTICAL CONDITIONS (HPLC) ***

HPLC TYPE : SHIMADZU LC-20AD
COLUMN : Hypersil GOLD 2.1 x 50 mm (5 um)
ELUENT : A; 20 mM Ammonium formate aq./formic acid (1000:1)
B; Acetonitrile
A/B(1:1)
FLOW RATE : 0.2 mL/min
COLUMN TEMP : 313 K
INJECT VOL : 5 uL

*** ANALYTICAL CONDITIONS (MS) ***

MS TYPE : Thermo Fisher Scientific TSQ Quantum Access MAX
IONIZATION : Negative ESI
VAPORIZER TEMP : 723 K
SPRAY VOLTAGE : 2500 V
SHEATH GAS : 50
AUX GAS : 15
ION SWEEP GAS : 0
CAPILLARY TEMP : 493 K
SCAN TYPE : SIM (m/z 180)



Reference 10 Water quality test

Analyzed at Institute of Ecotoxicology Co., Ltd.

<Quantitative analysis of the residual chlorine>
The quantification of the residual chlorine in water used in this study was performed according to JIS K0102 33.1 (*o*-Tolidine colorimetric method).
The concentrations of the residual chlorine during the experimental period were determined to be 0.01 mgL^{-1} or less.

Concentration of residual chlorine (mgL^{-1})	Date of quantitative analysis
<0.01	1.26.2011
<0.01	2. 2.2011
<0.01	2. 9.2011
<0.01	2.16.2011
<0.01	2.23.2011

<Quantitative analysis of copper>
The quantification of copper in water used in this study was performed according to JIS K0101 51.1 (Absorptiometry). The concentration of copper was confirmed to be 0.005 mgL^{-1} or less.

Copper (mgL^{-1})	Date of quantitative analysis
< 0.005	6.20.2010

<Quantitative analysis of dissolved organic carbon concentrations>
The quantitative analysis was performed using a total organic carbon meter TOC – 5000A (Shimadzu Corporation) (SOP/INS/4.9).
The dissolved organic carbon concentration was confirmed to be 2 mgL^{-1} or less.

Dissolved organic carbon concentration (mgL^{-1})	Date of quantitative analysis
< 2	2.14.2011

No. 106020
April 16, 2010

Report of the test results

To the Institute of Ecotoxicology Inc.

3-15-1 Komazawa, Setagaya-ku, Tokyo
Shinnihon Environmental Research Corporation
(Registration of Measurement Certification Business, Tokyo, No. 882)
Akira Kishi, Executive Director

The results of the test conducted on the sample as requested on April 1, 2010 were as follows.

Subject:	Analysis of water used for raising carp
Organization which collected the sample:	Institute of Ecotoxicology, Inc.
Type of sample:	Water quality
Date when sample was collected:	April 1, 2010

Sample names / Items analyzed		Water used for raising carp	Quantifiable lower limit	Test methods
Nitric nitrogen and nitrous nitrogen	(mg/L)	2.2	0.02	Service water test method (2001) VI–2 13.2
Potassium permanganate consumed	(mg/L)	1.5	0.5	Service water test method (2001) VI–1 17.2
Cyan	(mg/L)	Not detected*	0.001	Service water test method (2001) VI–2 22.5
Total mercury	(mg/L)	<0.0005	0.0005	Service water test method (2001) VI–3 25.2
Organic phosphorous	(mg/L)	Not detected*	0.05	1974 Environmental Agency Notification No. 64 Appendix 1
Iron	(mg/L)	<0.001	0.001	Service water test method (2001) VI–3 13.4
Manganese	(mg/L)	<0.001	0.001	Service water test method (2001) VI–3 12.4
Zinc	(mg/L)	0.014	0.001	Service water test method (2001) VI–3 16.4
Lead	(mg/L)	<0.001	0.001	Service water test method (2001) VI–3 27.2
Hexavalent chromium	(mg/L)	<0.005	0.005	Service water test method (2001) VI–3 11. Reference 2
Cadmium	(mg/L)	<0.001	0.001	Service water test method (2001) VI–3 21.4
Arsenic	(mg/L)	<0.001	0.001	Service water test method (2001) VI–3 17.2.2
Fluorine	(mg/L)	0.10	0.05	Service water test method (2001) VI–2 3.2
Total hardness	(mg/L)	63	0.02	Service water test method (2001) VI–1 15.2.3
Phenols	(mg/L)	<0.005	0.005	Service water test method (2001) VI–4 10.5
Anionic surfactant	(mg/L)	<0.02	0.02	Service water test method (2001) VI–4 11.2
pH ( ) indicating water temperature at the time of analysis		7.4 (21.1)	0.1	Service water test method (2001) VI–1 9.2

* "Not detected" means when measured by the analytical method listed above, the result was less than the quantifiable limit.

Reference 11 Records of observations of the conditions of the test fish (carp)

Records of observations of the conditions of the test fish	No. 1	Test substance		Test No.	C – 186

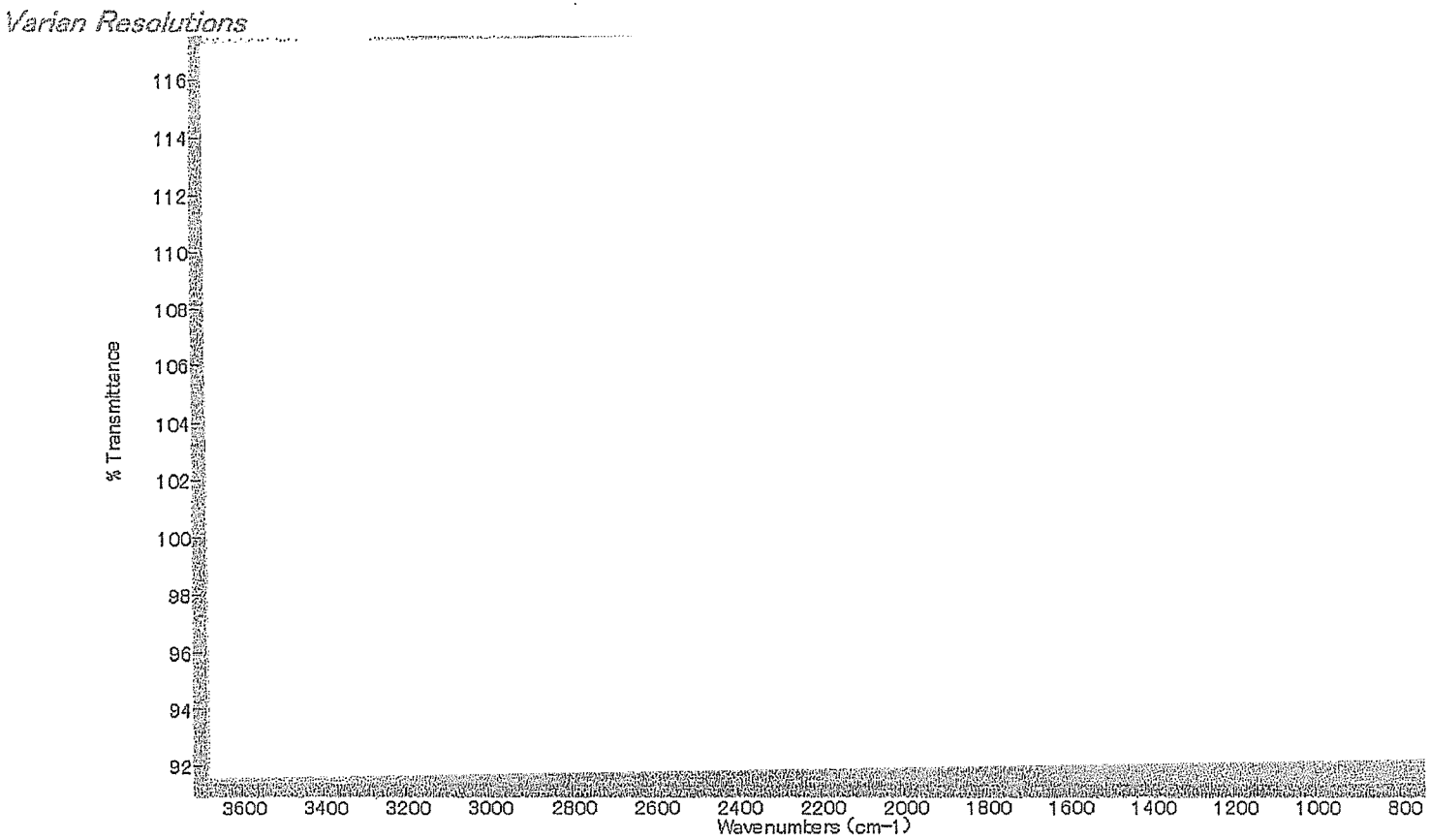
Date 2011	Feeding period (days)	Concentration Level 1 group (Water tank No. 9)					Concentration Level 2 group (Water tank No. 8)					Control group (Water tank No. 7)					Temperature/°C			Observed by
		Swim status	Food intake	Health	Water flow	Mortality	Swim status	Food intake	Health	Water flow	Mortality	Swim status	Food intake	Health	Water flow	Mortality	Level 1	Level 2	Control	
1.26	0	O.K.	–	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	24.8	25.1	25.5	Kaneko
1.27	1	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.1	25.5	25.6	Kaneko
1.28	2	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.2	25.3	25.5	Kaneko
1.29	3	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.3	25.3	25.5	Kaneko
1.30	4	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.3	25.5	Kaneko
1.31	5	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.4	25.1	25.5	Kaneko
2.1	6	O.K.	–	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	25.5	25.3	25.5	Kaneko
2.2	7	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.4	25.2	25.3	Kaneko
2.3	8	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.5	25.3	Kaneko
2.4	9	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.4	25.3	Kaneko
2.5	10	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.4	25.5	Kaneko
2.6	11	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.4	25.5	Kaneko
2.7	12	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.3	25.5	25.5	Kaneko
2.8	13	O.K.	–	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	25.5	25.5	25.5	Kaneko
2.9	14	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.5	25.4	Kaneko
		◈ Flow rate: Level 1: 300 ml/min, Level 2: 300 ml/min, Control: 300 ml/min to be confirmed.																		
		◈ Food intake: 0.050 g per fish ◈Feed provided: Swimmy (Japan Pet Food Corp., Lot No. 2013.09.E3)																		
		◈ Cumulative mortality at the end of the test: Level 1: 0%, Level 2: 0%, Control: 0%																		
		◈ Remarks (Situation at the time of abnormality and treatments taken): Not applicable																		

Records of observations of the conditions of the test fish	No. 2	Test substance		Test No.	C – 186

Date 2011	Feeding period (days)	Concentration Level 1 group (Water tank No. 9)					Concentration Level 2 group (Water tank No. 8)					Control group (Water tank No. 7)					Temperature/°C			Observed by
		Swim status	Food intake	Health	Water flow	Mortality	Swim status	Food intake	Health	Water flow	Mortality	Swim status	Food intake	Health	Water flow	Mortality	Level 1	Level 2	Control	
2.10	15	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.3	25.6	25.5	Kaneko
2.11	16	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.6	25.5	Kaneko
2.12	17	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.5	25.5	Kaneko
2.13	18	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.4	25.5	Kaneko
2.14	19	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.5	25.4	Kaneko
2.15	20	O.K.	–	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	25.5	25.6	25.4	Kaneko
2.16	21	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.1	25.4	25.1	Kaneko
2.17	22	O.K.	–	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	25.1	25.4	25.5	Kaneko
2.18	23	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.4	25.4	25.4	Kaneko
2.19	24	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.4	25.6	25.3	Kaneko
2.20	25	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.3	25.5	25.3	Kaneko
2.21	26	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.5	25.5	25.3	Kaneko
2.22	27	O.K.	–	O.K.	O.K.	0	O.K.	–.	O.K.	O.K.	0	O.K.	–	O.K.	O.K.	0	25.3	25.5	25.2	Kaneko
2.23	28	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	O.K.	O.K.	O.K.	O.K.	0	25.2	25.1	25.3	Kaneko

◈ Flow rate: Level 1: 300 ml/min, Level 2: 300 ml/min, Control: 300 ml/min to be confirmed.
◈ Food intake: 0.050 g per fish ◈Feed provided: Swimmy (Japan Pet Food Corp., Lot No. 2013.09.E3)
◈ Cumulative mortality at the end of the test: Level 1: 0%, Level 2: 0%, Control: 0%
◈ Remarks (Situation at the time of abnormality and treatments taken): Not applicable

Reference 12 **IR spectrum for the test substance (IR spectrum provided by the sponsor)**



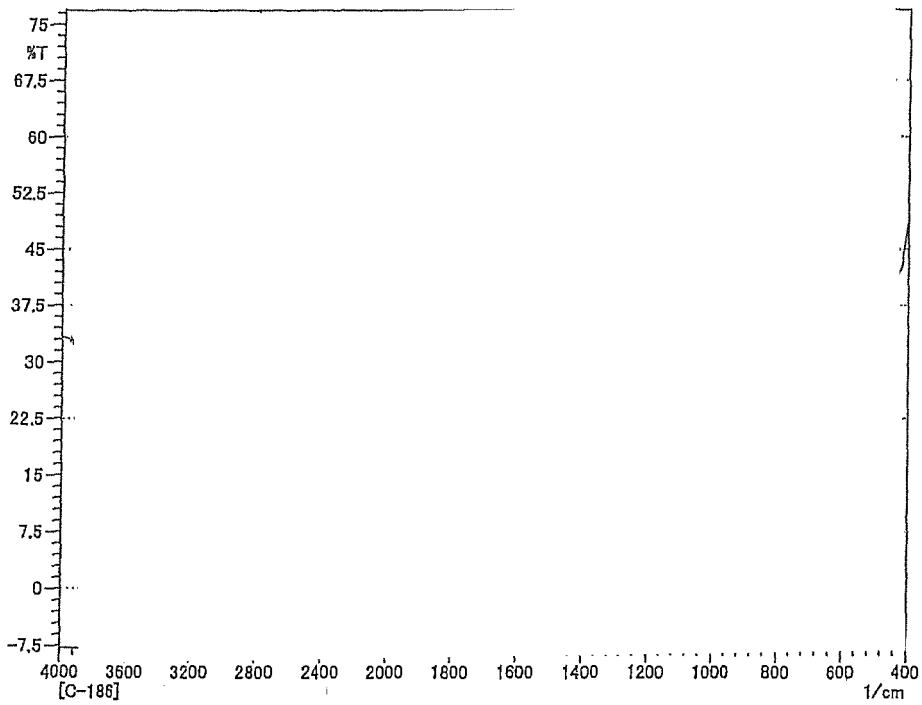
1. Name of the test substance:

2. Lot No.:

3. Date of measurement:

4. Operator: Yasunori Okumura

IR spectrum (at the beginning of the study) measured at our location



File Name:c186s5	Sample: [C-186]	
Date/Time: 2010/12/03 14:43:06		Operator: K. Kaneko
Type: IRPrestige-21	No. of Scans: 20	Method: KBr
Units: 1/cm	Ordinate: %T	Detector: standard
Min: 399.26	Max: 4000.36	Apodization: Happ-Genzel
No. of Datapoints: 1868	Data Interval: 1.93	Resolution: 4 [1/cm]
Gain: auto	Aperture: auto	SpeedStd: 2.8

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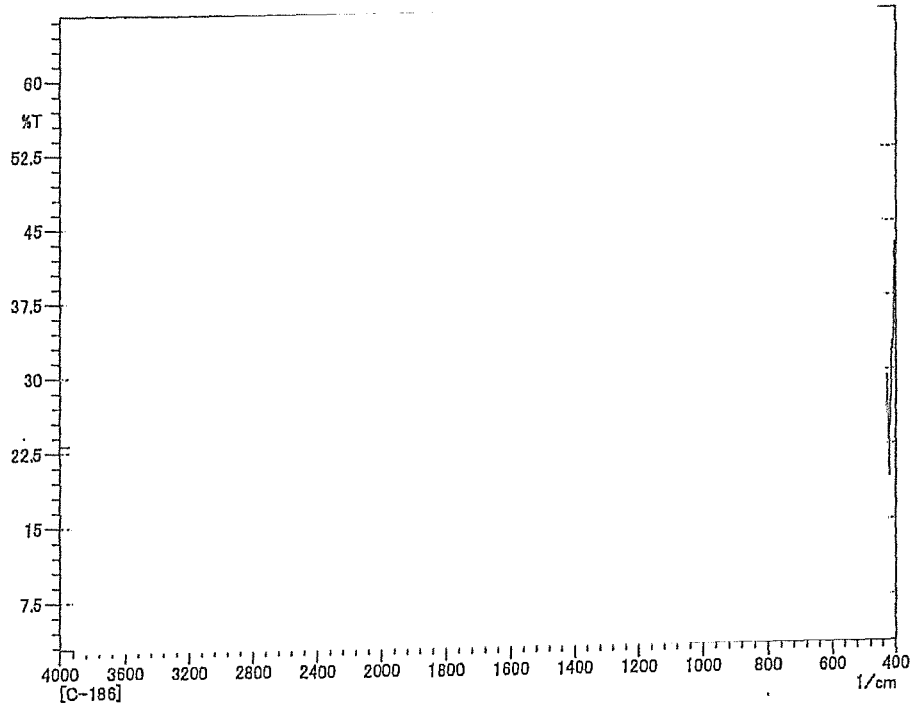
IR spectrum (at the end of the study) measured at our location



File Name:c186e1
Date/Time: 2011/02/25 9:39:17
Type: IRPrestige-21
Units: 1/cm
Min: 399.27
No. of Datapoints: 3735
Gain: auto

Sample: [C-186]
No. of Scans: 20
Ordinate: %T
Max: 4000.43
Data Interval: 0.96
Aperture: auto

Operator: K. Kaneko
Method: KBr
Detector: standard
Apodization: Happ-Genzel
Resolution: 2 [1/cm]
SpeedStd: 2.8

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