

Final Report

Bioconcentration Test of S510 in Carp, *Cyprinus carpio*

Report No. E4 -15022·C277·CH

July 15, 2015

Institute of Ecotoxicology Co., Ltd.

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This report includes 91 pages

Statement

Institute of Ecotoxicology Co., Ltd.

Sponsor: JNC Corporation

Title of the Test: Bioconcentration Test of S510 in Carp, *Cyprinus carpio*

Test No.: C-277

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

“Standards Concerning Testing Laboratories Implementing Tests Concerning New Chemical Substances” on Japanese GLP (Yakushokuhatsu 0331 No. 8, Director-General, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labor and Welfare, Heisei 23・03・31; Seikyoku No. 6, Director-General, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; and Kanpokiatsu No. 110331010, Joint Notice from the Director General of the Environmental Policy Bureau, Ministry of the Environment, Heisei 23・03・29), and “OECD Principles of Good Laboratory Practice” (as revised in 1997).

July 15, 2015

Study Director: _____

Signed in original

Takashi Sato (seal)

Quality Assurance Certificate

Title:	Bioconcentration Test of S510 in Carp, <i>Cyprinus carpio</i>
Sponsor:	JNC Corporation
Study Director:	Takashi Sato
Study Personnel:	Takashi Sato
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 the standard operation procedures, and the original data are accurately presented. The inspection and audit were performed as follows.

		Date of Inspection/audit	Report date to Test Facility Management and Study Director
Inspection of test performance	Water/fish analysis on Day 7 of exposure	June 17, 2015	June 17, 2015
	Water/fish analysis on Day 14 of exposure	June 24, 2015	June 24, 2015
	Water/fish analysis on Day 21 of exposure	July 1, 2015	July 1, 2015
Audit of test protocol	Protocol	May 11, 2015	May 11, 2015
	Revised protocol	June 8, 2015	June 8, 2015
	Alteration in revised protocol	July 13, 2015	July 13, 2015
Audit of final report	Final report	July 9, 2015	July 9, 2015
		July 15, 2015	July 15, 2015

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

Quality Assurance Director : _____

Signed in original
Mikiyasu Goto (seal)
Signature date: July 15, 2015
(Inspection/audit of study conduct, protocol and final report)

Quality Assurance : _____

Signed in original
Mitsuko Takamatsu (seal)
Signature date: July 15, 2015
(Inspection/audit of study conduct, protocol and final report)

Quality Assurance : _____

Signed in original
Miyuki Kuribara (seal)
Signature date: July 15, 2015
(Audit of protocol and final report)

Final Report

– Preface –

1. **Title** Bioconcentration Test of S510 in Carp, *Cyprinus carpio*
2. **Sponsor** JNC Corporation
2-2-1 Otemachi, Chiyoda-ku, Tokyo 100-8105, Japan
3. **Test facility** Institute of Ecotoxicology Co., Ltd.
2-28-1 Yoshino-cho, Kita-ku, Saitama City, 331-0811 Japan
Phone [REDACTED]
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.

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 0331 No.7, Director-General, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labor and Welfare, Heisei 23・03・31; Seikyoku No.5, Director-General, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; and Kanpokiatsu No.110331009, Joint Notice from the Director General of the Environmental Policy Bureau, Ministry of the Environment, Heisei 23・03・29); and OECD Guidelines for the Testing of Chemicals, “No. 305 Bioconcentration: Flow-through Fish Test” (adopted on October 2, 2012).

6. **GLP applied**

Japanese Chemical Substance GLP: “Standards Concerning Testing Laboratories Implementing Tests Concerning New Chemical Substances” (Yakushokuhatsu 0331 No. 8, Director-General, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labor and Welfare, Heisei 23・03・31; Seikyoku No. 6, Director-General, Manufacturing Industries Bureau, Ministry of Economy,

Trade and Industry; and Kanpokiatsu No. 110331010, Joint Notice from the Director General of the Environmental Policy Bureau, Ministry of the Environment, Heisei 23・03・29).
OECD GLP: “OECD Principles of Good Laboratory Practice” (as revised in 1997).

7. Name of the test substance

Chemical name : *trans* – 4–{[3,5–difluoro– 4–(trifluoromethyl)phenoxy]
(difluoro)methyl}–4’– *trans* –propyl–1,1’– bicyclohexyl
Abbreviation : S510 (Lot No. 14NA007)
CAS No. : 208338-86-7

8. Test periods

(1) Test initiation date : May 11, 2015
(2) Duration of the test
Arrival date of fish : May 18, 2015
Acclimation completion date : June 10, 2015
Experimental starting date : June 10, 2015
Experimental completion date : July 8, 2015
(3) Test completion date : July 15, 2015

9. Test results

A bioconcentration test of the test substance S510 in carp, *Cyprinus carpio*, was conducted. The bioconcentration factor (BCF) for 28 days of exposure was summarized in the following table.

Concentration level of exposure	High (Level 1) 0.02 mgL ⁻¹	Low (Level 2) 0.002 mgL ⁻¹
Bioconcentration factor (BCF)	12 to 30-fold	10 to 29-fold

10. Evaluation and discussion of the results and conclusion

The acute toxicity (96-h LC₅₀) of the test substance S510 in Zebrafish (*Danio rerio*) was greater than 25.6 mgL⁻¹. Since the solubility in water was 2.3 × 10⁻² mgL⁻¹, concentrations of the test substance in water were set at 0.02 mgL⁻¹ for the high exposure level (Level 1) and 0.002 mgL⁻¹ for the low exposure level (Level 2). According to the results, the bioconcentration factor (BCF) was less than 50-fold both in Levels 1 and Level 2. Thus, steady state was considered to have reached by day 28 of exposure in both levels. As a result, bioconcentration of the test substance in fish was confirmed to be low.

11. Storage of materials

The following records concerning the tests and samples will be stored for 10 years after receiving notification based on the provisions of CSCL (No. 117, 1973) Article 4, paragraphs 1 or 2, Article 5, paragraph 2, 3, or 8, Article 10, paragraph 3, or Article 14, paragraph 2, and the test substance, reference substance and other item will be stored for 10 years after receiving notification based on the provisions of the CSCL: Article 4, paragraph 1 or 2, Article 5, paragraph 2, 3, or 8, Article 10, paragraph 3, or Article 14, paragraph 2, or the period during which they can be stored stably without affecting their quality, whichever is shorter, at the Institute of Ecotoxicology Co., Ltd.. Storage after the period will be determined after separate discussion.

- (1) Protocol, and its modifications and changes
- (2) Records concerning the test substance, etc.
- (3) Experimental notes, raw data, and charts
- (4) Test substance, reference substance, and other items (at approximately 20°C in the dark)
- (5) Inspection and audit records by quality assurance personnel
- (6) Final report
- (7) Other necessary items

12. Environmental factors that may have affected data reliability

No particular abnormalities were observed.

13. Personnel related to the test and their assignments

Study Director: Takashi Sato

Study Personnel: Takashi Sato

- 96-h LC₅₀ test
- Measurement of water solubility
- Establishment of analysis method for test water
- Establishment of analysis method for fish
- Preparation of calibration curves
- Recovery tests from test water
- Recovery tests from test fish
- Investigation of dispersion method of the test substance in water
- Preliminary test to set the test schedule
- Analysis of test water and fish during the experiment period
- Preparation and maintenance of test solution during the experiment period
- Measurement of aquatic environment during the experiment period
- Measurement of lipid contents in fish body

- Measurement of IR
- Measurement of molar absorption coefficient
- Observation of fish rearing conditions
- Measurement of dissolved organic carbon concentration

Preparation of final report: Takashi Sato

14. Names and affiliations of experts involved in reporting the final report

None

15. Approval of the final report

Study Director: _____

Signed in original

Takashi Sato (seal)

Signature date: July 15, 2015

Main Subject

1. Test substance

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

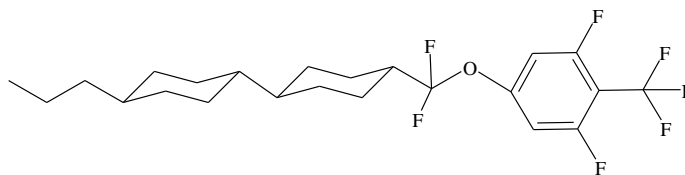
1.1 Name

Chemical name: *trans* – 4 – {[3,5 – difluoro – 4 – (trifluoromethyl)phenoxy] (difluoro)methyl} – 4' – *trans* – propyl – 1,1' – bicyclohexyl

Abbreviation: S510

CAS No.: 208338-86-7

1.2 Structural formula



Molecular formula: $C_{23}H_{29}F_7O$ (MW 454.46)

1.3 Supplier and Lot No.

(1) Supplier JNC Corporation

(2) Lot No. 14NA007

1.4 Identification

The IR spectrum of the test substance obtained before the test was compared with that from the sponsor, and their agreement was confirmed. Further, stability of the test substance during the test period was confirmed by the agreement of the IR spectrum obtained after the test with that measured before the test (see, Ref. 13).

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

Appearance*: White powder

Purity*: $\geq 99.9\%$

Impurities unknown $\leq 0.1\%$

Melting point*: 83°C

Solubility (our measurements at 25°C)

water	$2.3 \times 10^{-2} \text{ mgL}^{-1}$ (Flask method)
<i>n</i> -hexane	$> 1.0 \times 10^5 \text{ mgL}^{-1}$
dichloromethane	$> 1.0 \times 10^5 \text{ mgL}^{-1}$
acetone	$> 1.0 \times 10^5 \text{ mgL}^{-1}$

acetonitrile	$1.0 \times 10^4 \text{ mgL}^{-1}$
dimethylsulfoxide	$1.2 \times 10^3 \text{ mgL}^{-1}$

2. Test methods and analytical methods

This test was based on “Test Methods Concerning New Chemical Substances < Bioconcentration Test of Chemical Substances in the Bodies of Fish and Shellfish>” (Yakushokuhatsu No. 0331-7, Heisei 23/3/31; Seikyoku No. 5, Kanpokiatsu No. 110331009, Heisei 23/3/29; and OECD Guidelines for Testing of Chemicals, “No.305, Bioaccumulation in Fish: Aqueous and Dietary Exposure” (adopted on October 2, 2012).

2.1 Acute toxicity test (96-h LC₅₀) in Zebrafish (*Danio rerio*) for setting the substance concentration (SOP 2.6)

A 96-h LC₅₀ value was obtained at concentrations set based on the results of preliminary test (performed with reference to the method of OECD Test Guideline 203).

(1) Test fish

Zebrafish (*Danio rerio*)

Lot No. : 150310

Supplier: Kamihata Fish Ind., Ltd.

(292-1 Chigusa-cho, Hanamigawa-ku, Chiba City, 262-0012 Japan)

Date received: March 10, 2015

Mean total length: $3.4 \pm 0.1 \text{ cm}$

Mean bodyweight: $0.25 \pm 0.02 \text{ g}$

Acclimation period: from March 10 to May 12, 2015

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

(2) Test solution

Vehicle (Dispersant): Polyoxyethylene Sorbitan Monooleate (Tween 80)

Preparation of a stock solution: A test substance and 4 times the amount of Tween 80 as the test substance were weighed into a glass sample tube and mixed while heating. Boiling ultrapure water was added to the mixture. After cooling to room temperature with stirring, ultrapure water was added to prepare a 1000 mgL⁻¹ stock solution. This stock solution was diluted with test for water (see (6)) to obtain test water.

(3) Test temperature $25.1 \pm 0.1^\circ\text{C}$

(4) Test aquarium Glass with 4 L capacity

(5) Exposure method Semi-static method (replacing water after 48 hours)

(6) Water for test (water for dilution)

Dechlorinated city water of Saitama (hereafter, referred to as “water”)

As a result of the water quality test, it was confirmed that the items shown in Reference 10 comply with the “water quality standards for fisheries water”.

(7) **Number of fish** 10 fish/4 L test aquarium

(8) **Experiment period** from May 12 to May 16, 2015

(9) **Sensitivity test of *Danio rerio***

A sensitivity test was conducted using $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ as a standard substance, and fish from the same lot that passed our specified value (96-h LC_{50} : 0.08 to 0.3 mgL^{-1}) were used as test fish.

(10) **Test results**

The test substance was dispersed using a 4-times amount of Tween 80. When the concentration of the test substance should be set so that Tween 80 concentration does not exceed 100 mgL^{-1} , the upper limit of test substance concentration is 25 mgL^{-1} . Since the 96-h LC_{50} value in the preliminary test was greater than 25 mgL^{-1} , a limit test with a maximum concentration of 25 mgL^{-1} was applied. As a result, the 96-h LC_{50} value was confirmed to be greater than 25.6 mgL^{-1} . The concentration of the test substance is the initial measured value determined by HPLC (see the table below).

Test water		Mortality (%)			
Compound	Concentration (mgL^{-1})	24 h	48 h	72 h	96 h
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (sensitivity test)	0.5	50	80	100	100
	0.05	0	0	0	0
Test substance	25.6	0	0	0	0
Control (water with Tween 80)	100	0	0	0	0
Control (water)	—	0	0	0	0

2.2 Performance of the bioconcentration test

(1) **Test fish**

To confirm the bioconcentration in fish, the carp (*Cyprinus carpio*), representative fish species in Japan, was selected (SOP 2. 1-2) as the test fish.

Common carp: *Cyprinus carpio* (Lot No. 150518 No.3)

Supplier: Kitamura Carp Breeding Farm (12-388 Gunchiku, Yashiro-shi, Kumamoto Prefecture, Japan)

Date purchased: May 18, 2015

Feeding and acclimation:

After being received by the Institute of Ecotoxicology Co., Ltd., the fish with a total length of approx. 8.0 ± 4.0 cm were selected (SOP 2.4). After treatment with Elevage® (4-[2-(5-nitro-2-furanyl)ethenyl]benzoic acid, sodium salt) and rock salt, acclimation was started in acclimation tank No. 1 (SOP2.3, SOP2.5).

Finally, they were acclimatized at water temperature $25 \pm 2.0^{\circ}\text{C}$ for 7 days and used for the test, after confirming that the mortality rate was less than 5%.

Test fish:

Mean body weight	4.45 ± 0.33 g (SOP 2.8)
Mean total length	7.8 ± 0.1 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	4.1% (Mean value, n=2) (SOP 2.9)
After experiment	3.8% (Mean value, n=2) (SOP 2.9)

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

(2) Feeding pellet

Feed type: Maruha-jirushi compound feed for juvenile carp C-2

Crude proteins	42.0%
Crude lipids	3.0%

Manufacturer: Hayashikane Sangyo Co., Ltd.

Feeding method: fed every day in an amount of approximately 1.5% of the bodyweight, except the day before sampling on which no feed was given.

(3) Conduct of the test

Water for test: as described in Section 2.1 (6).

Solution of the test substance

Preparation of a stock solution:

A test substance and 4 times its amount of Tween 80 were weighed into a glass sample tube and mixed while heating. Boiling ultrapure water was added to the mixture. After cooling to room temperature with stirring, ultrapure water was added to prepare a 1000 mgL^{-1} stock solution. This stock solution was diluted with water to obtain test water.

High exposure level (Level 1):

The stock solution was diluted with water to 2.0 mgL^{-1} . This was further diluted with water and continuously supplied to the test aquarium.

Low exposure level (Level 2):

The stock solution was diluted with water to 0.2 mgL^{-1} . This was further diluted with water and continuously supplied to the test aquarium.

Control: A 8 mgL⁻¹ solution of Tween 80 was diluted with water and continuously supplied to the test aquarium.

Bioconcentration test equipment: see Ref. 1

Test aquarium: “Flow-through system”

Glass with 100 L capacity (amount of test water 50 L)

Flow rate: 300 mL min⁻¹ (432 L day⁻¹)

No. 1: High exposure level (Level 1), No. 2: Low exposure Level (Level 2),

No. 3: Control

Test concentration:

The concentration of the test substance was set below its solubility in water i.e. 2.3×10^{-2} mgL⁻¹. Since the 96-h LC₅₀ in the zebrafish was greater than 25.6 mgL⁻¹, 1/1280 and 1/12800 were used for safety factors, and the concentrations were set as shown in the following table.

Exposure level	High (Level 1)	Low (Level 2)	Control
Concentration of the test substance	0.02 mgL ⁻¹	0.002 mgL ⁻¹	–
Concentration of Tween 80	0.08 mgL ⁻¹	0.008 mgL ⁻¹	0.08 mgL ⁻¹

Test condition:

Temperature of test water: $24.7 \pm 0.3^{\circ}\text{C}$

pH of test water: 7.6 ± 0.1

DO of test water: $7.36 \pm 0.22 \text{ mgL}^{-1}$

(Temperature, pH and DO on each analytical day were shown in Ref. 2-2)

Total organic carbon concentrations in the test water (SOP 2.12):

High exposure level (Level 1) 0.4886 mgL⁻¹

Low exposure level (Level 2) 0.1364 mgL⁻¹

Control (with dispersant) 0.2262 mgL⁻¹

(10 mgL⁻¹ or less in all 3 groups).

Light type: UV- cut fluorescent lamp

Photoperiod: Approximately 10 hours per day

Experiment period: from June 10 to July 8, 2015

Analysis schedule: shown in Section 2.8.

Number of test fish: High exposure level (Level 1) 45

Low exposure level (Level 2) 45

Control 15

Number of analyzed fish and number of repetitions:

2 fish were paired for a single analysis with 2 repetitions (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)

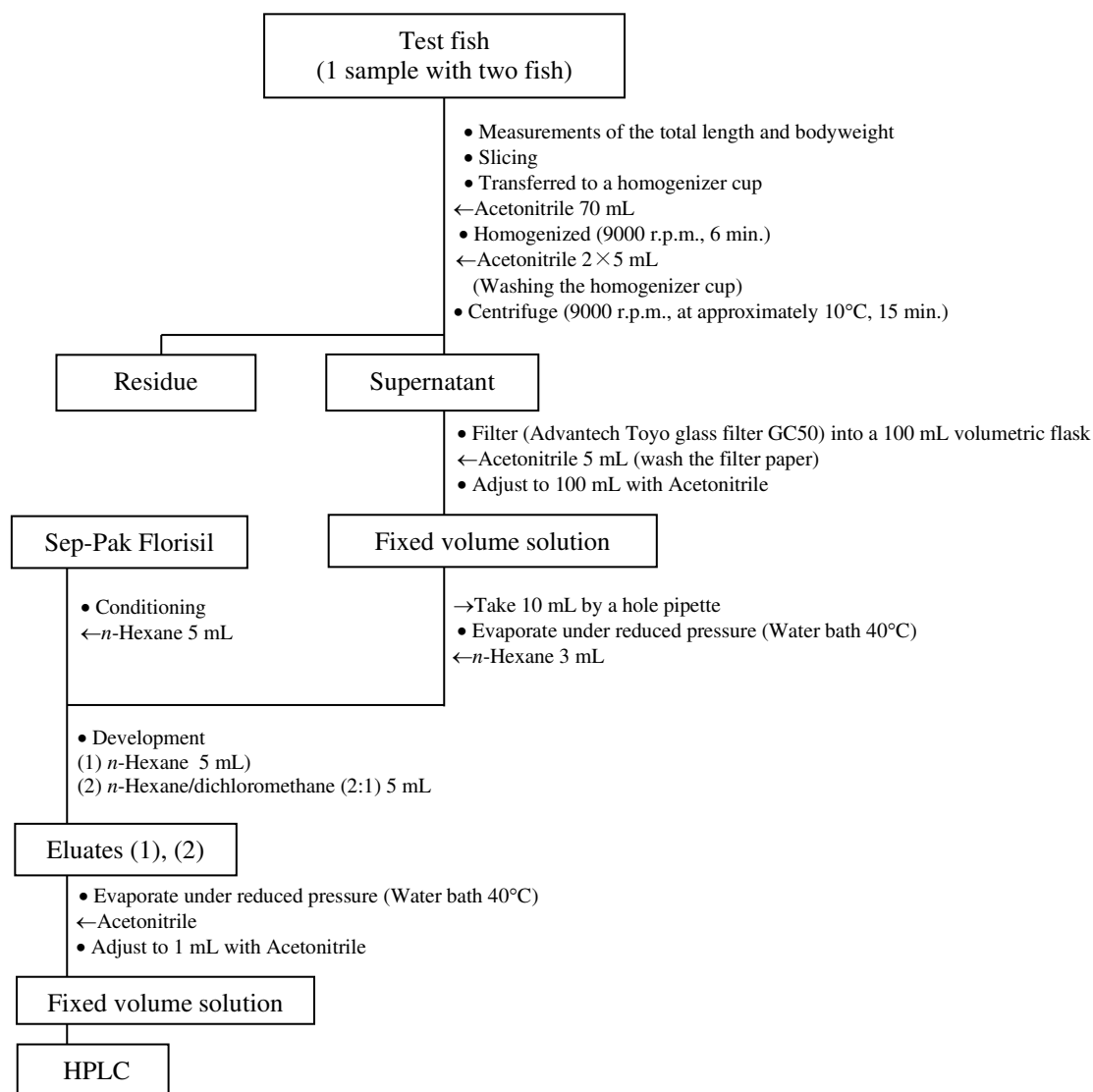
Pretreatment:

On each analysis day, 6 fish each from Level 1 and Level 2, and 2 fish from Control are sampled using a landing net. After immersing them in ice water and suspending them, the surface of the fish was washed with tap water and then lightly wiped with gauze. Body weight was measured to the nearest 0.01 g on a balance (SOP/INS/4.2.2), and the total length, from the tip of the mouth to the tip of the tail fin, to the nearest 0.1 cm. In each test group, 2 fish were used as one sample in duplicate analysis. Remaining fish are put into a plastic container and stored in a freezer after wrapped separately with aluminum-foil and packed in a plastic bag (SOP 2.15). Fish were put into a homogenizer cup after slicing. After adding 70 mL of acetonitrile, the mixture was homogenized at 9000 r.p.m. for 6 min (SOP/INS/4.17.2). The homogenate was transferred to a centrifuge tube. The homogenizer cup was rinsed twice with 5 mL of acetonitrile and the washings were added to the centrifuge tube. After centrifugation for 15 min (9000 r.p.m., at approximately 10°C) (SOP/INS/4.16.3), the supernatant was filtered (Advantech Toyo glass filter GC50) into a 100 mL measuring flask. The filter paper was rinsed with 5 mL of acetonitrile, and the volume was adjusted to 100 mL with acetonitrile. 10 mL was aliquoted to a 50 mL round-bottom flask and dried under reduced pressure using a rotary evaporator (SOP/INS/4.14.3.2) (water bath 40°C). The residue was dried by blowing air.

Separately, a Sep-Pak Florisil (Waters, Milford, MA, USA) was conditioned by eluting 5 mL of *n*-hexane. The fish extract in the round-bottom flask was dissolved in 3 mL of *n*-hexane and injected into the conditioned Sep-Pak florisil, and processed in the following order.

(1) The inside of the round-bottom flask was washed with 5 mL of *n*-hexane, and the washing was injected. (2) 5 mL of *n*-hexane/dichloromethane (2:1, v/v) was injected into the Sep-Pak, and the eluates (1) and (2) were received in a 50 mL round-bottom flask. The flow rate was approximately 3 mL/min throughout (1) and (2). The eluate was dried using a rotary evaporator (water bath 40°C). The dried content was dissolved in a small amount of acetonitrile, and transferred to a 1 mL measuring flask while washing. The volume was adjusted to 1 mL and used for high performance liquid chromatography (HPLC) measurement.

The pretreatment process is shown in the following flowsheet.

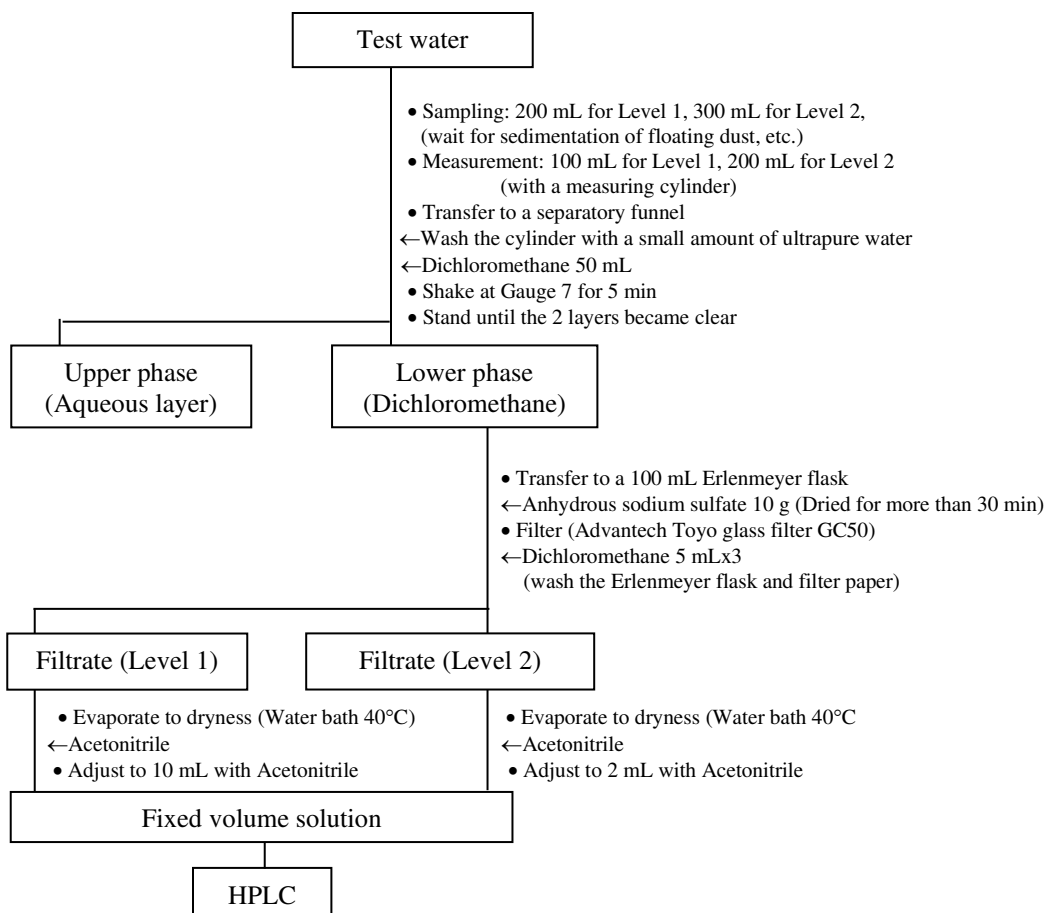


2.3.2 Analytical method for test water (SOP 2.10)

Pretreatment:

Test water, approximately 200 mL and 300 mL from Level 1 and 2, respectively, was sampled from the central area of each aquarium. After waiting for sedimentation of floating dust and etc., a supernatant, 100 mL and 200 mL for Level 1 and 2, respectively, was measured using a measuring cylinder, and transferred to a 200 mL and a 300 mL separatory funnel, respectively. The cylinder was rinsed with a small amount of ultrapure water and the washings were added to the separatory funnel. Dichloromethane (50 mL each for Level 1 and Level 2) was added and the contents were shaken vigorously for 5 min (SOP/INS/4.15). After standing until the two layers become transparent, the lower layer (dichloromethane) was transferred to a 100 mL Erlenmeyer flask. 10 g of anhydrous sodium sulfate was added and allowed to stand for 30 min or longer to

remove water. After that, sodium sulfate was removed by filtering (Advantech Toyo glass filter GC50) into a 100 mL round-bottom flask. The filter paper and the flask were washed three times with 5 mL of dichloromethane, and washings were also filtered to the round-bottom flask. The filtrate was evaporated to dryness (water bath 40°C) using a rotary evaporator (SOP/INS/4.14.3.2). The dried content was dissolved in a small amount of acetonitrile and transferred to the measuring flask (10 mL for Level 1 and 2 mL for Level 2) while washing. After adjusting to each volume, resulting solution was used for HPLC measurement. The pretreatment process is shown in the following flowsheet.



The water analysis for Control at the start of the test followed the method for Level 2.

2.3.3 Quantification method

[for fish analysis]

<Apparatus>

High performance liquid chromatography LC-10A (Shimadzu Corporation)

System No. 6 (SOP/INS/4.11.5)

<Analytical conditions>

Column	Asahipak C8-50 4E 4.6 × 250 mm (5 μm) (Lot No. P103321) Showa Denko K.K.
Mobile phase	Acetonitrile/water (85:15)
Flow rate	1.0 mL/min
Temperature	40°C
Detector	UV-VIS 214 nm
Injection rate	20 μL

<Quantitative calculation>

Instrument	LCsolution (Shimadzu Corporation)
Calculation method	2-point calibration method (Absolute calibration method)

The quantification of samples was performed on the analysis day by calibration using two standard solutions.

[For water analysis]

< Apparatus >

High performance liquid chromatography LC-10A (Shimadzu Corporation)
System No. 6 (SOP/INS/4.11.5)

<Analytical conditions>

Column	InertSustain C18 4.6 × 250 mm (5 μm) (Lot No. 1BR98005) GL Sciences Inc.
Mobile phase	Acetonitrile
Flow rate	1.0 mL/min
Temperature	40°C
Detector	UV-VIS 214 nm
Injection rate	20 μL

<Quantitative calculation>

Instrument	LCsolution (Shimadzu Corporation)
Calculation method	Two-point calibration method (Absolute calibration method)

The quantification of samples was performed on the analysis day by calibration using two standard solutions.

- The UV/visible absorption spectrum of the test substance is shown in Ref. 12.

2.4 Calibration curve by HPLC for fish analysis

2.4.1 Preparation of standard solutions

The test substance, each 4.90 mg and 3.10 mg, was weighed with a balance, Mettler Toledo XS105DU (SOP/INS/4.1.4), into a 50 mL measuring flask, and the volume was adjusted with acetonitrile. The concentrations of these solutions were 97.9020 mgL⁻¹ and 61.9380 mgL⁻¹, respectively, when considering the purity. Each solution was diluted with acetonitrile to prepare standard solutions for calibration curve.

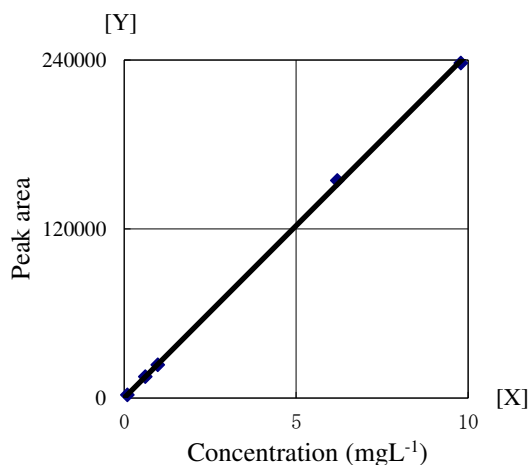
2.4.2 Preparation of calibration curve

A calibration curve was prepared using the standard solutions prepared in Section 2.4.1 to confirm the linearity for quantitative measurement.

Solution No.	Concentration [X] (mg L ⁻¹)	Peak area [Y]
1	0.0979	2151
2	0.6194	15037
3	0.9790	23500
4	6.1938	154396
5	9.7902	237859

Correlation formula: $Y = 24459.1955 X + 99.4172$
(by least square method)

Correlation coefficient: $r = 1.000$



- The chromatograms for preparing the calibration curve is shown in Ref. 6-1.

2.5 Calibration curve by HPLC for water analysis

2.5.1 Preparation of standard solutions

The test substance, each 4.82 mg and 3.16 mg, was weighed with a balance, Mettler Toledo XS105DU (SOP/INS/4.1.4), into a 50 mL measuring flask, and the volume was adjusted with acetonitrile. The concentrations of these solutions were 96.3036 mgL⁻¹ and 63.1368 mgL⁻¹, respectively, when considering the purity. Each solution was diluted with acetonitrile to prepare standard solutions for calibration curve.

2.5.2 Preparation of calibration curve

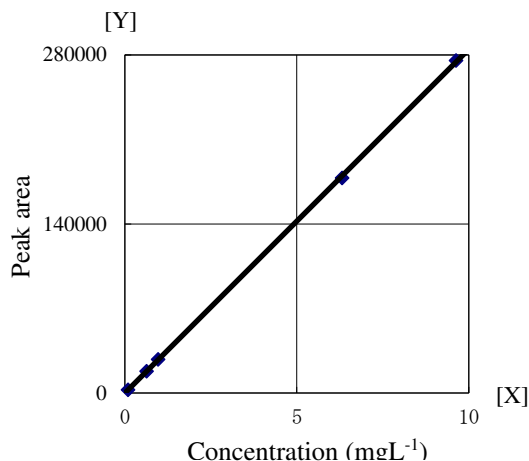
A calibration curve was prepared using the standard solutions prepared in Section 2.5.1 to confirm the linearity for quantitative measurement.

Solution No.	Concentration [X] (mg L ⁻¹)	Peak area [Y]
1	0.0963	2745
2	0.6314	18011
3	0.9630	27867
4	6.3137	178107
5	9.6304	275278

Correlation formula: $Y = 28482.0941 X - 53.6066$

(by least square method)

Correlation coefficient: $r = 1.000$



- The chromatograms for preparing the calibration curve is shown in Ref. 6-2.

2.6 Recovery test and blank test for the test substance

2.6.1 Recovery from fish (SOP 2.11)

Fish bodies (two fish for one sample) were crashed with a blender after mincing. After adding the acetonitrile solution (0.2 mL) of the test substance, pretreatment was processed according to “2.3.1 Analytical method for fish” for HPLC analysis. The recovery rates were shown in the following table. The amount of test substance added, 1.964 μg , was calculated to be 0.376 $\mu\text{g g}^{-1}$ for a fish weight of 2.61 g (5.22 g/2 fish), which is equivalent to a bioconcentration factor (BCF) of approximately 188-fold in Level 2 (0.002 mg L^{-1}) group.

Added group

Sample No.	Fish weight (g)	Fixed volume (mL)	Concentration factor	Amount added		Amount recovered (measured area)	Recovery rate (%)
				Measured area	Calculated concentration (mg L^{-1})		
1	2.47 2.73	100	1/10	4594	0.1964	3936	85.7
2	2.54 2.55	100	1/10	4594	0.1964	3912	85.2
3	2.66 2.59	100	1/10	4594	0.1964	3613	78.6

Mean recovery rate: $83.2 \pm 3.2\%$

<Background of the fish>

After measuring weight and total length, fish bodies (two fish as one sample) were crashed with a blender after mincing. After adding 0.2 mL of acetonitrile, pretreatment was processed

according to “2.3.1 Analytical method for fish” for HPLC analysis. No peak corresponding to the test substance was detected.

Control group

Sample No.	Fish bodyweight (g)	Fixed volume (mL)	Concentration factor	Measured area
1	3.01 2.46	100	1/10	N.D.
2	2.40 2.73	100	1/10	N.D.
3	2.84 2.44	100	1/10	N.D.

- The chromatograms for calculating the recovery rates from fish are shown in Ref. 7-1.

2.6.2 Recovery from test water (SOP 2.10)

Water for fish breeding was collected, and the test substance solution (dispersed with Tween 80) was added. Pretreatment was processed according to “2.3.2 Analytical method for water” for HPLC analysis. As shown in the following table, the mean recovery rates of the test substance were $91.8 \pm 1.2\%$ and $97.8 \pm 1.4\%$ for Level 1 and Level 2, respectively.

Recovery rate for Level 1 (Added group)

Sample No.	Amount of water sampled (mL)	Fixed volume (mL)	Amount added		Amount recovered (measured area)	Recovery rate (%)
			Measured area	Calculated concentration (mgL ⁻¹)		
1	100	10	5521	0.1932	5027	91.0
2	100	10	5521	0.1932	5005	90.7
3	100	10	5521	0.1932	5160	93.5

Mean recovery rate: $91.8 \pm 1.2\%$

Recovery rate for Level 2 (Added group)

Sample No.	Amount of water sampled (mL)	Fixed volume (mL)	Amount added		Amount recovered (measured area)	Recovery rate (%)
			Measured areas	Calculated concentration (mgL ⁻¹)		
1	200	2	5590	0.1975	5394	96.5
2	200	2	5590	0.1975	5433	97.2
3	200	2	5590	0.1975	5578	99.8

Mean recovery rate: $97.8 \pm 1.4\%$

<Background of the test water>

Water for fish breeding was collected, and Tween 80 was added. Pretreatment was processed according to “2.3.2 Analytical method for water” for HPLC analysis. No peak corresponding to the test substance was detected for both Level 1 and Level 2.

Control for Level 1				Control for Level 2			
Sample No.	Sampling volume (mL)	Fixed volume (mL)	Measured area	Sample No.	Sampling volume (mL)	Fixed volume (mL)	Measured area
1	100	10	N.D.	1	200	2	N.D.
2	100	10	N.D.	2	200	2	N.D.
3	100	10	N.D.	3	200	2	N.D.

■ The chromatograms for calculating the recovery rates of the test substance from water are shown in Ref. 7-2.

2.7 Quantification limit

2.7.1 Quantification limit for fish

In the HPLC chromatogram of fish analysis, no background peak was observed at the position of the test substance. The following table shows the quantifiable bioconcentration factor when the peak area of 300 was set as the detection limit.

Concentration set for exposure	Detection limit concentration ¹⁾ (mgL ⁻¹)	Quantification limit ²⁾ (μgg ⁻¹)	Bioconcentration factor ³⁾	
			Level 1 (0.02 mgL ⁻¹)	Level 2 (0.002 mgL ⁻¹)
Level 1 0.02 mgL ⁻¹	0.0082	0.0189	1-fold or greater	9-fold or greater
Level 2 0.002 mgL ⁻¹				

1) The detection limit concentration was calculated from the calibration curve in Section 2.4.2.

2) The quantification limit for fish was calculated by the following formula.

Calculations were performed with a fish weight of 5.22 g (2.61 g per fish, 2 fish for 1 sample), a recovery rate of 83.2%, a fixed volume of 100 mL, and a concentration factor of 1/10.

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

$$3) \text{ Bioconcentration factor (BCF)} = \frac{\text{Quantification limit (}\mu\text{gg}^{-1}\text{)}}{\text{Concentration set for exposure (theoretical value) (mgL}^{-1}\text{)}}$$

2.7.2 Quantification limit for test water

In the HPLC chromatogram of water analysis, no background peak was observed at the position of the test substance. The following table shows the quantification limit when the peak area of 300 was set as the detection limit.

Concentration set for exposure		Detection limit concentration ¹⁾ (mgL ⁻¹)	Quantification limit ²⁾ (mgL ⁻¹)	
Level 1	Level 2		Level 1 (0.02 mgL ⁻¹)	Level 2 (0.002 mgL ⁻¹)
0.02 mgL ⁻¹	0.002 mgL ⁻¹	0.0124	0.00135	0.000127

- 1) The detection limit concentration was calculated from the calibration curve in Section 2.5.2.
- 2) The quantification limit for the test water was calculated by the following formula.
Calculations were performed with fixed volume: 10 mL for Level 1 and 2 mL for Level 2, recovery rate: 91.8% for Level 1 and 97.8% for Level 2, and amount of water sampled: 100 mL for Level 1 and 200 mL for Level 2.

$$\text{Quantification limit (mgL}^{-1}\text{)} = \frac{\text{Detection limit (mgL}^{-1}\text{)} \times \text{Fixed volume (mL)}}{\text{Recovery rate (\%)} \times \text{Amount of water sampled (mL)}} \times 100$$

2.8 Preliminary test

Test fish (*Cyprinus carpio*) were exposed to the concentration of Level 1 (0.002 mgL⁻¹) under the flow-through system. On day 7 of exposure, test water and fish (2 fish for 1 sample, n=2) were collected and analyzed according to "2.3.1 Analytical method for fish" and "2.3.2 Analytical method for water". As a result, BCFs in duplicate of the test substance were found to be 62-fold and 51-fold.

2.9 Schedule of analyses for fish and test water

Analyses were conducted according to the following schedule:

	Sampling date	Days after exposure	Analysis for Test group	Analysis for Control group
Exposure period (28 days)	June 10, 2015	0	water	water and fish lipid contents
	June 17, 2015	7	water and fish	—
	June 24, 2015	14	water and fish	—
	July 1, 2015	21	water and fish	—
	July 6, 2015	26	water and fish	—
	July 8, 2015	28	water and fish	fish lipid contents

On the day of water analysis, pH, DO (set to approximately 7 mgL⁻¹) and water temperature in each aquarium were measured and recorded (pH meter: SOP/INS/4.3.3, DO meter: SOP/INS/4.4.4).

Water analysis was performed according to "2.3.2 Test water analysis method" in compliance with SOP 2.10 (measurement for concentration of the test substance in test water). The variation was confirmed to be within $\pm 20\%$ of the average measured value during the test period.

Fish analysis was performed according to "2.3.1 Analysis method for fish" in compliance with SOP 2.11 (measurement of concentration of test substance in test fish). The lipid content was determined by the "chloroform-methanol extraction method" in compliance with SOP 2.9 (Determination of lipid content of test fish). The variation at the start and end of the experiment was confirmed to be within $\pm 25\%$.

Storage of fish before analysis and disposal of waste during analysis were performed according to SOP 2.15 (storage before analysis of fish) and SOP 2.16 (disposal of waste during analysis of fish).

3. Test results

3.1 Concentration of the test substance in test water

Concentrations of the test substance in test water (mgL^{-1}) are shown in the following table.

Test group \ Days after exposure	0	7	14	21	26	28
Level 1	0.0179	0.0201	0.0203	0.0181	0.0202	0.0198
Level 2	0.00176	0.00200	0.00198	0.00175	0.00185	0.00185

■ Analytical data for test water are shown in Ref. 5.

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

Level 1: $0.0194 \pm 0.00100 \text{ mgL}^{-1}$

Level 2: $0.00186 \pm 0.0000967 \text{ mgL}^{-1}$

Calculation was performed by the following formula.

Mean

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

Standard deviation

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

■ The time-dependent changes in concentration in water, water temperature, pH and DO are shown in Ref. 2. The chromatograms of analysis on days 0, 7, 14, 21, 26 and 28 of exposure are shown in Ref. 9-1, and those for Control group are shown in Ref. 9-2.

3.2 Bioconcentration factor (BCF)

Results of this test confirmed that the bioconcentration factor (BCF) of the test substance reached a steady state by 28th day of exposure in both Level 1 and Level 2. Therefore, the exposure was terminated on day 28. The following table shows the BCF of the test substance for each analysis day.

Days after exposure	Level 1		Level 2	
	Sample No. (n=2)	Bioconcentration factor (BCF)	Sample No. (n=2)	Bioconcentration factor (BCF)
7	No. 1	22	No. 1	20
	No. 2	16	No. 2	23
14	No. 1	14	No. 1	14
	No. 2	18	No. 2	14
21	No. 1	12	No. 1	14
	No. 2	18	No. 2	10
26	No. 1	15	No. 1	29
	No. 2	21	No. 2	20
28	No. 1	12	No. 1	22
	No. 2	30	No. 2	< 7

(Note 1) Analysis was performed in duplicate (n=2), No.1 and No.2, respectively, where 2 fish were used as 1 sample.

(Note 2) The fish weight and other data of analysis are shown in Ref. 4, and the chromatograms of analysis on days 7, 14, 21, 26 and 28 are shown in Ref. 8-1. The chromatograms for Control group are shown in Ref. 8-2.

(Note 3) The time-dependent change in the BCF is shown in Ref. 3.

(1) Mean and standard deviation of bioconcentration factor (BCF)

The mean and standard deviation of BCF during the experiment period are:

Level 1: 18 ± 5 (n=10)

Level 2: 18 ± 6 (n=9)

Calculated as:

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

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 steady state

Since the BCFs were less than 50-fold throughout the exposure period, both Level 1 and Level 2 groups were considered to have reached steady state, and the exposure was terminated on day 28.

Variation (%) of the BCF in three measurements at intervals of 48 h or longer in Level 1 group was as follows:

$$\text{Variation (\%)} = \frac{6}{18} \times 100 = 33$$

(Calculated from the BCFs on 21, 26, and 28 days after exposure)

In Level 2, variation (%) of the BCF was not calculated, because the concentration in fish on day 28 (1 out of 2 samples) was below the detection limit.

$$\text{Variation (\%)} = \frac{\text{Standard deviation of BCF for 3 consecutive time points}}{\text{Mean BCF for 3 consecutive time points}}$$

< Reference items>

The test fish in Control group (n=1) were analyzed on day 0, before the start of exposure, and on day 28, after the end of exposure. No peaks were detected at the position of the test substance in the HPLC chromatograms, as observed in the recovery test for Control group. The chromatograms are shown in Ref. 8-2.

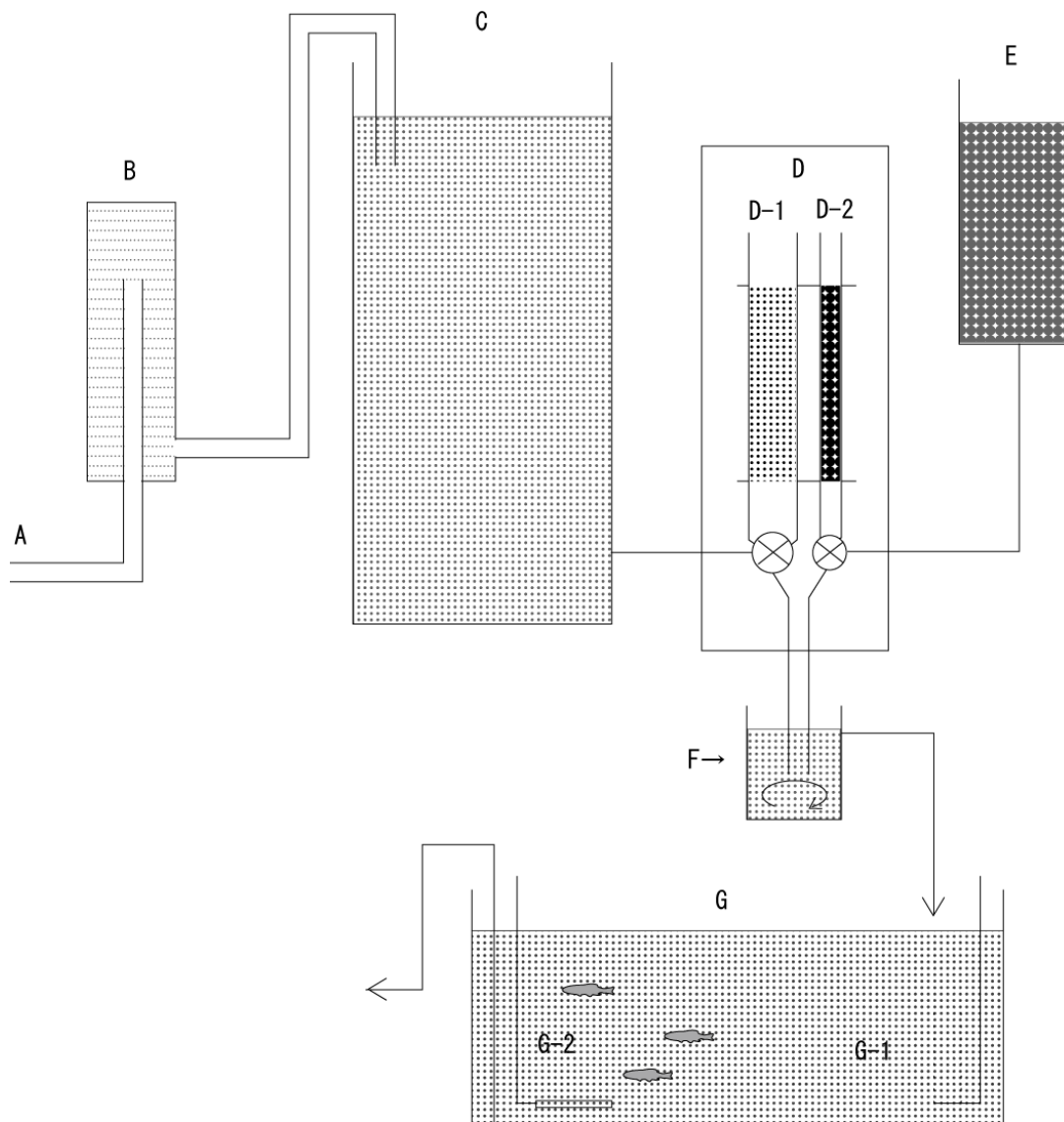
3.3 Evaluation and discussion of the results and conclusion

The 96-h LC₅₀ of the test substance S510 in Zebrafish (*Danio rerio*) was greater than 25.6 mgL⁻¹. Since the solubility in water was 2.3 × 10⁻² mgL⁻¹, concentrations of the test substance in water were set at 0.02 mgL⁻¹ for the high exposure level (Level 1) and 0.002 mgL⁻¹ for the low exposure level (Level 2). According to the results, the bioconcentration factor (BCF) was less than 50-fold both in Levels 1 and 2. Thus, steady state was considered to have reached by day 28 of exposure in both levels. As a result, bioconcentration of the test substance in fish was confirmed to be low.

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Reference 1 Outline of equipment for bioconcentration test



A: Tap water
B: Filter/Activated charcoal tank
(Dechlorination equipment)

C: Water tank for water
(Dechlorinated water)

D: Regulator for flow rate and mixing ratio
D-1: Water for test
D-2: Test substance stock solution

E: Tank for stock solution of test substance
(10L)

F: Mixing tank (2L) (Mechanical stirrer)

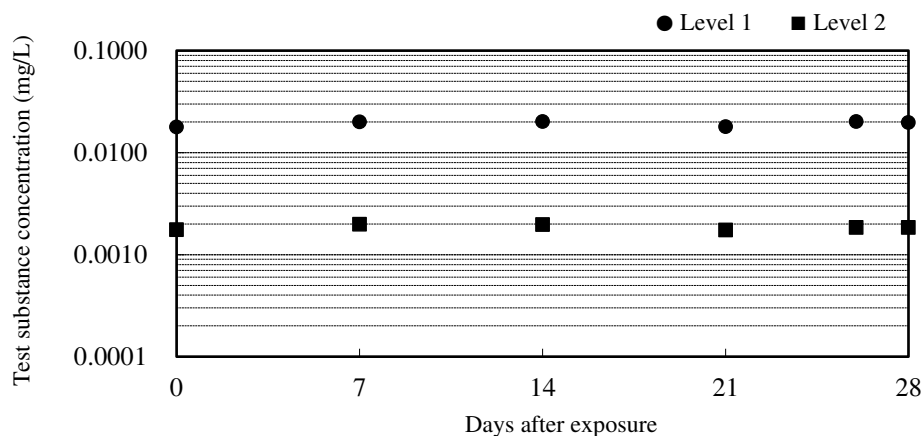
G: Test aquarium (60L)

G-1: Aeration

G-2: Temperature regulator/heater

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

2-1 Concentration of the test substance in water

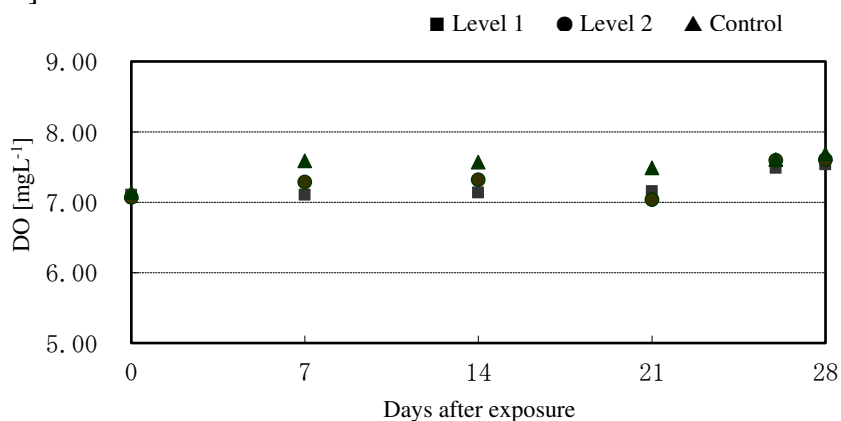


2-2 Measurement of water temperature, pH and DO in water

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

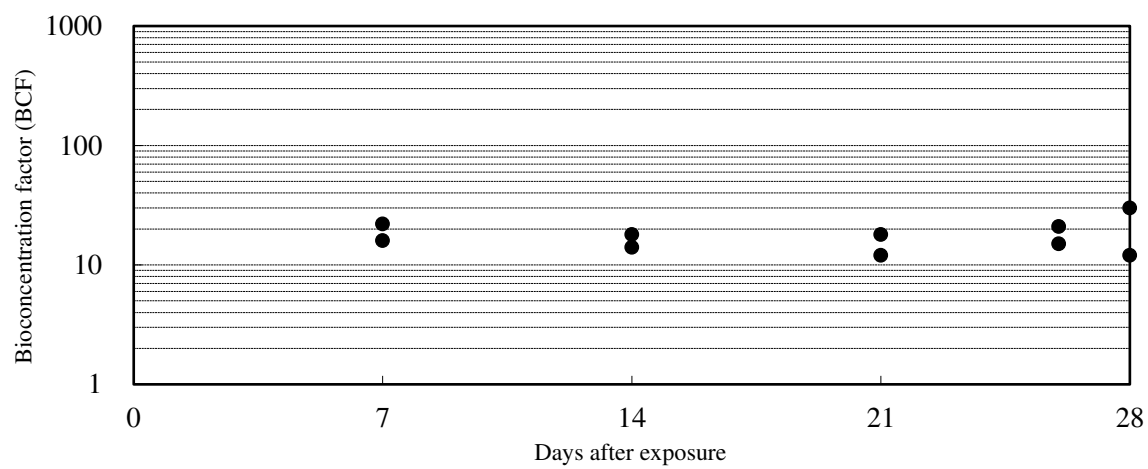
Days of exposure	Level 1			Level 2			Control		
	Water temperature (°C)	pH	DO (mgL ⁻¹)	Water temperature (°C)	pH	DO (mgL ⁻¹)	Water temperature (°C)	pH	DO (mgL ⁻¹)
0	24.5	7.5	7.11	24.5	7.5	7.07	24.5	7.5	7.14
7	24.8	7.6	7.11	24.3	7.5	7.29	24.4	7.6	7.59
14	25.3	7.6	7.14	24.9	7.6	7.32	24.9	7.6	7.57
21	24.8	7.7	7.16	24.5	7.7	7.04	24.4	7.7	7.49
26	25.2	7.6	7.49	25.0	7.6	7.60	24.7	7.6	7.61
28	24.6	7.6	7.54	24.7	7.6	7.61	24.6	7.7	7.69

[DO in water]

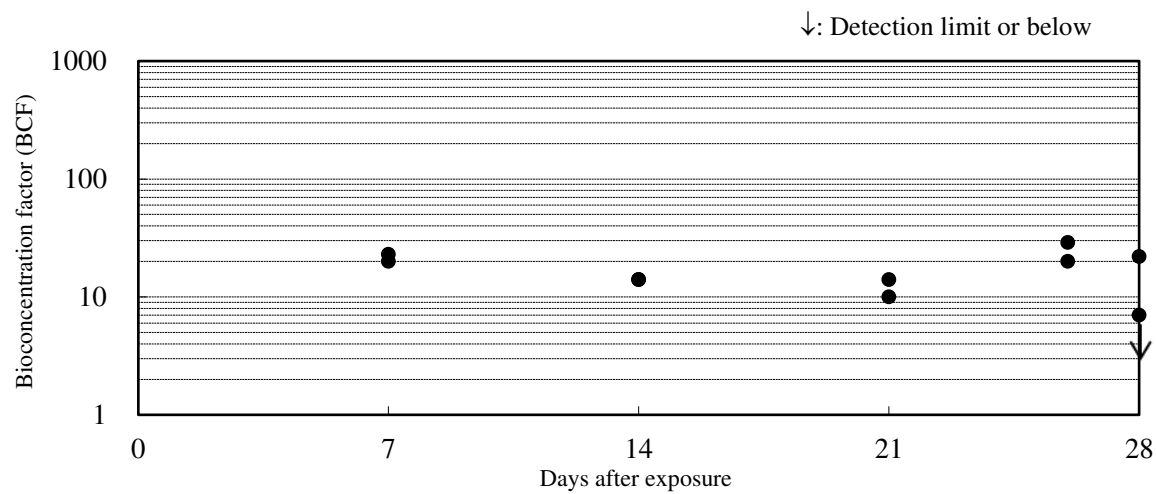


Reference 3 Time-dependent changes in bioconcentration factor

[Level 1]



[Level 2]



Reference 4 Fish analysis data

[Level 1]

Item Sample (n=2)	A Fixed volume (mL)	B Concentration factor in preparation	C Peak area	D Concentration from 2-point calibration curve (mgL ⁻¹)	E Amount deposited in fish (μg)	F Fish weight (g)	G Recovery rate (%)	H Concentration in fish (μgg ⁻¹)	I Concentration in water (mgL ⁻¹)	J Concentration factor (BCF)	K Date of measurement
Day 7 No.1	100	1/10	8558	0.3541	3.541	9.42 [4.68, 4.74]	83.2	0.452	0.0201	22	6.17.2015
Day 7 No.2	100	1/10	5860	0.2450	2.450	9.18 [4.88, 4.30]	83.2	0.321	0.0201	16	
Day 14 No.1	100	1/10	5096	0.2069	2.069	8.90 [4.36, 4.54]	83.2	0.279	0.0203	14	6.24.2015
Day 14 No.2	100	1/10	6776	0.2747	2.747	8.85 [4.26, 4.59]	83.2	0.373	0.0203	18	
Day 21 No.1	100	1/10	3990	0.1571	1.571	9.04 [4.62, 4.42]	83.2	0.209	0.0181	12	7.1.2015
Day 21 No.2	100	1/10	6267	0.2482	2.482	9.38 [4.64, 4.74]	83.2	0.318	0.0181	18	
Day 26 No.1	100	1/10	5893	0.2343	2.343	8.99 [4.52, 4.47]	83.2	0.313	0.0202	15	7.6.2015
Day 26 No.2	100	1/10	8049	0.3185	3.185	9.21 [5.10, 4.11]	83.2	0.416	0.0202	21	
Day 28 No.1	100	1/10	3890	0.1561	1.561	8.18 [4.51, 3.67]	83.2	0.229	0.0198	12	7.8.2015
Day 28 No.2	100	1/10	10446	0.4184	4.148	8.45 [4.47, 3.98]	83.2	0.590	0.0198	30	

Calculation methods:

Amount deposited in fish

$$E = A \times B \times D$$

Concentration in fish

$$H = \frac{E \times 100}{F \times G}$$

F: total weight for 2 fish

Concentration factor (BCF)

$$J = \frac{H}{I}$$

(Note) Analysis was performed in duplicate (n=2), No.1 and No.2, respectively, where 2 fish were used as 1 sample.
The weight of each two fish is shown in [] of column F.

[Level 2]

Items Samples	A Fixed volume (mL)	B Concentration Factor in preparation	C Peak area	D Concentration from two-point calibration curve (mgL ⁻¹)	E Amount deposited in fish (μg)	F Fish weight (g)	G Recovery rate (%)	H Concentration in fish (μgg ⁻¹)	I Concentration in water (mgL ⁻¹)	J Concentration factor (BCF)	K Date of measurement
Day 7 No.1	100	1/10	524	0.0292	0.292	8.98 [4.97, 4.01]	83.2	0.0391	0.00200	20	6.17. 2015
Day 7 No.2	100	1/10	633	0.0336	0.336	8.91 [4.28, 4.63]	83.2	0.0453	0.00200	23	
Day 14 No.1	100	1/10	463	0.0199	0.199	8.82 [4.48, 4.34]	83.2	0.0271	0.00198	14	6.24. 2015
Day 14 No.2	100	1/10	467	0.0200	0.200	8.65 [4.37, 4.28]	83.2	0.0278	0.00198	14	
Day 21 No.1	100	1/10	499	0.0175	0.175	8.49 [4.12, 4.37]	83.2	0.0248	0.00175	14	7.1. 2015
Day 21 No.2	100	1/10	370	0.0123	0.123	8.45 [4.21, 4.24]	83.2	0.0175	0.00175	10	
Day 26 No.1	100	1/10	1009	0.0436	0.436	9.72 [4.66, 5.06]	83.2	0.0539	0.00185	29	7.6. 2015
Day 26 No.2	100	1/10	642	0.0292	0.292	9.51 [4.59, 4.92]	83.2	0.0369	0.00185	20	
Day 28 No.1	100	1/10	649	0.0283	0.283	8.29 [3.91, 4.38]	83.2	0.0410	0.00185	22	7.8. 2015
Day 28 No.2	100	1/10	N.D.	< 0.0082	< 0.082	8.09 [3.81, 4.28]	83.2	< 0.0122	0.00185	< 7	

Calculation methods:

Amount deposited in fish

$$E = A \times B \times D$$

Concentration in fish

$$H = \frac{E \times 100}{F \times G}$$

F: total weight for 2 fish

Concentration factor (BCF)

$$J = \frac{H}{I}$$

(Note) Analysis was performed in duplicate (n=2), No.1 and No.2, respectively, where 2 fish were used as 1 sample.

The weight of each two fish is shown in [] of column F.

In column C, the peak area of 300 was set as the detection limit, and samples below the detection limit were indicated as N.D.

E for N.D. was calculated by substituting the detection limit (0.0082 mgL⁻¹) into D in the formula.

[Control]

Items Samples	A Fixed volume (mL)	B Concentration Factor in preparation	C Peak area	D Concentration from 2-point calibration curve (mgL ⁻¹)	E Amount deposited in fish (µg)	F Fish weight (g)	G Recovery rate (%)	H Concentration in fish (µgg ⁻¹)	I Concentration in water (mgL ⁻¹)	J Concentration factor (BCF)	K Date of measurement
Day 0 (at start of exposure)	100	1/10	N.D.	–	–	9.05 [4.02, 5.03]	–	–	–	–	6.10.2015
Day 28 (at end of exposure)	100	1/10	N.D.	–	–	9.16 [4.35, 4.81]	–	–	–	–	7.8. 2015

(Note) The peak area of 300 was set as the detection limit, and samples below the detection limit were indicated as N.D.

Reference 5 Water analysis data

[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 sampled (mL)	F Recovery rate (%)	G Concentration in water tank (mgL ⁻¹)	H Date of measurement
Day 0	10	4063	0.1644	1.644	100	91.8	0.0179	6.10. 2015
Day 7	10	4718	0.1842	1.842	100	91.8	0.0201	6.17. 2015
Day 14	10	4758	0.1864	1.864	100	91.8	0.0203	6.24. 2015
Day 21	10	4167	0.1664	1.664	100	91.8	0.0181	7.1. 2015
Day 26	10	4625	0.1857	1.857	100	91.8	0.0202	7.6. 2015
Day 28	10	4543	0.1821	1.821	100	91.8	0.0198	7.8. 2015

Calculation methods: Absolute amount in the fixed volume of solution

$$D = A \times C$$

Concentration in the aquarium

$$G = \frac{D \times 100}{E \times F}$$

[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 sampled (mL)	F Recovery rate (%)	G Concentration in water tank (mgL ⁻¹)	H Date of measurement
Day 0	2	4262	0.1724	0.3448	200	97.8	0.00176	6.10. 2015
Day 7	2	5004	0.1959	0.3918	200	97.8	0.00200	6.17. 2015
Day 14	2	4938	0.1938	0.3876	200	97.8	0.00198	6.24. 2015
Day 21	2	4276	0.1707	0.3414	200	97.8	0.00175	7.1. 2015
Day 23	2	4503	0.1810	0.3620	200	97.8	0.00185	7.6. 2015
Day 28	2	4522	0.1813	0.3626	200	97.8	0.00185	7.8. 2015

Calculation methods: Absolute amount in the fixed volume of solution

$$D = A \times C$$

Concentration in the aquarium

$$G = \frac{D \times 100}{E \times F}$$

[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 sampled (mL)	F Recovery rate (%)	G Concentration in water tank (mgL ⁻¹)	H Date of measurement
Day 0	2	N.D.	—	—	200	—	—	6.10.2015

(Note) The peak area of 300 was set as the detection limit, and samples below the detection limit were indicated as N.D.

Reference 6 Chromatograms for preparing calibration curve

6-1 For fish analysis

Calibration curve
(fish analysis)
Standard solution
0.0979 mgL⁻¹

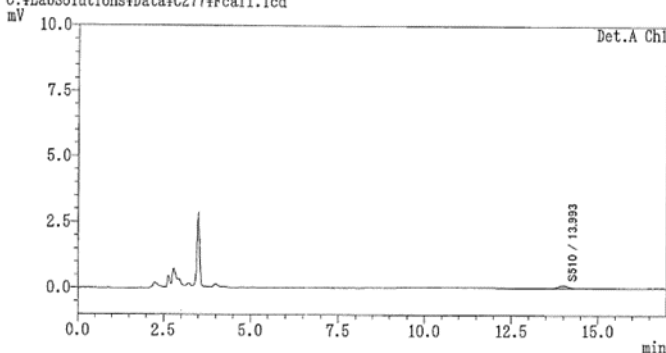
DATE: 2015/05/18

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve (Fish analysis)
Sample ID : Std. 0.0979 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\Data\FC277\Fcal1.1cd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	13.992	2151	S510
Total		2151	

Calibration curve
(fish analysis)
Standard solution
0.6194 mgL⁻¹

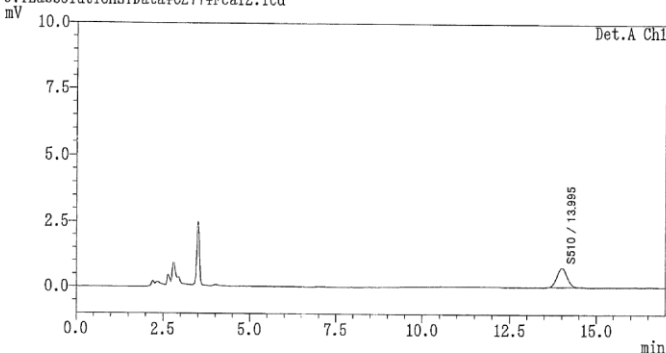
DATE: 2015/05/18

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve (Fish analysis)
Sample ID : Std. 0.6194 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\Data\FC277\Fcal2.1cd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	13.995	15037	S510
Total		15037	

Calibration curve
(fish analysis)
Standard solution
0.9790 mgL⁻¹

DATE: 2015/05/18

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve (Fish analysis)
Sample ID : Std. 0.9790 mg/L

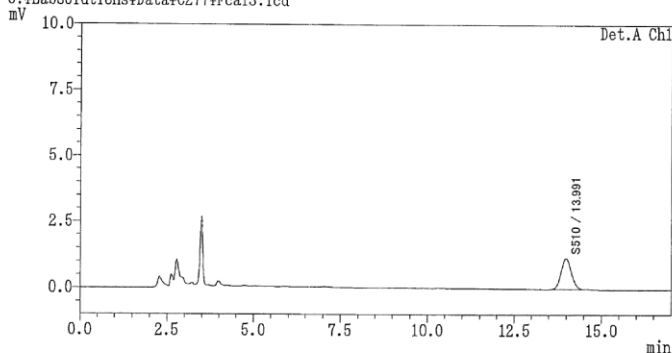
*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***

C:\LabSolutions\Data\VC277\Fcal3.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	13.991	23500	S510
Total		23500	

Calibration curve
(fish analysis)
Standard solution
6.1938 mgL⁻¹

DATE: 2015/05/18

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve (Fish analysis)
Sample ID : Std. 6.1938 mg/L

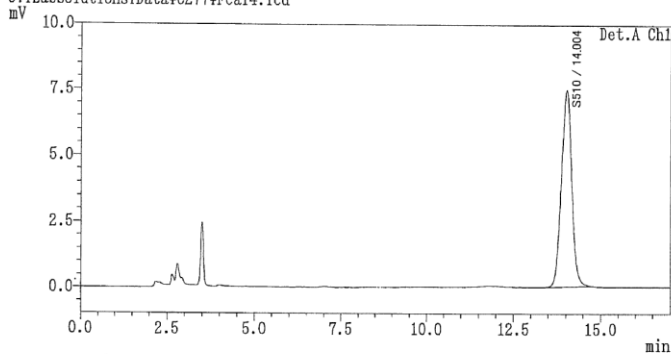
*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***

C:\LabSolutions\Data\VC277\Fcal4.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	14.004	154396	S510
Total		154396	

Calibration curve
(fish analysis)
Standard solution
9.7902 mgL⁻¹

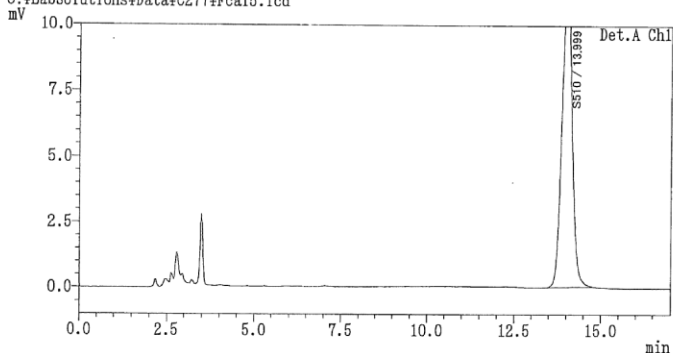
DATE: 2015/05/18

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve (Fish analysis)
Sample ID : Std. 9.7902 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\Data\C277\Fcal5.1cd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	13.999	237859	S510
Total		237859	

Calibration curve
(fish analysis)
Chromatogram of
solvent for
adjustment

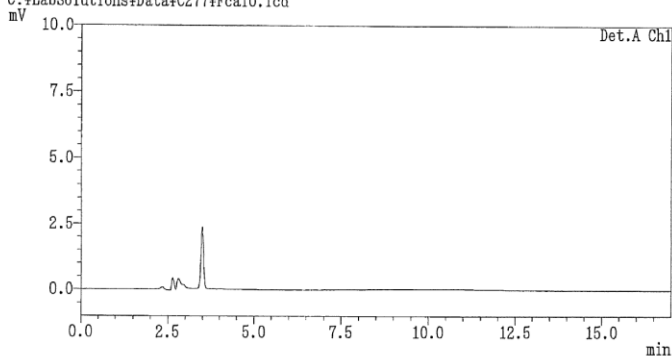
DATE: 2015/05/18

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve (Fish analysis)
Sample ID : solvent

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\Data\C277\Fcal0.1cd



*** Peak Table ***
Detector A Ch1 214nm

6-2 For water analysis

Calibration curve
(water analysis)
Standard solution
0.0963 mgL⁻¹

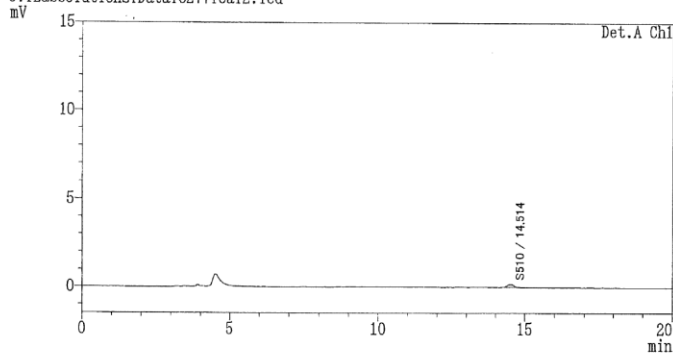
DATE: 2015/05/11

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve
Sample ID : Std. 0.0963 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\Data\C277\Cal2.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	14.513	2745	S510
Total		2745	

Calibration curve
(water analysis)
Standard solution
0.6314 mgL⁻¹

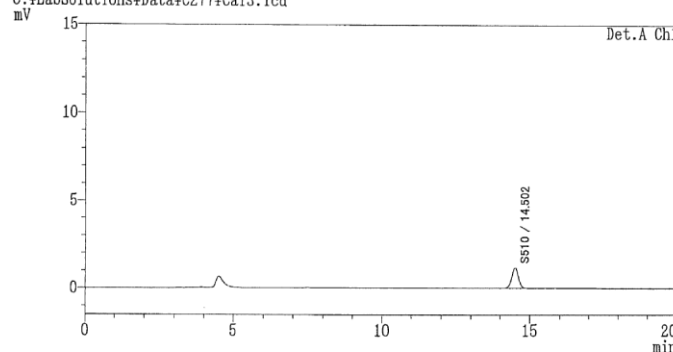
DATE: 2015/05/11

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve
Sample ID : Std. 0.6314 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\Data\C277\Cal3.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	14.501	18011	S510
Total		18011	

Calibration curve
(water analysis)
Standard solution
0.9630 mgL⁻¹

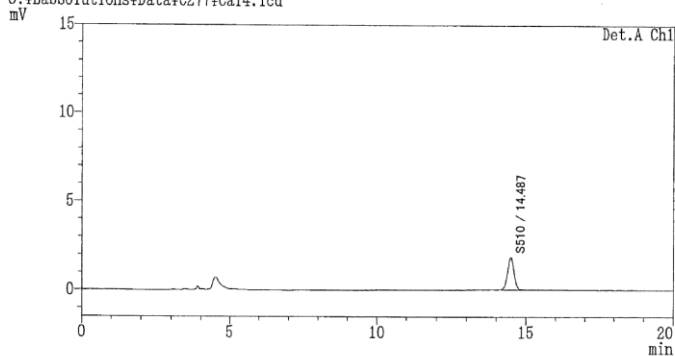
DATE: 2015/05/11

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve
Sample ID : Std. 0.9630 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\Data\C277\Cal4.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	14.487	27867	S510
Total		27867	

Calibration curve
(water analysis)
Standard solution
6.3137 mgL⁻¹

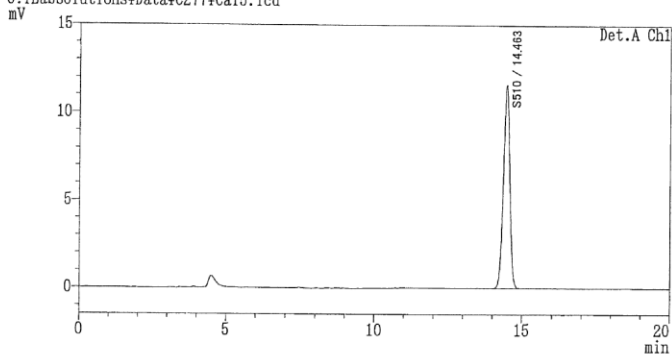
DATE: 2015/05/11

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve
Sample ID : Std. 6.3137 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\Data\C277\Cal5.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	14.463	178107	S510
Total		178107	

Calibration curve
(water analysis)
Standard solution
9.6304 mgL⁻¹

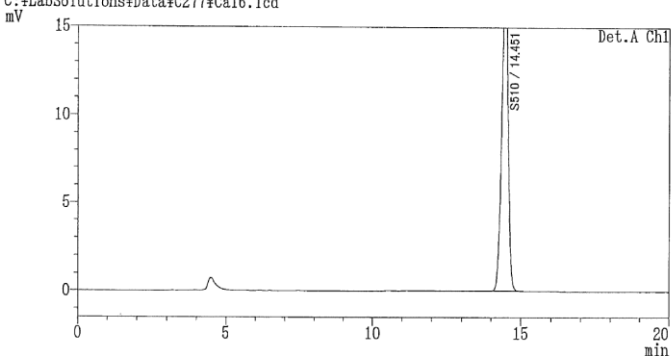
DATE: 2015/05/11

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve
Sample ID : Std. 9.6304 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\YData\YC277\Cal6.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	14.450	275278	S510
Total		275278	

Calibration curve
(water analysis)
Chromatogram of
solvent for
adjustment

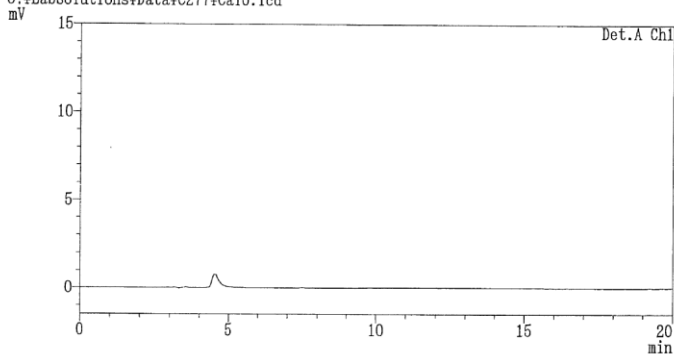
DATE: 2015/05/11

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Calibration curve
Sample ID : solvent

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\YData\YC277\Cal10.lcd



*** Peak Table ***
Detector A Ch1 214nm

7 Chromatograms for recovery rate of the test substance

7-1 For recovery from fish

Recovery test
(Fish analysis)

Standard solution
0.0979 mgL⁻¹

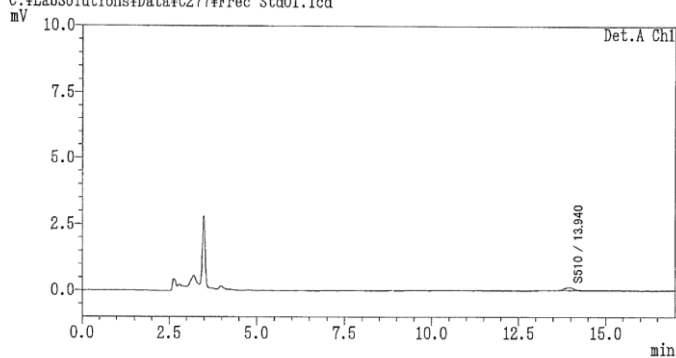
DATE: 2015/05/26

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Fish recovery test
Sample ID : Std. 0.0979 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\WC277\Frec Std01.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	13.939	2171	S510
Total		2171	

Recovery test
(Fish analysis)

Standard solution
0.6194 mgL⁻¹

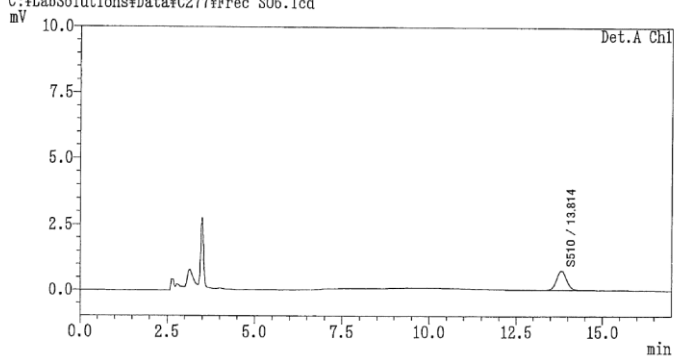
DATE: 2015/05/26

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Fish recovery test
Sample ID : Std. 0.6194 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\WC277\Frec S06.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	13.813	15002	S510
Total		15002	

Recovery test
(Fish analysis)

Added Standard
solution

DATE: 2015/05/26

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Fish recovery test
Sample ID : Added Std.

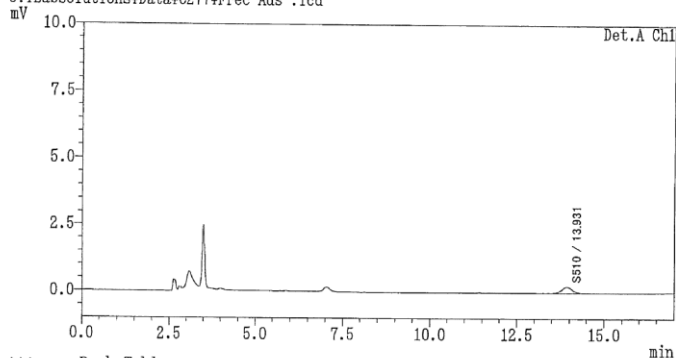
*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***

C:\LabSolutions\YData\YC277\Frec Ads .lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	13.931	4594	0.1964	S510
Total		4594		

Recovery test
(Fish analysis)

Added group
No.1

DATE: 2015/05/26

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Fish recovery test
Sample ID : Added No.1

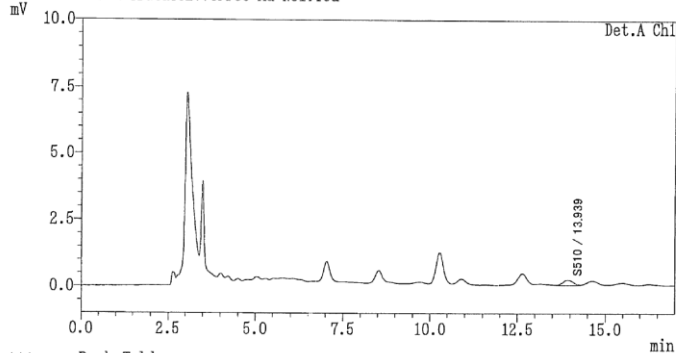
*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***

C:\LabSolutions\YData\YC277\Frec Ad No1.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	13.939	3936	0.1697	S510
Total		3936		

Recovery test
(Fish analysis)

Added group
No.2

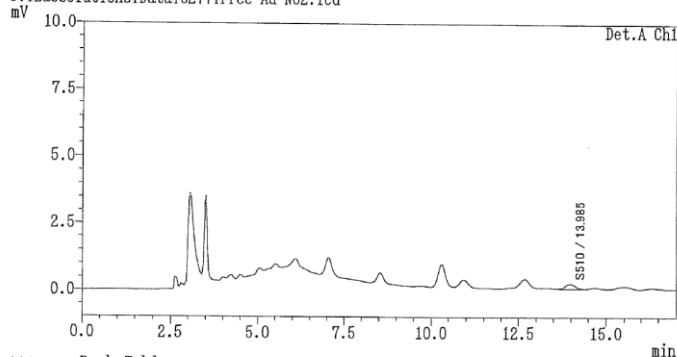
DATE: 2015/05/26

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Fish recovery test
Sample ID : Added No.2

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\Frec Ad No2.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	13.985	3912	0.1687	S510
Total		3912		

Recovery test
(Fish analysis)

Added group
No.3

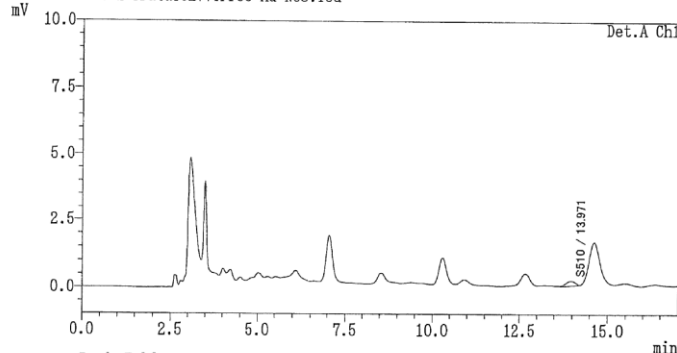
DATE: 2015/05/26

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Fish recovery test
Sample ID : Added No.3

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\Frec Ad No3.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	13.971	3613	0.1565	S510
Total		3613		

Recovery test
(Fish analysis)

Control group
No.1

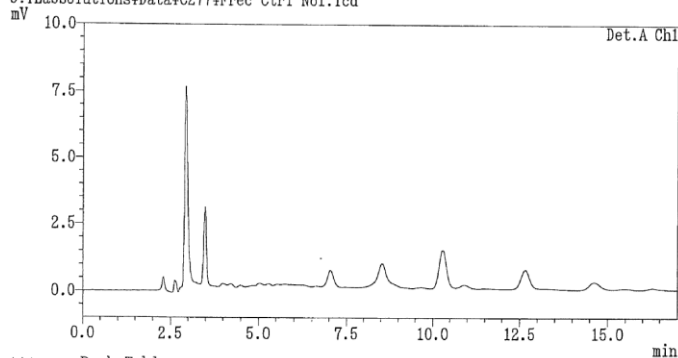
DATE: 2015/05/26

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Fish recovery test
Sample ID : Control No.1

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\Frec Ctrl No1.lcd



*** Peak Table ***
Detector A Ch1 214nm

Recovery test
(Fish analysis)

Control group
No.2

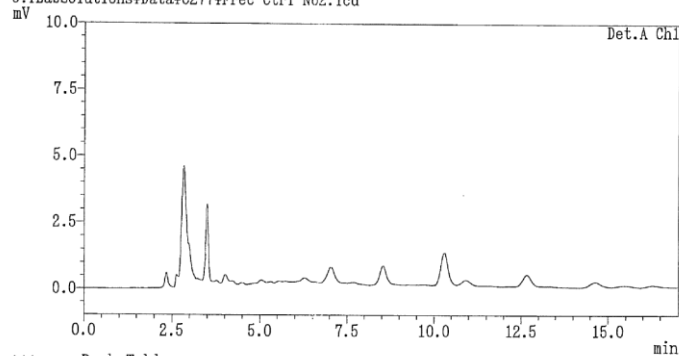
DATE: 2015/05/26

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Fish recovery test
Sample ID : Control No.2

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\Frec Ctrl No2.lcd



*** Peak Table ***
Detector A Ch1 214nm

Recovery test
(Fish analysis)

Control group
No.3

DATE: 2015/05/26

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Fish recovery test
Sample ID : Control No.3

*** ANALYTICAL CONDITIONS ***

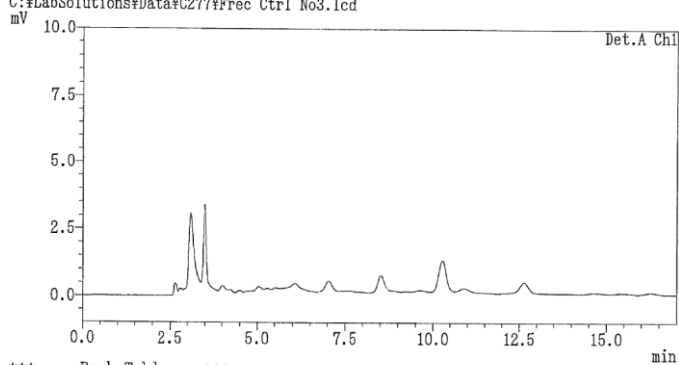
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR :

T. Sato

*** Chromatgram ***

C:\LabSolutions\¥Data\¥C277\Frec Ctrl No3.1cd



*** Peak Table ***
Detector A Ch1 214nm

7-2 For recovery from water

Recovery test
(Water analysis)

Standard solution
 0.0963 mgL^{-1}

DATE: 2015/05/15

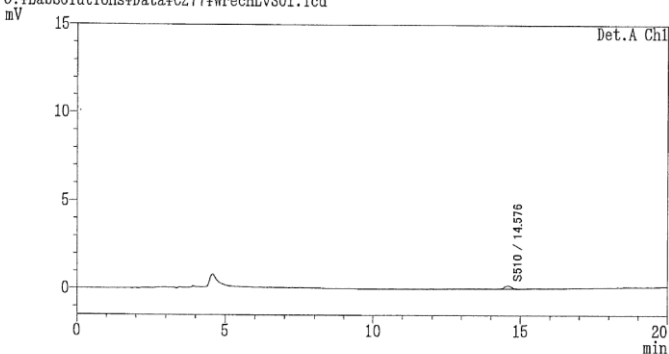
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Std. 0.0963 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5 μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\0277\WrecHLvS01.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	14.576	2765	S510
Total		2765	

Recovery test
(Water analysis)

Standard solution
 0.6314 mgL^{-1}

DATE: 2015/05/15

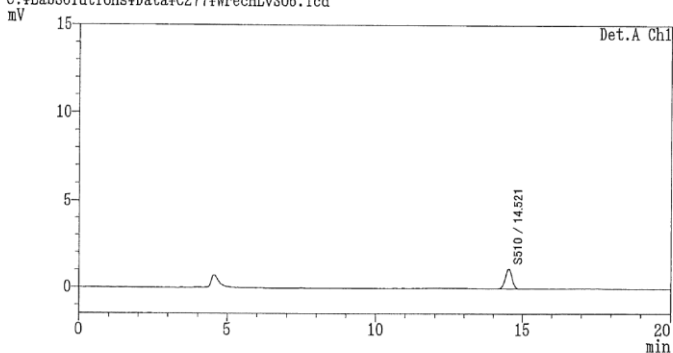
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Std. 0.6314 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5 μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\0277\WrecHLvS06.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	14.520	17986	S510
Total		17986	

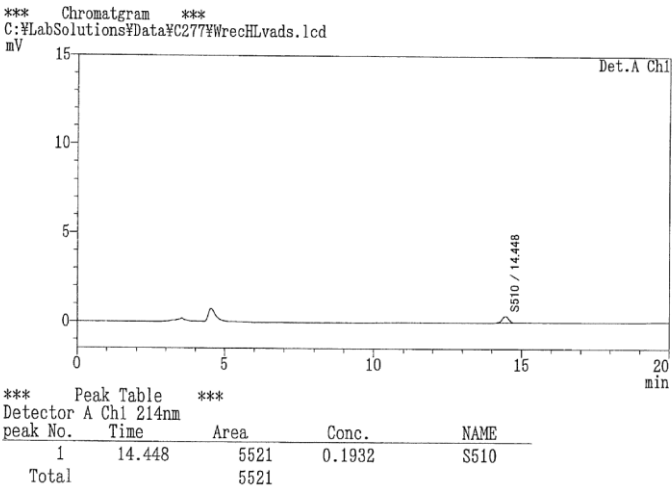
Recovery test
(Water analysis)

High exposure level
Added Standard
solution

DATE: 2015/05/15

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : High level added std.

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



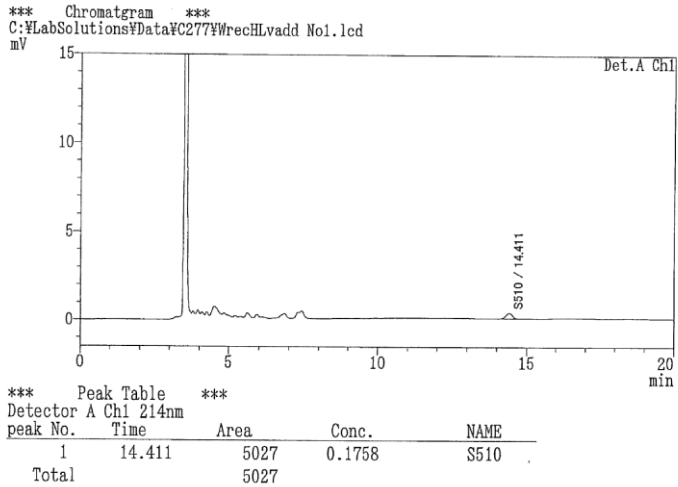
Recovery test
(Water analysis)

High exposure level
Added group No.1

DATE: 2015/05/15

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : High level added No.1

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



Recovery test
(Water analysis)

High exposure level
Added group No.2

DATE: 2015/05/15

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : High level added No.2

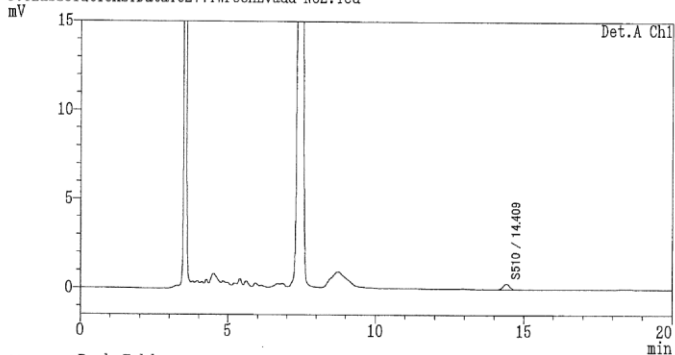
*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***

C:\LabSolutions\Data\C277\FWrecHLvadd No2.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.409	5005	0.1750	S510
Total		5005		

Recovery test
(Water analysis)

High exposure level
Added group No.3

DATE: 2015/05/15

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : High level added No.3

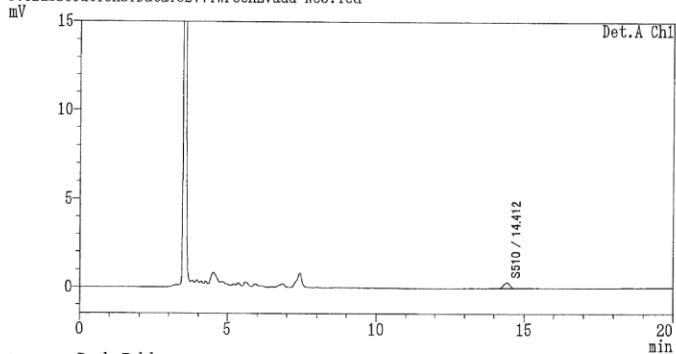
*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***

C:\LabSolutions\Data\C277\FWrecHLvadd No3.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.412	5160	0.1805	S510
Total		5160		

Recovery test
(Water analysis)

High exposure level
Control group No.1

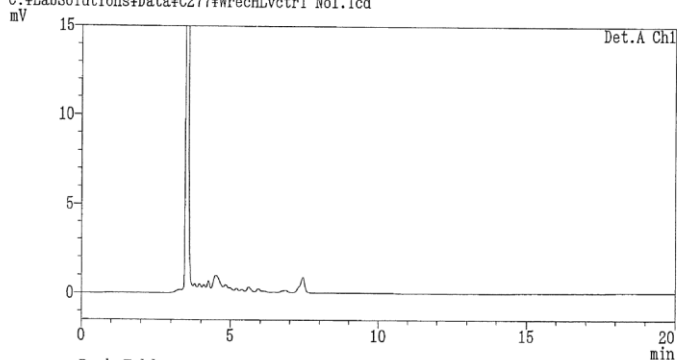
DATE: 2015/05/15

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : High level control No.1

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\WrecHLvctrl No1.lcd



*** Peak Table ***
Detector A Ch1 214nm

Recovery test
(Water analysis)

High exposure level
Control group No.2

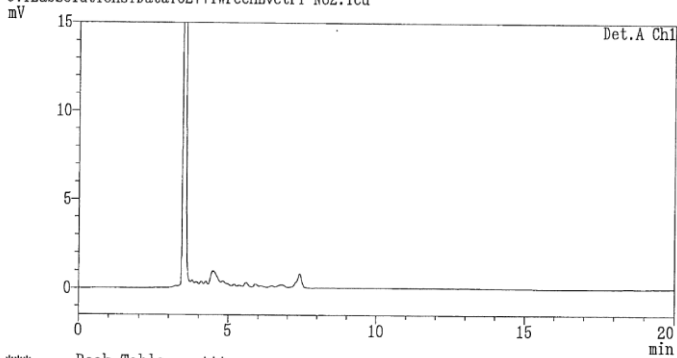
DATE: 2015/05/15

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : High level control No.2

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\WrecHLvctrl No2.lcd



*** Peak Table ***
Detector A Ch1 214nm

Recovery test
(Water analysis)

High exposure level
Control group No.3

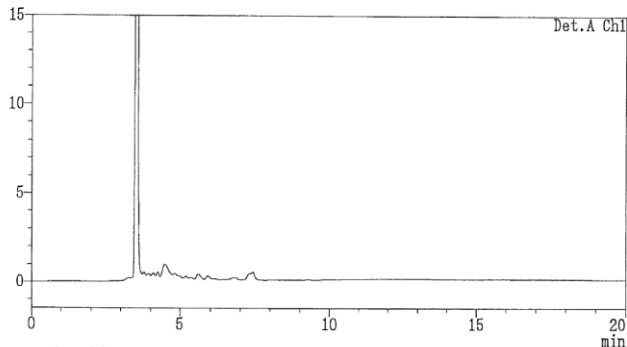
DATE: 2015/05/15

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : High level control No.3

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\4Data\4C277\4WrecHLvctrl No3.lcd
mV



*** Peak Table ***
Detector A Ch1 214nm

Recovery test
(Water analysis)

Standard solution
0.0963 mgL⁻¹

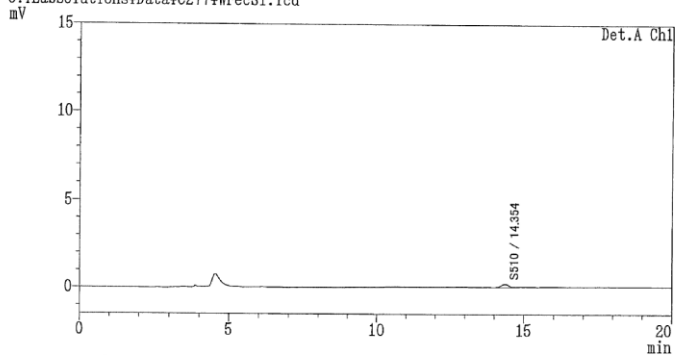
DATE: 2015/05/14

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Std. 0.0963 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\4Data\4C277\4WrecS1.lcd
mV



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	14.354	2739	S510
Total		2739	

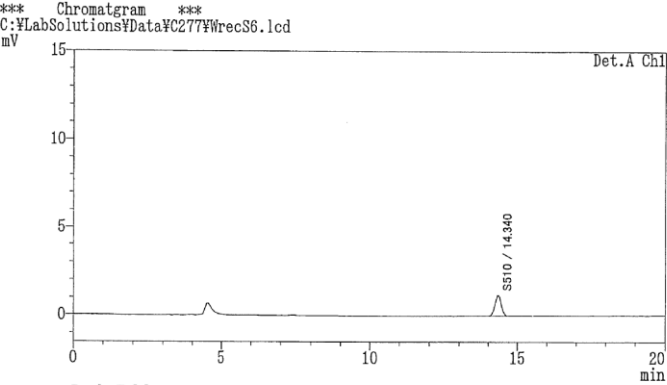
Recovery test
(Water analysis)

Standard solution

0.6314 mgL⁻¹

DATE: 2015/05/14
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Std. 0.6314 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	14.340	17807	S510
Total		17807	

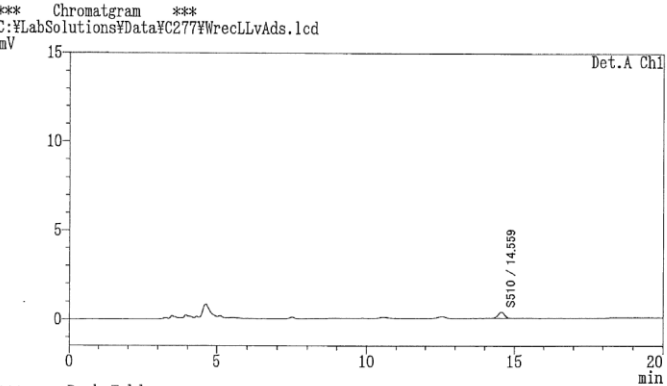
Recovery test
(Water analysis)

Low exposure level
Added Standard

solution

DATE: 2015/05/14
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Low level added Std.

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.559	5590	0.1975	S510
Total		5590		

Recovery test
(Water analysis)

Low exposure level
Added group No.1

DATE: 2015/05/14

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Low level added No.1

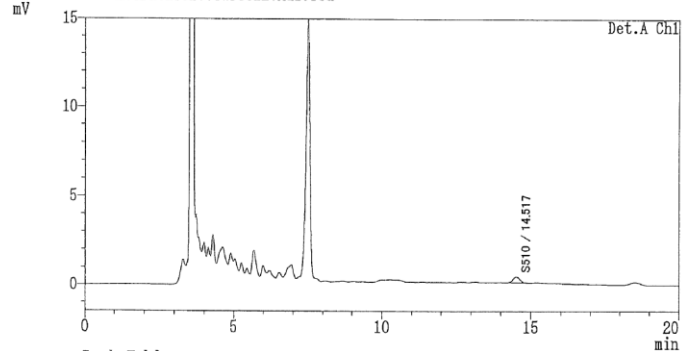
*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***

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*** Peak Table ***

peak No.	Time	Area	Conc.	NAME
1	14.517	5394	0.1906	S510
Total		5394		

Recovery test
(Water analysis)

Low exposure level
Added group No.2

DATE: 2015/05/14

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Low level added No.2

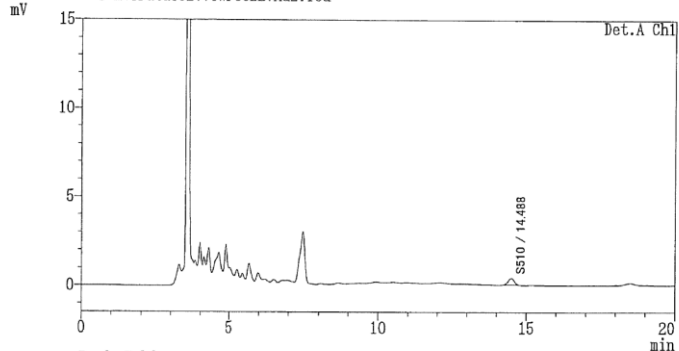
*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***

C:\LabSolutions\FData\YC277\FWrecLvAd2.lcd



*** Peak Table ***

peak No.	Time	Area	Conc.	NAME
1	14.488	5433	0.1920	S510
Total		5433		

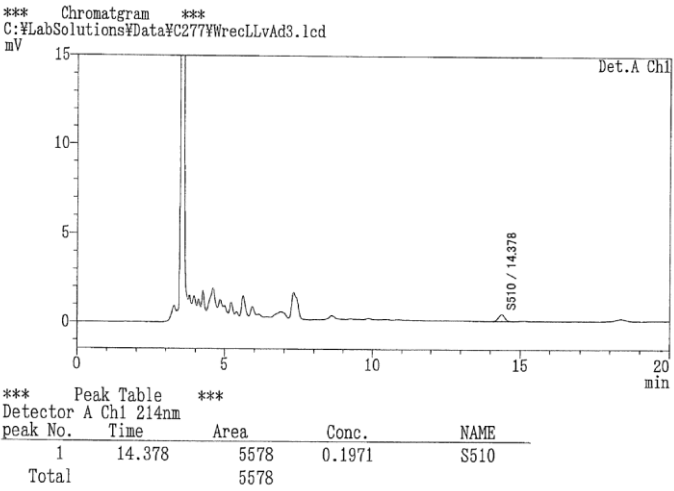
Recovery test
(Water analysis)

Low exposure level
Added group No.3

DATE: 2015/05/14

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Low level added No.3

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



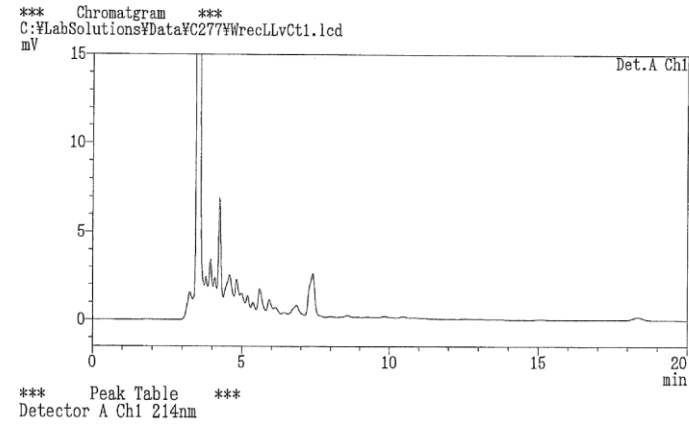
Recovery test
(Water analysis)

Low exposure level
Control group No.1

DATE: 2015/05/14

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Low level control No.1

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato

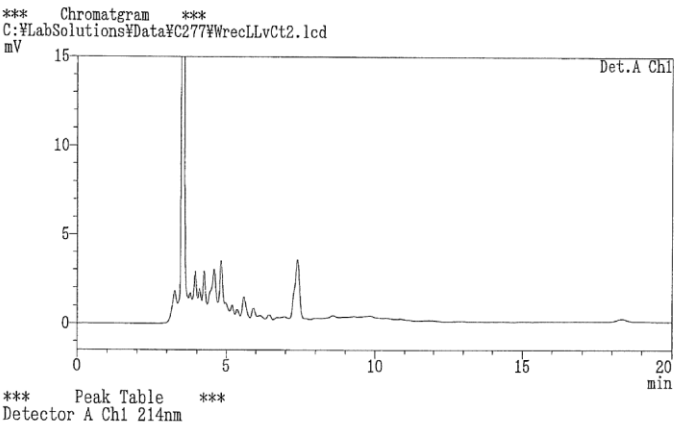


Recovery test
(Water analysis)

Low exposure level
Control group No.2

DATE: 2015/05/14
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Low level control No.2

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL
OPERATOR : T. Sato

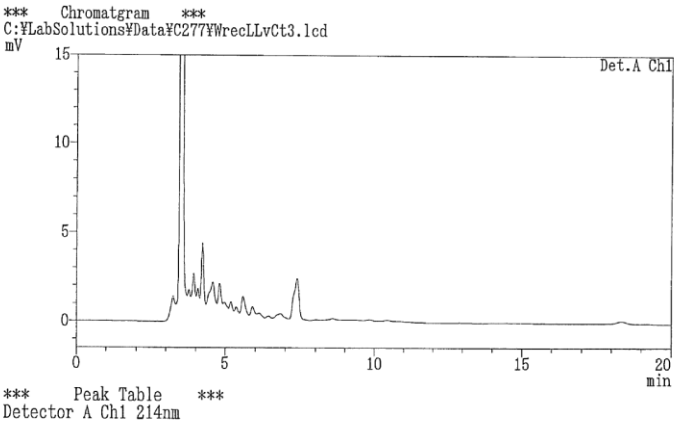


Recovery test
(Water analysis)

Low exposure level
Control group No.3

DATE: 2015/05/14
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Low level control No.3

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL
OPERATOR : T. Sato



8 Chromatograms of fish analysis

8-1 Test group

Fish analysis
Day 7
Standard solution
0.0979 mgL⁻¹

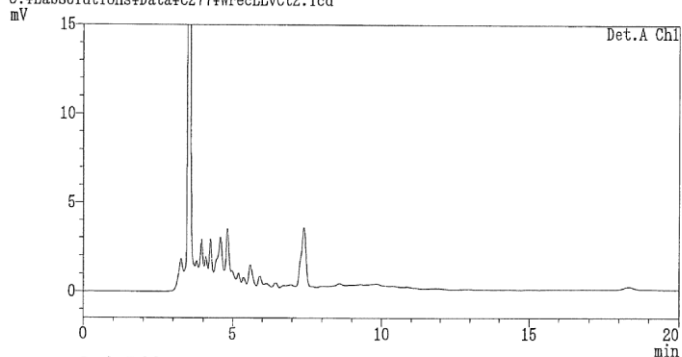
DATE: 2015/05/14

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Low level control No.2

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\YData\VC277\WrecLLvCt2.lcd



*** Peak Table ***
Detector A Ch1 214nm

Fish analysis
Day 7
Standard solution
0.6194 mgL⁻¹

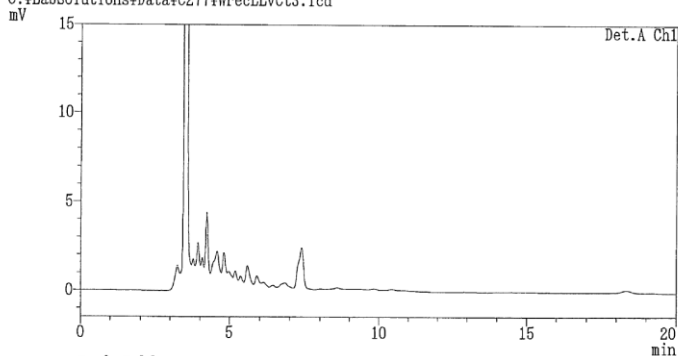
DATE: 2015/05/14

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Water recovery test
Sample ID : Low level control No.3

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\YData\VC277\WrecLLvCt3.lcd



*** Peak Table ***
Detector A Ch1 214nm

Fish analysis
Day 7

High exposure level

No.1

DATE: 2015/06/17

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 7 day
Sample ID : High level No.1

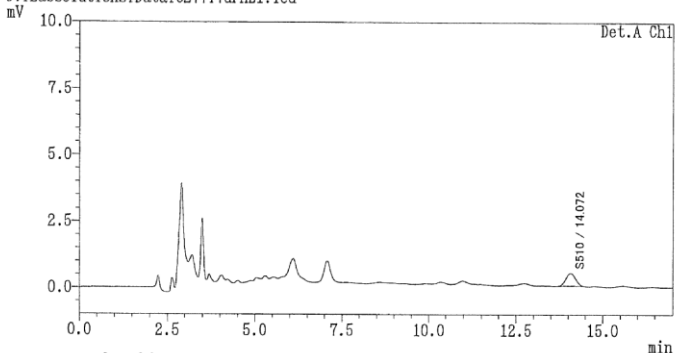
*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***

C:\LabSolutions\YData\YC277\7dFHL1.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.072	8558	0.3541	S510
Total		8558		

Fish analysis
Day 7

High exposure level

No.2

DATE: 2015/06/17

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 7 day
Sample ID : High level No.2

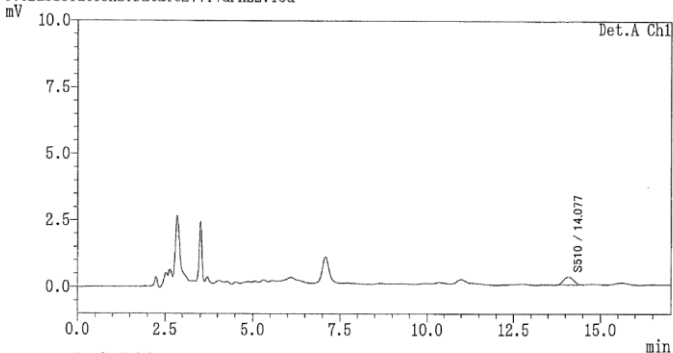
*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***

C:\LabSolutions\YData\YC277\7dFHL2.lcd



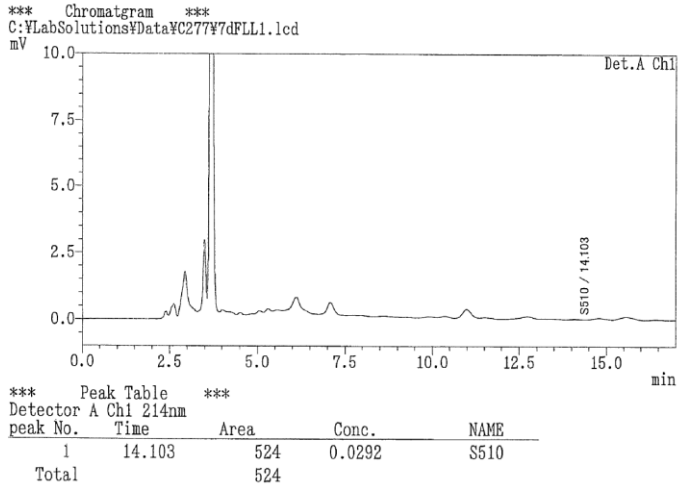
*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.077	5860	0.2450	S510
Total		5860		

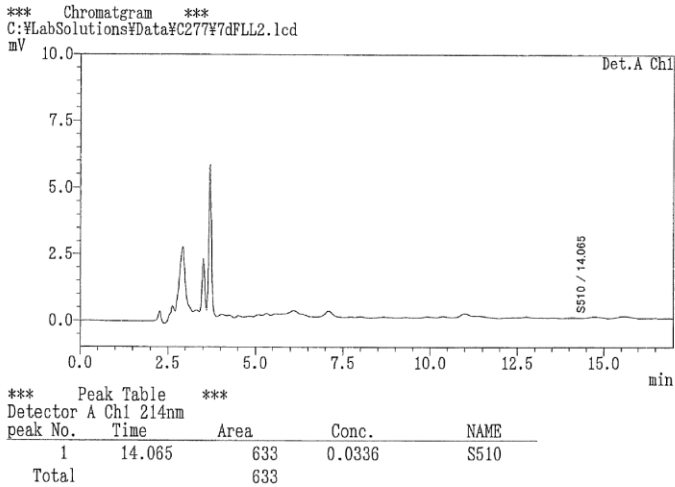
Fish analysis
Day 7
Low exposure level
No.1

DATE: 2015/06/17
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 7 day
Sample ID : Low level No.1
*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL
OPERATOR : T. Sato



Fish analysis
Day 7
Low exposure level
No.2

DATE: 2015/06/17
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 7 day
Sample ID : Low level No.2
*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL
OPERATOR : T. Sato

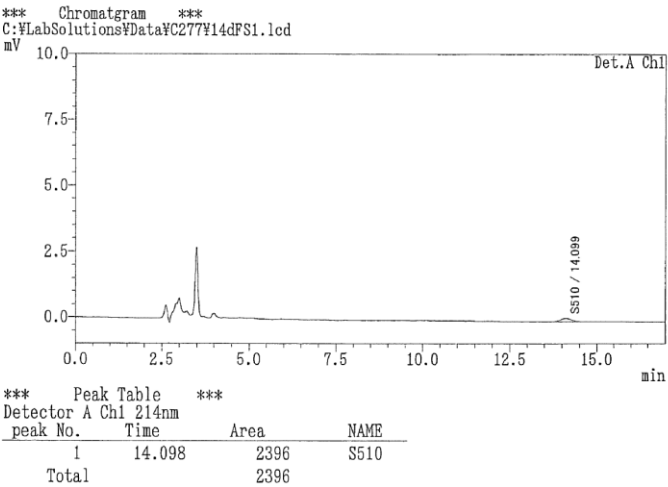


Fish analysis
Day 14

Standard solution
0.0979 mgL⁻¹

DATE: 2015/06/24
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 14 day
Sample ID : Std. 0.0979 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL
OPERATOR : T. Sato

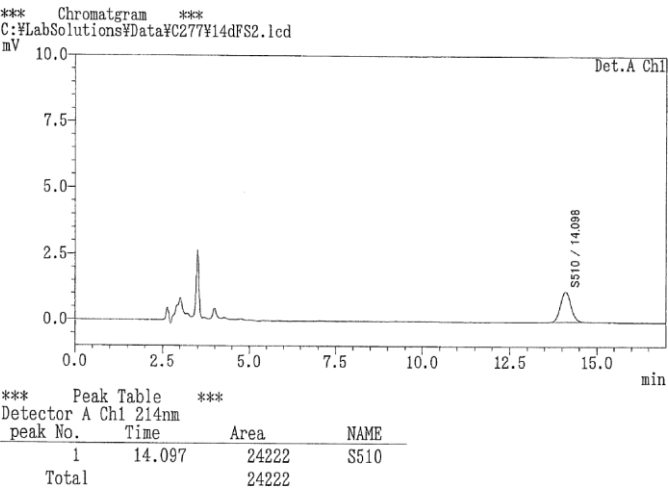


Fish analysis
Day 14

Standard solution
0.9790 mgL⁻¹

DATE: 2015/06/24
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 14 day
Sample ID : Std. 0.9790 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL
OPERATOR : T. Sato



Fish analysis
Day 14
High exposure level
No.1

DATE: 2015/06/24

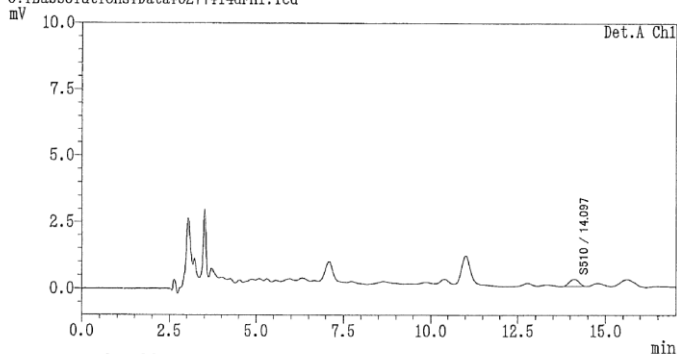
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 14 day
Sample ID : High level No.1

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5 μ m)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μ L

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\C277\14dFH1.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.097	5096	0.2069	S510
Total		5096		

Fish analysis
Day 14
High exposure level
No.2

DATE: 2015/06/24

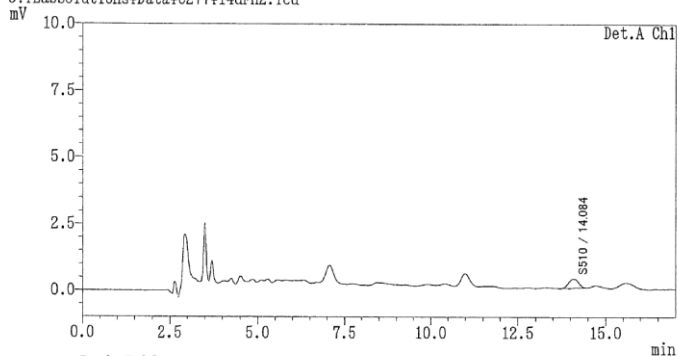
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 14 day
Sample ID : High level No.2

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5 μ m)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μ L

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\C277\14dFH2.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.084	6776	0.2747	S510
Total		6776		

Fish analysis
Day 14

Low exposure level

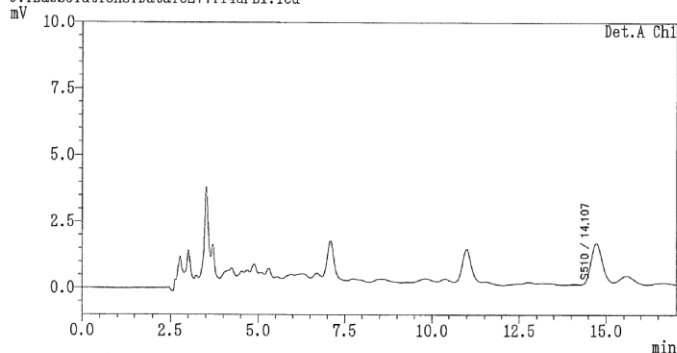
No.1

DATE: 2015/06/24
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 14 day
Sample ID : Low level No.1

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\14dFL1.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.107	463	0.0199	S510
Total		463		

Fish analysis
Day 14

Low exposure level

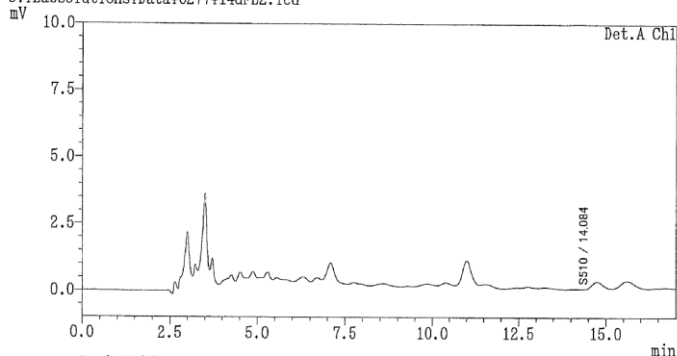
No.2

DATE: 2015/06/24
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 14 day
Sample ID : Low level No.2

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\14dFL2.lcd



*** Peak Table ***
Detector A Ch1 214nm

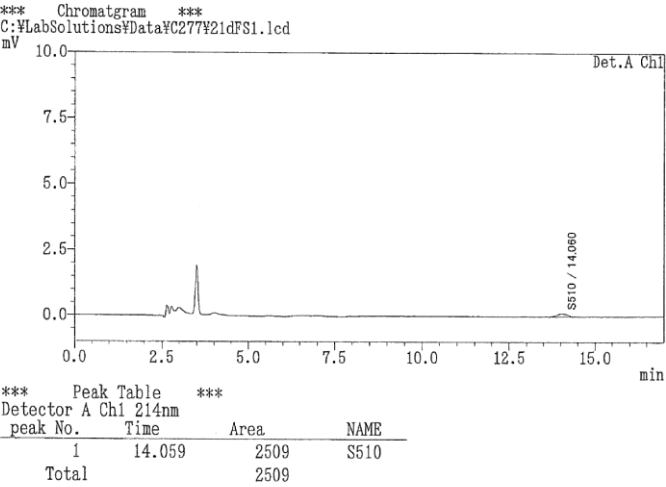
peak No.	Time	Area	Conc.	NAME
1	14.084	467	0.0200	S510
Total		467		

Fish analysis
Day 21

Standard solution
0.0979 mgL⁻¹

DATE: 2015/07/01
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 21 day
Sample ID : Std. 0.0979 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato

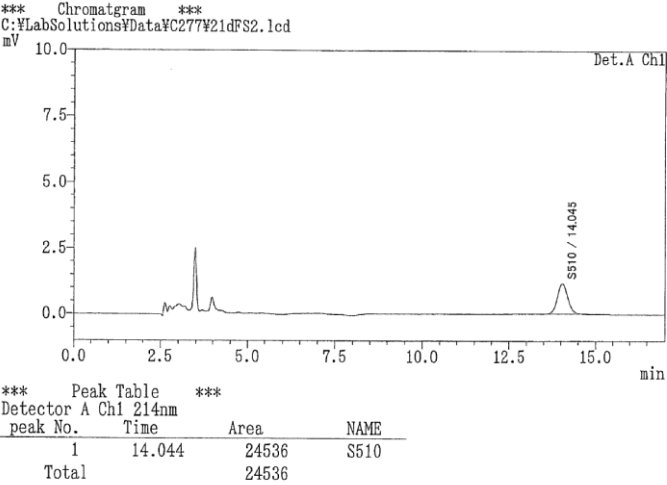


Fish analysis
Day 21

Standard solution
0.9790 mgL⁻¹

DATE: 2015/07/01
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 21 day
Sample ID : Std. 0.9790 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



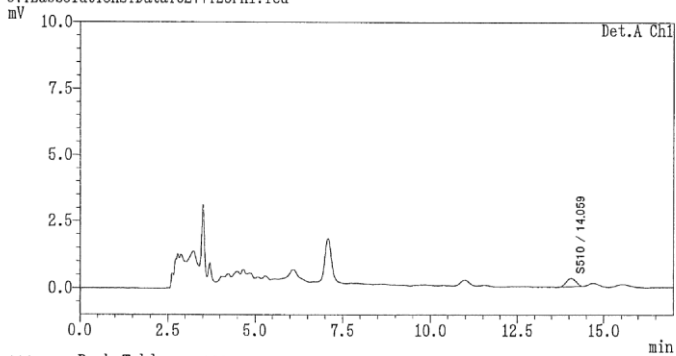
Fish analysis
Day 21
High exposure level
No.1

DATE: 2015/07/06

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : High level No.1

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\C277\26FH1.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.059	5893	0.2343	S510
Total		5893		

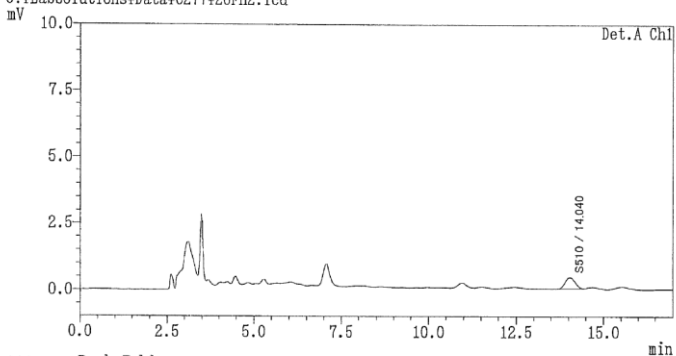
Fish analysis
Day 21
High exposure level
No.2

DATE: 2015/07/06

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : High level No.2

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\C277\26FH2.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.040	8049	0.3185	S510
Total		8049		

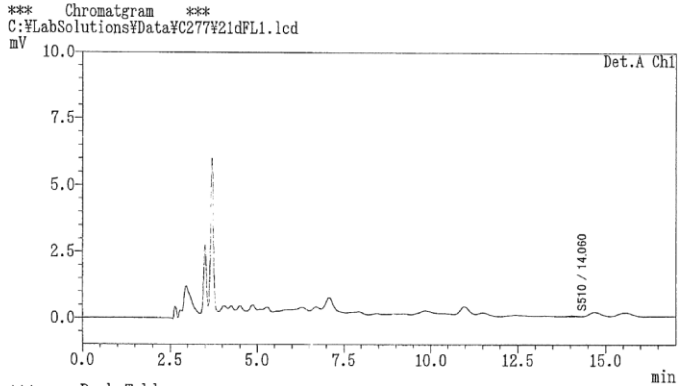
Fish analysis
Day 21

Low exposure level

No.1

DATE: 2015/07/01
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 21 day
Sample ID : Low level No.1

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.060	499	0.0175	S510
Total		499		

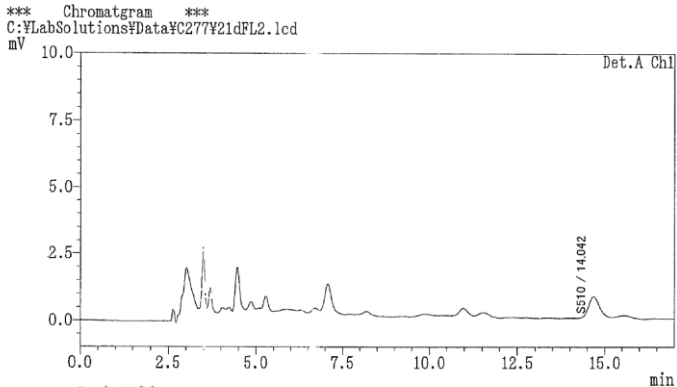
Fish analysis
Day 21

Low exposure level

No.2

DATE: 2015/07/01
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 21 day
Sample ID : Low level No.2

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.042	370	0.0123	S510
Total		370		

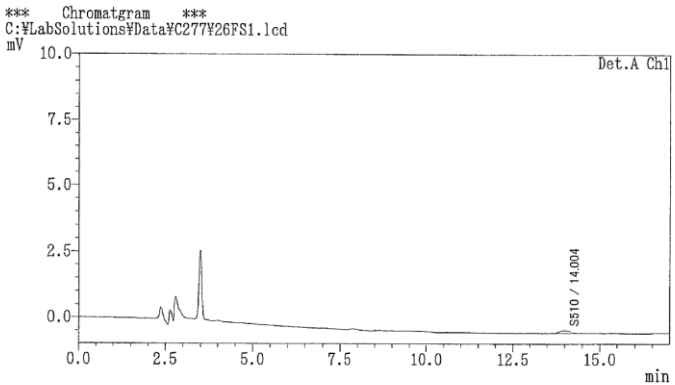
Fish analysis
Day 26

Standard solution
0.0979 mgL⁻¹

DATE: 2015/07/06

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : Std. 0.0979 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	14.003	2400	S510
Total		2400	

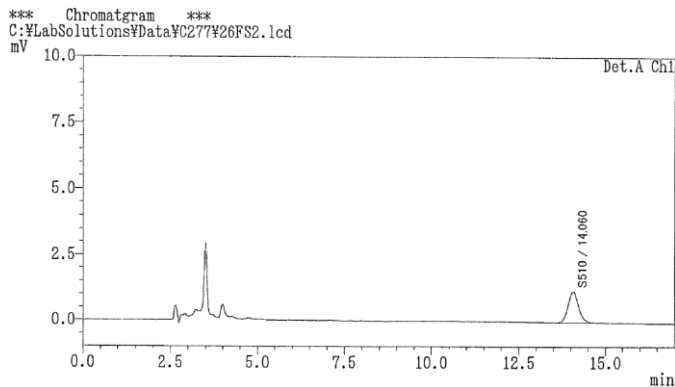
Fish analysis
Day 26

Standard solution
0.9790 mgL⁻¹

DATE: 2015/07/06

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : Std. 0.9790 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	14.059	24959	S510
Total		24959	

Fish analysis
Day 26

High exposure level
No.1

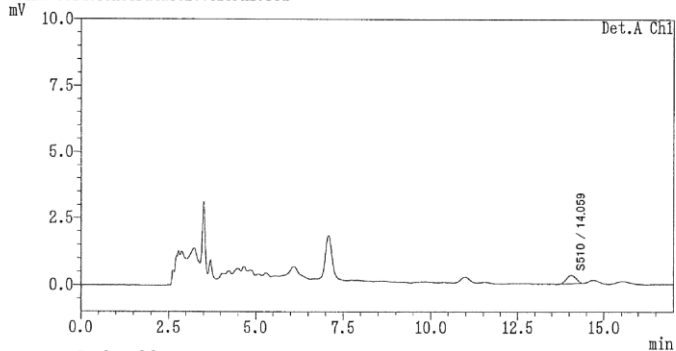
DATE: 2015/07/06

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : High level No.1

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\VC277\26FH1.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.059	5893	0.2343	S510
Total		5893		

Fish analysis
Day 26

High exposure level
No.2

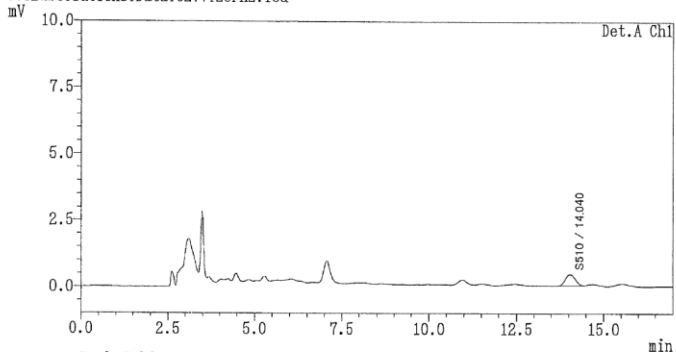
DATE: 2015/07/06

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : High level No.2

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\VC277\26FH2.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	14.040	8049	0.3185	S510
Total		8049		

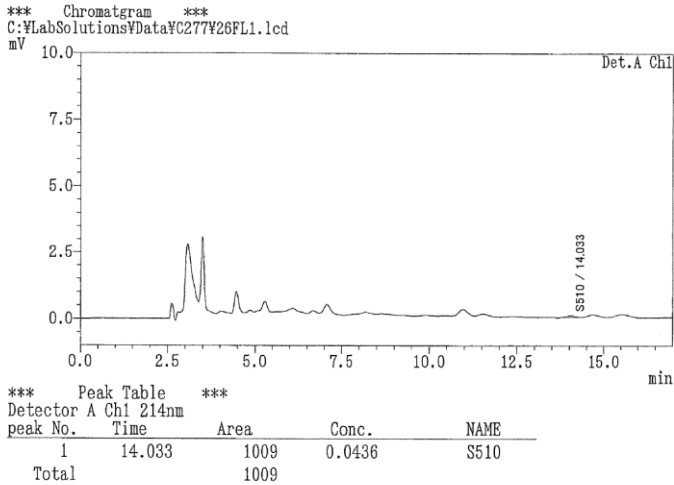
Fish analysis
Day 26

Low exposure level

No.1

DATE: 2015/07/06
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : Low level No.1

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



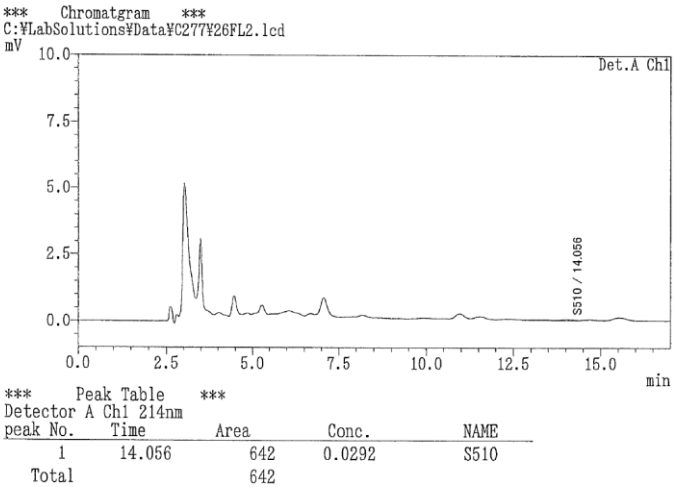
Fish analysis
Day 26

Low exposure level

No.2

DATE: 2015/07/06
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : Low level No.2

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato

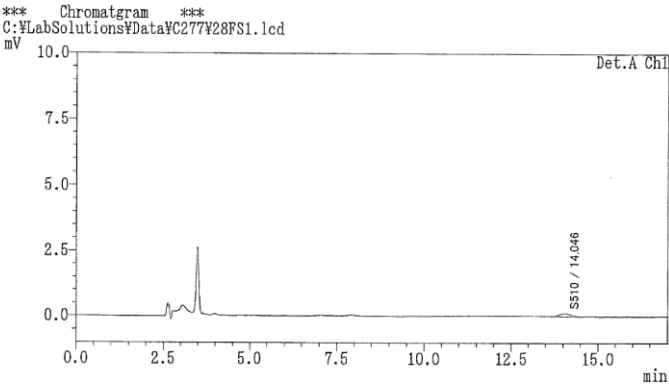


Fish analysis
Day 28

Standard solution
0.0979 mgL⁻¹

DATE: 2015/07/08
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : Std. 0.0979 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL
OPERATOR : T. Sato



*** Peak Table ***
Detector A Ch1 214nm

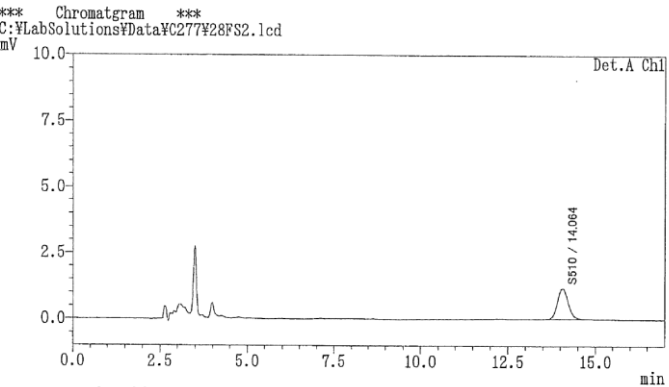
peak No.	Time	Area	NAME
1	14.046	2413	S510
Total		2413	

Fish analysis
Day 28

Standard solution
0.9790 mgL⁻¹

DATE: 2015/07/08
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : Std. 0.9790 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL
OPERATOR : T. Sato



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	14.064	24747	S510
Total		24747	

Fish analysis
Day 28
High exposure level
No.1

DATE: 2015/07/08

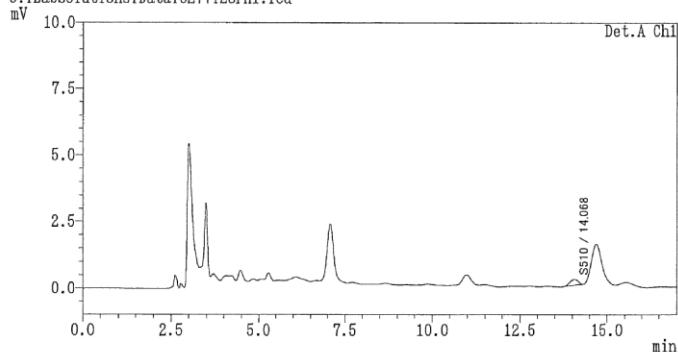
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : High level No.1

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\Y28FH1.lcd



*** Peak Table ***

peak No.	Time	Area	Conc.	NAME
1	14.068	3890	0.1561	S510
Total		3890		

Fish analysis
Day 28
High exposure level
No.2

DATE: 2015/07/08

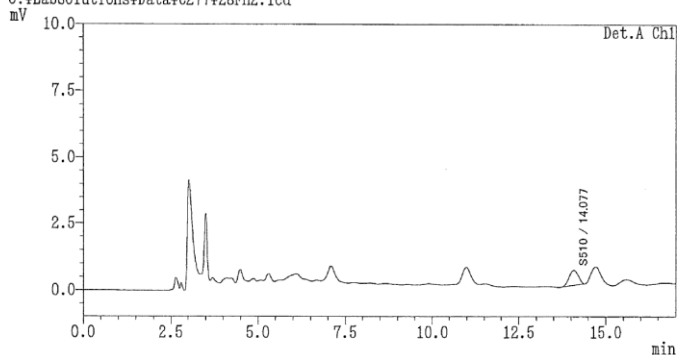
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : High level No.2

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\Y28FH2.lcd

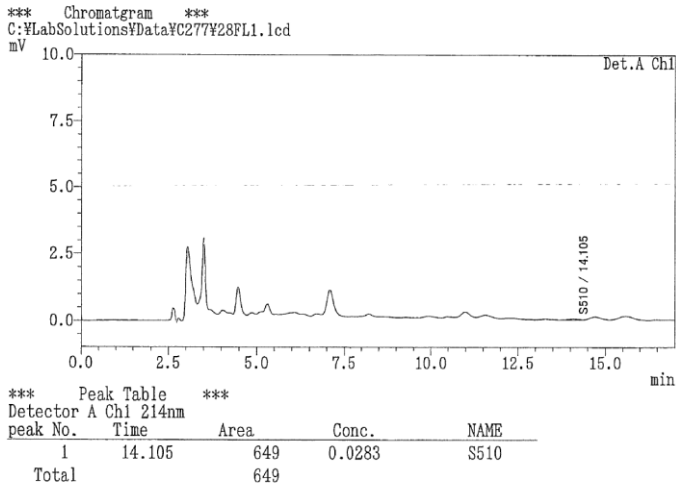


*** Peak Table ***

peak No.	Time	Area	Conc.	NAME
1	14.077	10446	0.4148	S510
Total		10446		

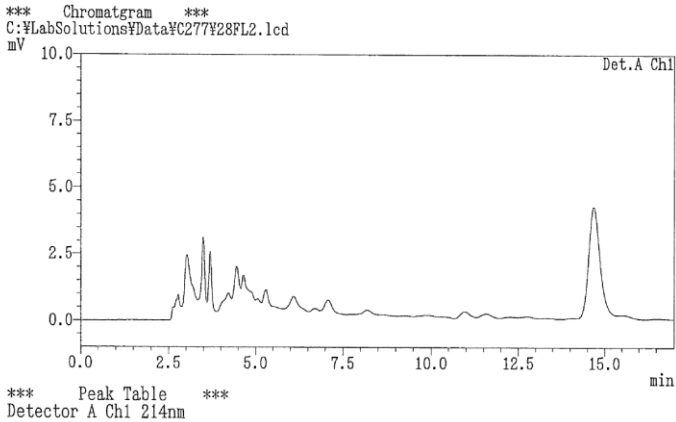
Fish analysis
Day 28
Low exposure level
No.1

DATE: 2015/07/08
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : Low level No.1
*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



Fish analysis
Day 28
Low exposure level
No.2

DATE: 2015/07/08
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : Low level No.2
*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL OPERATOR : T. Sato



8-2 Control group

Fish analysis
Day 0

Standard solution
0.0979 mgL⁻¹

DATE: 2015/06/10

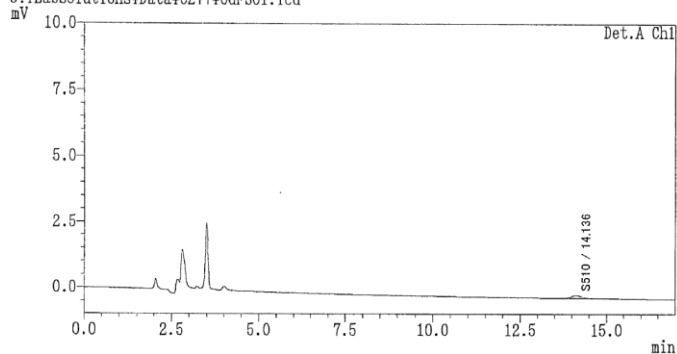
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 0 day
Sample ID : Std. 0.0979 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\YodFS01.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	14.136	2143	S510
Total		2143	

Fish analysis
Day 0

Standard solution
0.6194 mgL⁻¹

DATE: 2015/06/10

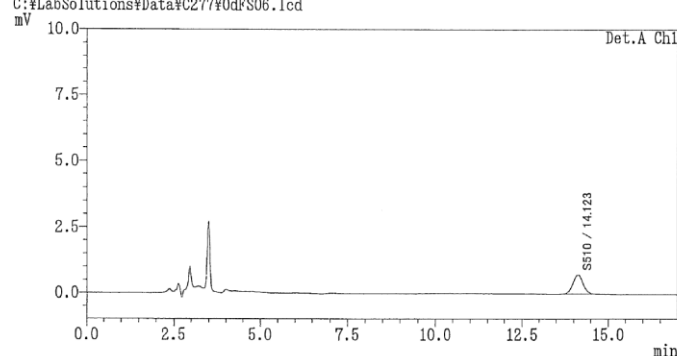
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 0 day
Sample ID : Std. 0.6194 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\YodFS06.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	14.122	15180	S510
Total		15180	

Fish analysis
Day 0
(before starting
exposure)
Control group

DATE: 2015/06/10

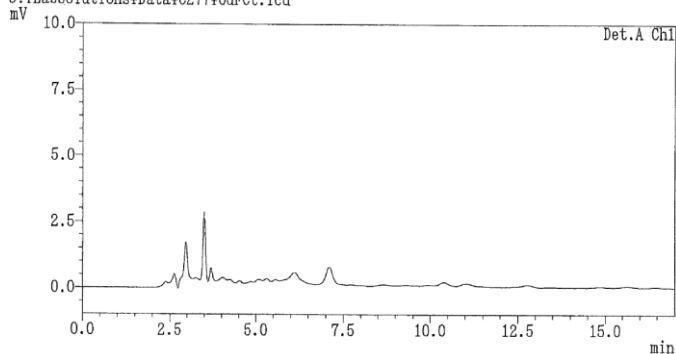
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 0 day
Sample ID : Fish control

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\VC277\F0dFct.lcd



*** Peak Table ***
Detector A Ch1 214nm

Fish analysis
Day 28
Standard solution
0.0979 mgL⁻¹

DATE: 2015/07/08

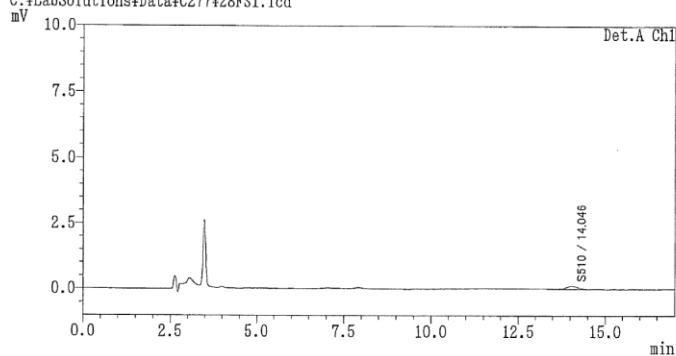
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : Std. 0.0979 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\VC277\Y28FS1.lcd



*** Peak Table ***

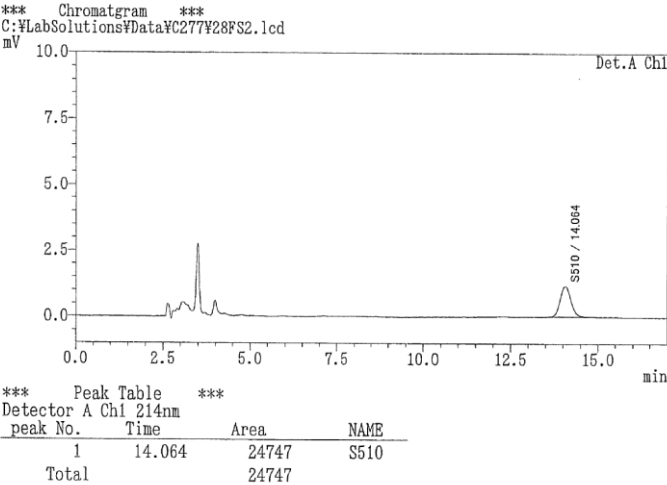
peak No.	Time	Area	NAME
1	14.046	2413	S510
Total		2413	

Fish analysis
Day 28

Standard solution
0.9790 mgL⁻¹

DATE: 2015/07/08
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : Std. 0.9790 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL
OPERATOR : T. Sato

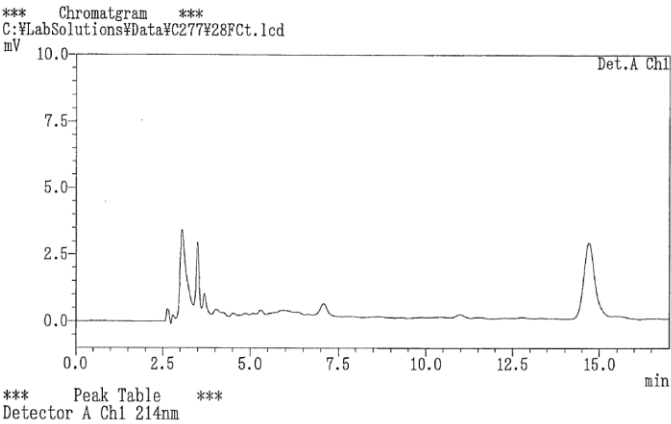


Fish analysis
Day 28
(at the termination
of exposure)

Control group

DATE: 2015/07/08
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : Control

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : Asahipak C8-50 4E 4.6×250 mm (5μm)
ELUENT : MeCN/Water (85:15)
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL
OPERATOR : T. Sato



9 Chromatograms of water analysis

9-1 Test group

Water analysis
Day 0

Standard solution
0.0963 mgL⁻¹

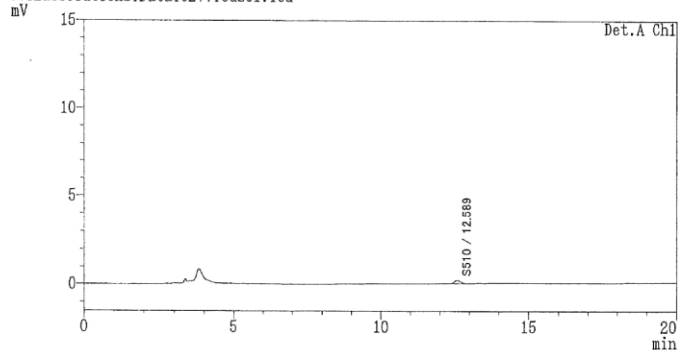
DATE: 2015/06/10

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 0 day
Sample ID : Std. 0.0963 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\Data\F027740dS01.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	12.589	2379	S510
Total		2379	

Water analysis
Day 0

Standard solution
0.6314 mgL⁻¹

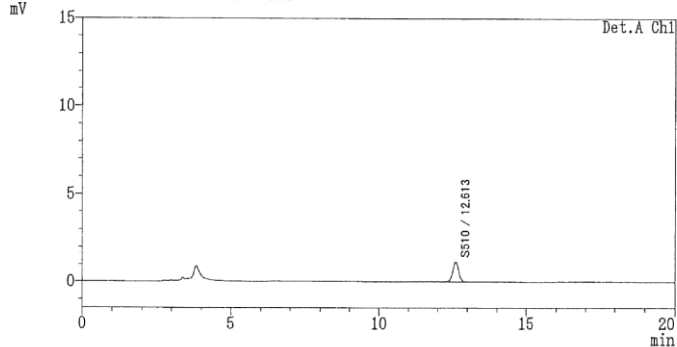
DATE: 2015/06/10

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 0 day
Sample ID : Std. 0.6314 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatogram ***
C:\LabSolutions\Data\F027740dS06.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	12.613	15617	S510
Total		15617	

Water analysis
Day 0

High exposure level

DATE: 2015/06/10

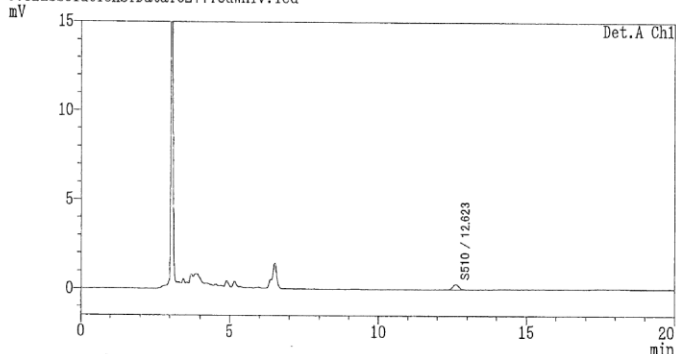
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 0 day
Sample ID : High exposure level

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\Y0dWHlv.lcd



*** Peak Table ***

peak No.	Time	Area	Conc.	NAME
1	12.623	4063	0.1644	S510
Total		4063		

Water analysis
Day 0

Low exposure level

DATE: 2015/06/10

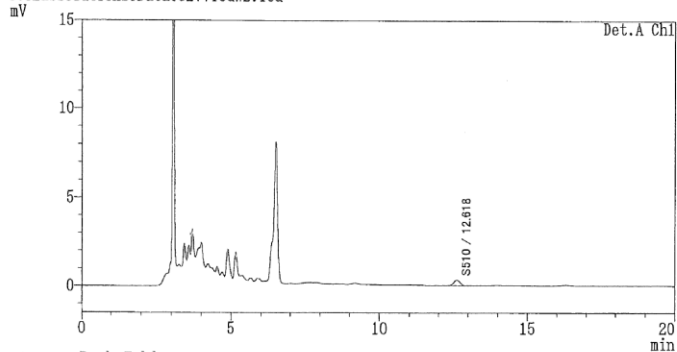
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 0 day
Sample ID : Low exposure level

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\Y0dWL.lcd



*** Peak Table ***

peak No.	Time	Area	Conc.	NAME
1	12.618	4262	0.1724	S510
Total		4262		

Water analysis
Day 7

Standard solution
0.0963 mgL⁻¹

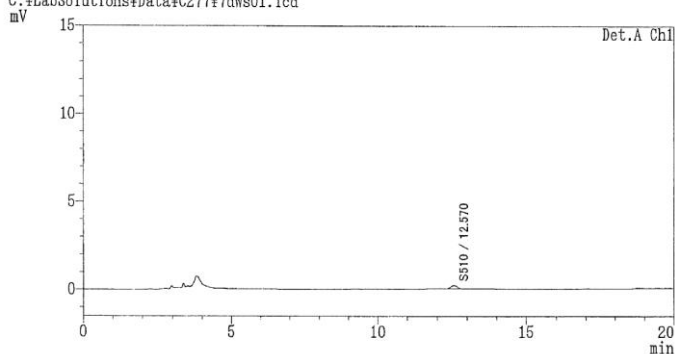
DATE: 2015/06/17

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 7 day
Sample ID : Std. 0.0963 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\VC277\7dWs01.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	12.570	2564	S510
Total		2564	

Water analysis
Day 7

Standard solution
0.6314 mgL⁻¹

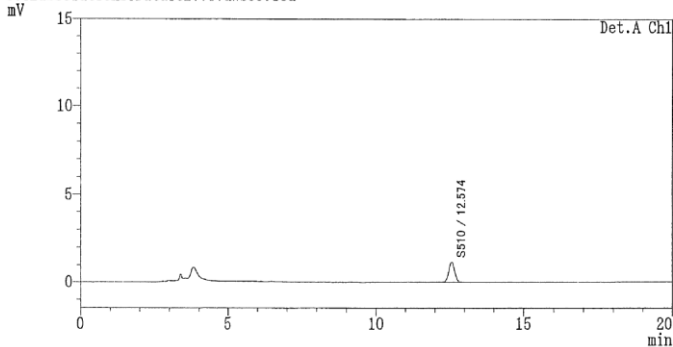
DATE: 2015/06/17

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 7 day
Sample ID : Std. 0.6314 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\VC277\7dWs06.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	12.573	15678	S510
Total		15678	

Water analysis
Day 7

High exposure level

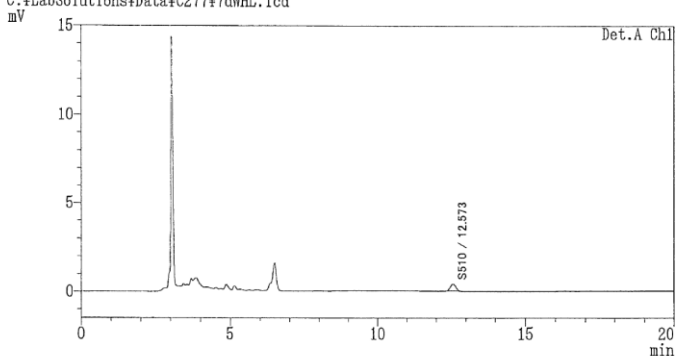
DATE: 2015/06/17

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 7 day
Sample ID : High exposure level

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\C277\F7dWHL.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	12.573	4718	0.1842	S510
Total		4718		

Water analysis
Day 7

Low exposure level

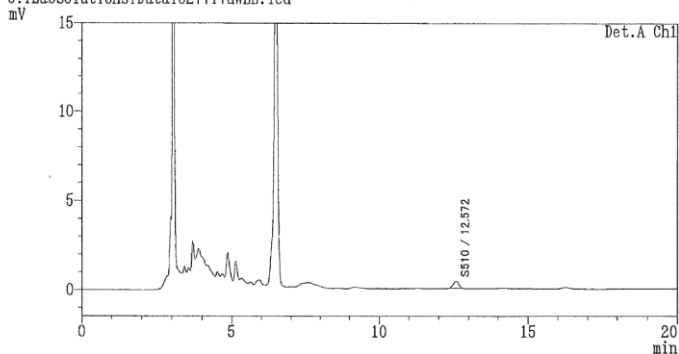
DATE: 2015/06/17

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 7 day
Sample ID : Low exposure level

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\C277\F7dWLL.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	12.572	5004	0.1959	S510
Total		5004		

Water analysis
Day 14

Standard solution
0.0963 mgL⁻¹

DATE: 2015/06/24

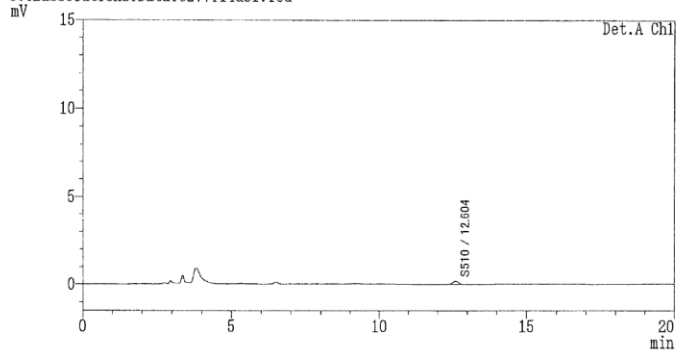
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 14 day
Sample ID : Std. 0.0963 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\14dS1.lcd
mV



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	12.603	2577	S510
Total		2577	

Water analysis
Day 14

Standard solution
0.6314 mgL⁻¹

DATE: 2015/06/24

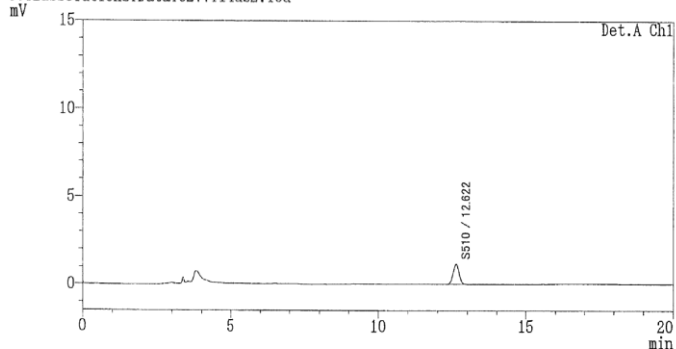
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 14 day
Sample ID : Std. 0.6314 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\14dS2.lcd
mV



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	12.622	15533	S510
Total		15533	

Water analysis
Day 14

High exposure level

DATE: 2015/06/24

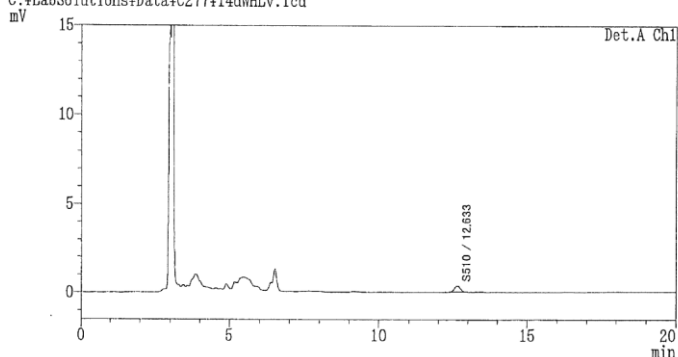
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 14 day
Sample ID : High exposure level

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\Y14d\WHLv.lcd



*** Peak Table ***

peak No.	Time	Area	Conc.	NAME
1	12.633	4758	0.1864	S510
Total		4758		

Water analysis
Day 14

Low exposure level

DATE: 2015/06/24

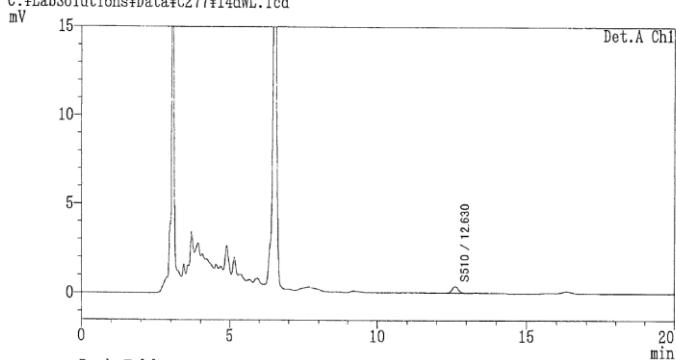
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 14 day
Sample ID : Low exposure level

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\Y14d\WL.lcd



*** Peak Table ***

peak No.	Time	Area	Conc.	NAME
1	12.630	4938	0.1938	S510
Total		4938		

Water analysis
Day 21

Standard solution
0.0963 mgL⁻¹

DATE: 2015/07/01

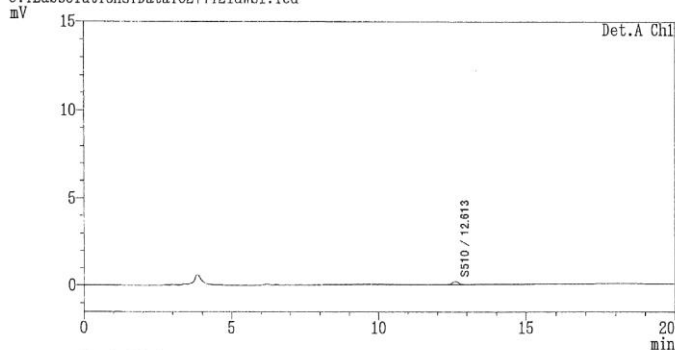
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 21 day
Sample ID : Std. 0.0963 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\277\21dWS1.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	12.612	2386	S510
Total		2386	

Water analysis
Day 21

Standard solution
0.6314 mgL⁻¹

DATE: 2015/07/01

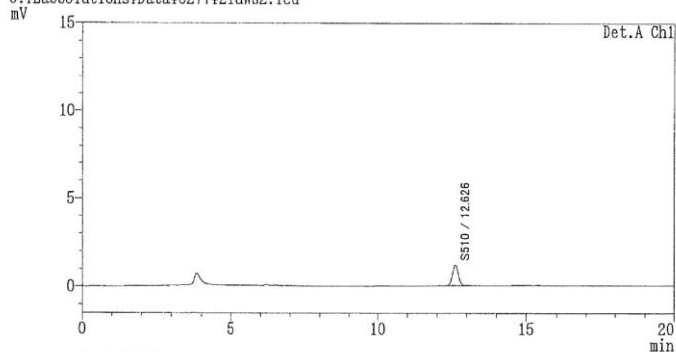
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 21 day
Sample ID : Std. 0.6314 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\277\21dWS2.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	12.625	15985	S510
Total		15985	

Water analysis
Day 21

High exposure level

DATE: 2015/07/01

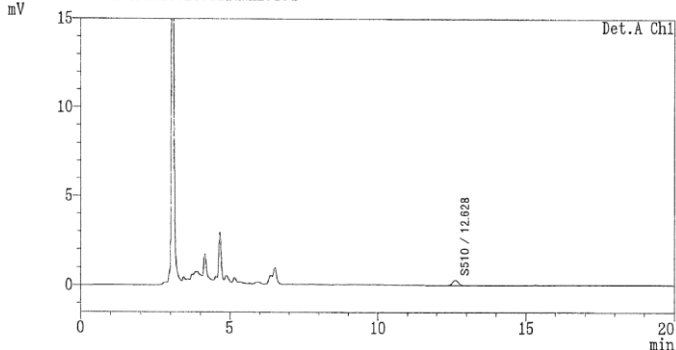
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 21 day
Sample ID : High exposure level

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\4Data\4C277\21dWHL.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	12.628	4167	0.1664	S510
Total		4167		

Water analysis
Day 21

Low exposure level

DATE: 2015/07/01

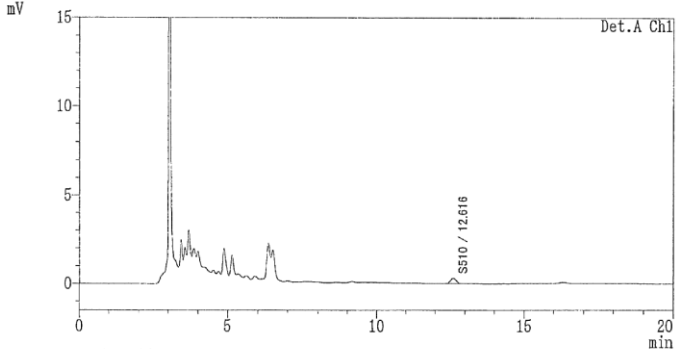
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 21 day
Sample ID : Low exposure level

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\4Data\4C277\21dWLLv.lcd



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	12.616	4276	0.1707	S510
Total		4276		

Water analysis
Day 26

Standard solution
0.0963 mgL⁻¹

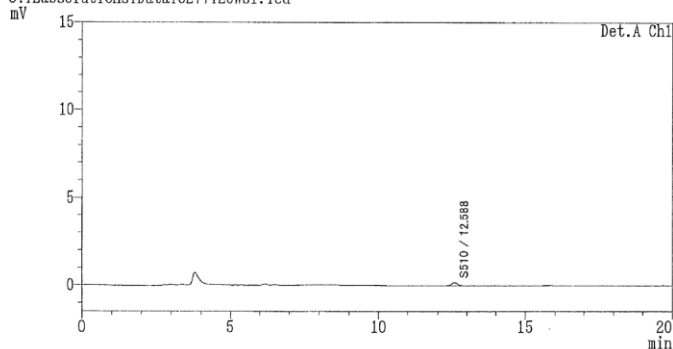
DATE: 2015/07/06

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : Std. 0.0963 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\Y26WS1.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	12.587	2340	S510
Total		2340	

Water analysis
Day 26

Standard solution
0.6314 mgL⁻¹

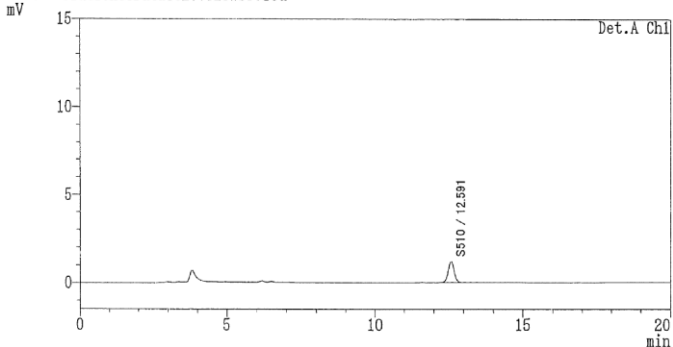
DATE: 2015/07/06

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : Std. 0.6314 mg/L

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\Y26WS6.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	12.590	16010	S510
Total		16010	

Water analysis
Day 26

High exposure level

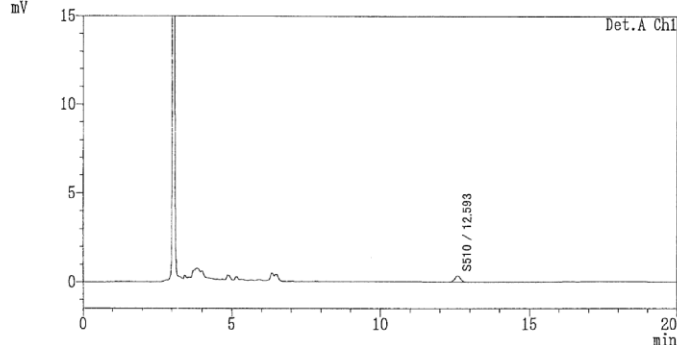
DATE: 2015/07/06

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : High exposure level

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\Y277Y26WH.lcd
mV



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	12.593	4625	0.1857	S510
Total		4625		

Water analysis
Day 26

Low exposure level

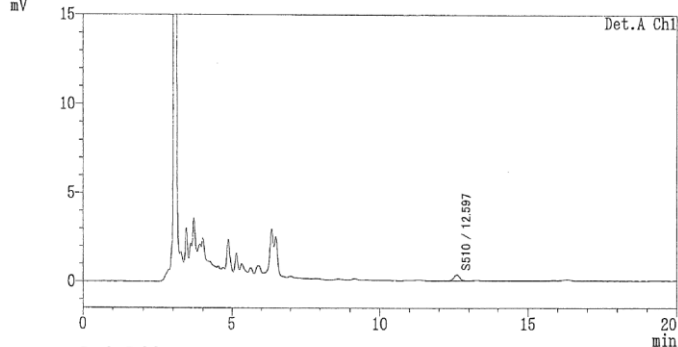
DATE: 2015/07/06

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 26 day
Sample ID : Low exposure level

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\Y277Y26WL.lcd
mV



*** Peak Table ***

Detector A Ch1 214nm

peak No.	Time	Area	Conc.	NAME
1	12.597	4503	0.1810	S510
Total		4503		

Water analysis
Day 28

Standard solution
0.0963 mgL⁻¹

DATE: 2015/07/08

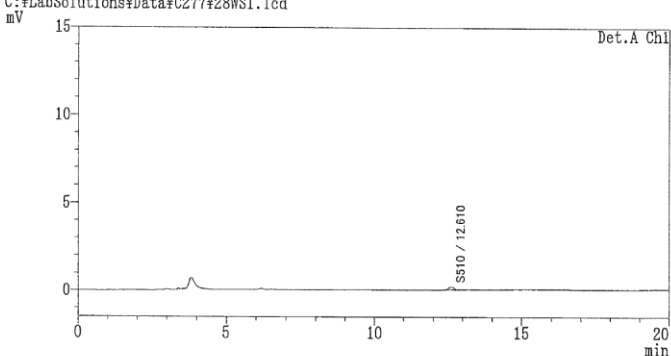
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : Std. 0.0963 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\28WS1.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	12.610	2358	S510
Total		2358	

Water analysis
Day 28

Standard solution
0.6314 mgL⁻¹

DATE: 2015/07/08

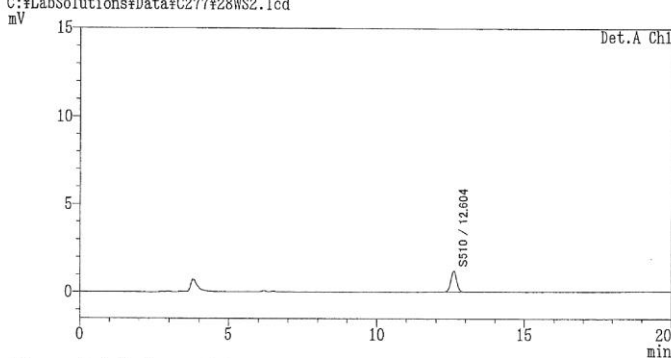
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : Std. 0.6314 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\YData\YC277\28WS2.lcd



*** Peak Table ***

peak No.	Time	Area	NAME
1	12.603	15985	S510
Total		15985	

Water analysis
Day 28

High exposure level

DATE: 2015/07/08

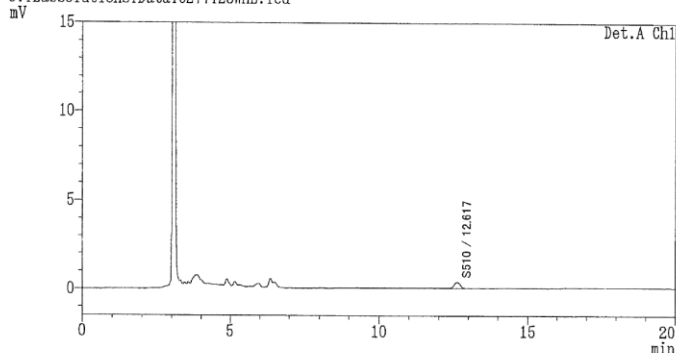
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : High exposure level

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\28WHL.lcd



*** Peak Table ***

peak No.	Time	Area	Conc.	NAME
1	12.617	4543	0.1821	S510
Total		4543		

Water analysis
Day 28

Low exposure level

DATE: 2015/07/08

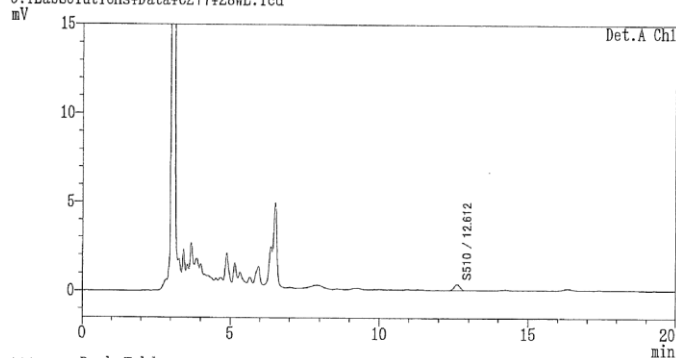
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 28 day
Sample ID : Low exposure level

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\YC277\28WL.lcd



*** Peak Table ***

peak No.	Time	Area	Conc.	NAME
1	12.612	4522	0.1813	S510
Total		4522		

9-2 Control group

Water analysis
Day 0

Standard solution
0.0963 mgL⁻¹

DATE: 2015/06/10

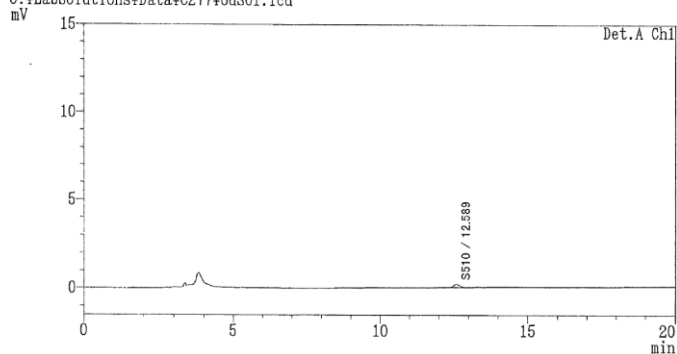
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 0 day
Sample ID : Std. 0.0963 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\C277\0dS01.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	12.589	2379	S510
Total		2379	

Water analysis
Day 0

Standard solution
0.6314 mgL⁻¹

DATE: 2015/06/10

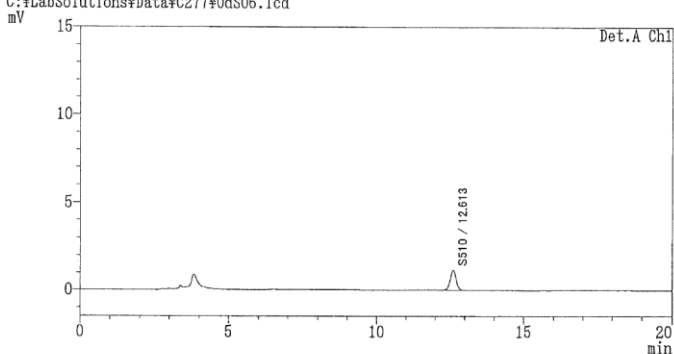
SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 0 day
Sample ID : Std. 0.6314 mg/L

*** ANALYTICAL CONDITIONS ***

HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-Vis 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\Data\C277\0dS06.lcd



*** Peak Table ***
Detector A Ch1 214nm

peak No.	Time	Area	NAME
1	12.613	15617	S510
Total		15617	

Water analysis
Day 0

Control group

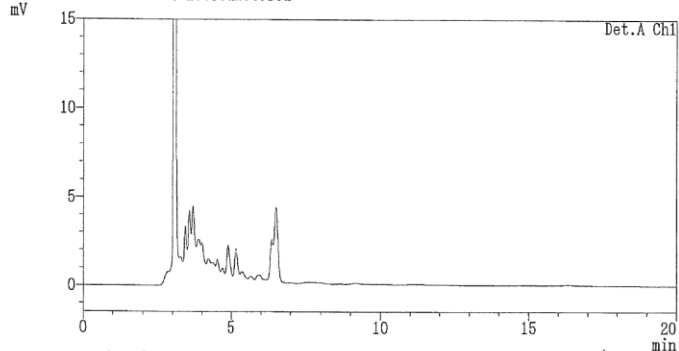
DATE: 2015/06/10

SAMPLE : [C-277] Bioconcentration test on S510
REMARK : Analysis 0 day
Sample ID : Control

*** ANALYTICAL CONDITIONS ***
HPLC TYPE : SHIMADZU LC-10A
COLUMN : InertSustain C18 4.6×250 mm (5μm)
ELUENT : MeCN
FLOW RATE : 1.0 mL/min
COLUMN TEMP : 40 °C
WAVELENGTH : UV-VIS 214 nm
INJECT VOL. : 20 μL

OPERATOR : T. Sato

*** Chromatgram ***
C:\LabSolutions\¥Data\¥C277\¥0dWCT.lcd
mV



*** Peak Table ***
Detector A Ch1 214nm

Reference 10 Water quality test

Analyzed at Institute of Ecotoxicology, Inc.

<Quantitative analysis of the residual chlorine>

The quantification of 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	6. 3. 2015
<0.01	6. 10. 2015
<0.01	6. 17. 2015
<0.01	6. 24. 2015
<0.01	7. 1. 2015
<0.01	7. 8. 2015

<Quantitative analysis of copper>

The quantification of copper in water used in this study was performed according to JIS K0101 51.1 (Absorptimetry). The concentration of copper was confirmed to be 0.005 mgL⁻¹ or less.

Copper (mgL ⁻¹)	Date of quantitative analysis
< 0.005	6. 30. 2015

<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	6. 29. 2015

Report of the test results

To the Institute of Ecotoxicology Inc.

3-15-1 Komazawa, Setagaya-ku, Tokyo
Shinnihon Environmental Research Corporation
(Measurement Certification Industry Registration,
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 7, 2015

Sample Names	Water used for raising carp	Quantifiable lower limit	Test methods
Items analyzed			
Nitric nitrogen and nitrous nitrogen (mg/L)	1.5	0.02	Service water test method (2001) VI-2 13.2
Potassium permanganate consumed (mg/L)	1.2	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)	0.011	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)	0.08	0.05	Service water test method (2001) VI-2 3.2
Total hardness (mg/L)	66	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 (water temperature at time of analysis)	7.1 (20.4)	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 Observation record of the test fish (carp)

Observation record of the test fish	No 1	Test Substance	S510	Test No.	C-277
-------------------------------------	------	----------------	------	----------	-------

Date	Feeding period (days)	Concentration Level 1 Group (Water tank No. 4)					Concentration Level 2 Group (Water tank No. 5)					Control Group (Water tank No. 6)					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	
2015																				
6.10	0	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	24.5	24.5	24.5	T.S
11	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	24.4	24.5	24.4	T.S
12	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	24.2	24.3	24.5	T.S
13	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	24.2	24.6	24.2	T.S
14	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	24.5	24.9	24.4	T.S
15	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	24.5	24.7	24.4	T.S
16	6	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	24.9	24.7	24.4	T.S
17	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	24.8	24.3	24.4	T.S
18	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	24.5	24.8	24.6	T.S
19	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	24.6	24.9	24.9	T.S
20	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	24.5	24.2	24.6	T.S
21	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	24.2	24.2	24.7	T.S
22	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	24.6	24.7	24.9	T.S
23	13	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	25.3	24.6	25.1	T.S
24	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	25.3	24.9	24.9	T.S
		◆ Flow rate: Level 1: 300 ml/min, Level 2: 300 ml/min, Control: 300 ml/min to be confirmed.																		
		◆ Food intake: 0.06 g per fish ◆Feed provided: Maruha-jirushi compound feed for juvenile carp C-2 (Hayashikane Sangyo Co., Ltd. Lot 01374)																		
		◆ 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.																		

Observation record of the test fish	No 2	Test Substance	S510	Test No.	C-277
-------------------------------------	------	----------------	------	----------	-------

Date	Feeding period (days)	Concentration Level 1 Group (Water tank No. 4)					Concentration Level 2 Group (Water tank No. 5)					Control Group (Water tank No. 6)					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	
2015																				
6.25	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	25.1	24.7	24.5	T.S
26	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	24.6	24.5	24.4	T.S
27	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	24.9	24.3	24.5	T.S
28	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	24.4	24.9	24.7	T.S
29	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	24.9	24.7	24.9	T.S
30	20	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	24.9	24.9	24.4	T.S
7. 1	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	24.8	24.5	24.4	T.S
2	22	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	24.6	25.2	24.2	T.S
3	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	24.6	25.0	24.5	T.S
4	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	24.7	25.0	24.4	M.T
5	25	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	24.5	25.5	24.5	T.S
6	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	25.2	25.0	24.7	M.T
7	27	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	O.K.	—	O.K.	O.K.	0	24.8	24.8	24.4	T.S
8	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	24.6	24.7	24.6	T.S
		◆ Flow rate: Level 1: 300 ml/min, Level 2: 300 ml/min, Control: 300 ml/min to be confirmed. ◆ Food intake: 0.06 g per fish ◆ Feed provided: Maruha-jirushi compound feed for juvenile carp C-2 (Hayashikane Sangyo Co., Ltd. Lot 01374) ◆ 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 UV/VIS absorption spectrum of the test substance

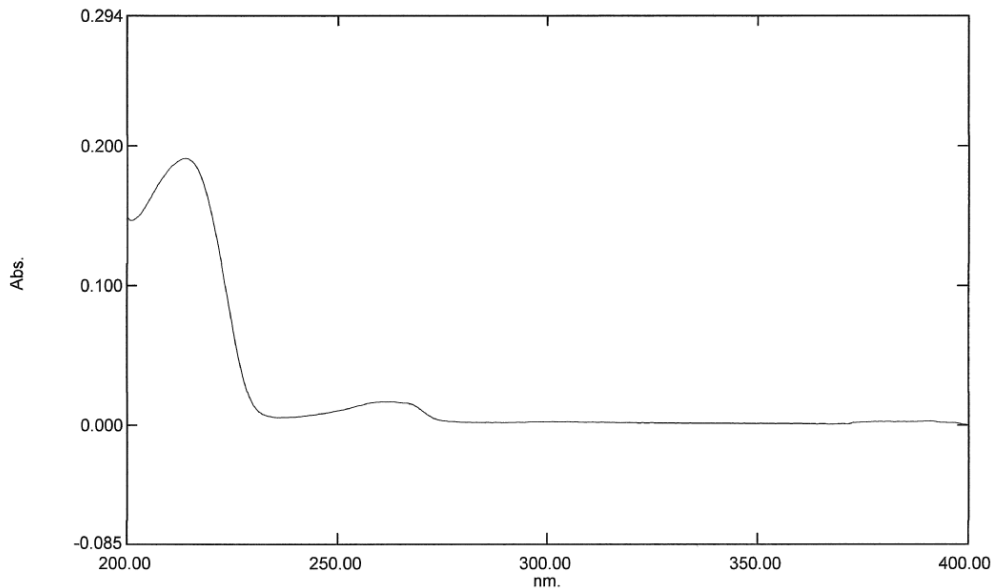
UV (Acetonitrile)

$\lambda_{\max}$ (log ϵ) 214.00 nm (3.95)

Spectrum Point Pick Report

04/24/2015 04:46:15 PM

Data Set: C277 S510 - RawData



[Measurement Properties]
Wavelength Range (nm.): 190.00 to 400.00
Scan Speed: Fast
Sampling Interval: 0.2
Auto Sampling Interval: Enabled
Scan Mode: Single

No.	Wavelength	Absorbance	Description
1	214.00	0.191	
2			

[Instrument Properties]
Instrument Type: UV-2600 Series
Measuring Mode: Absorbance
Slit Width: 2.0
Accumulation time: 0.1 sec.
Light Source Change Wavelength: 323.0 nm
Detector Unit: Direct
S/R Exchange: Normal
Stair Correction: OFF

[Attachment Properties]
Attachment: None

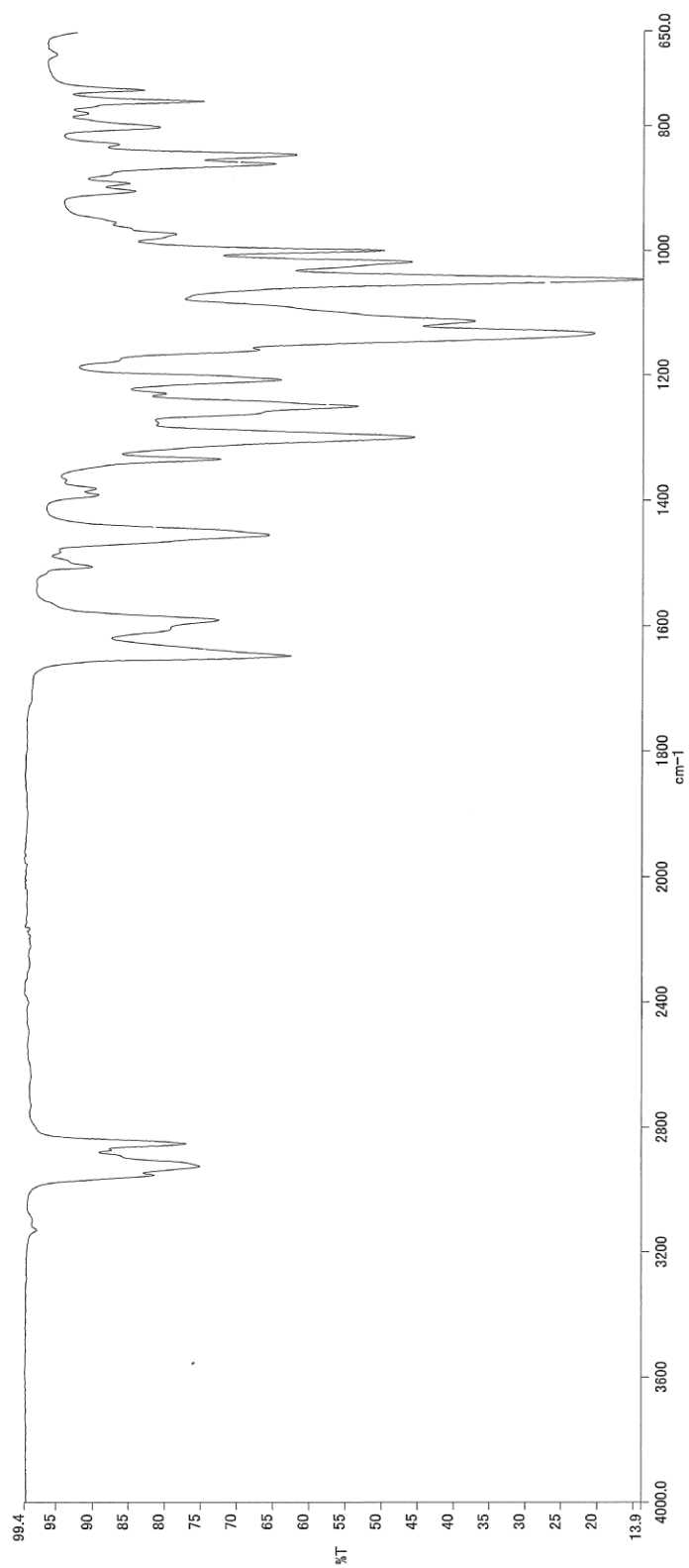
[Operation]
Threshold: 0.1000000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

Standard solution: 9.63mg/L (MeCN)

T. Sato

Reference 13 IR spectra for the test substance
IR spectrum provided by the sponsor



File name: 3HHXFFCF3

Resolution: 4 cm-1

No. of scan: 4

Apodization: strong

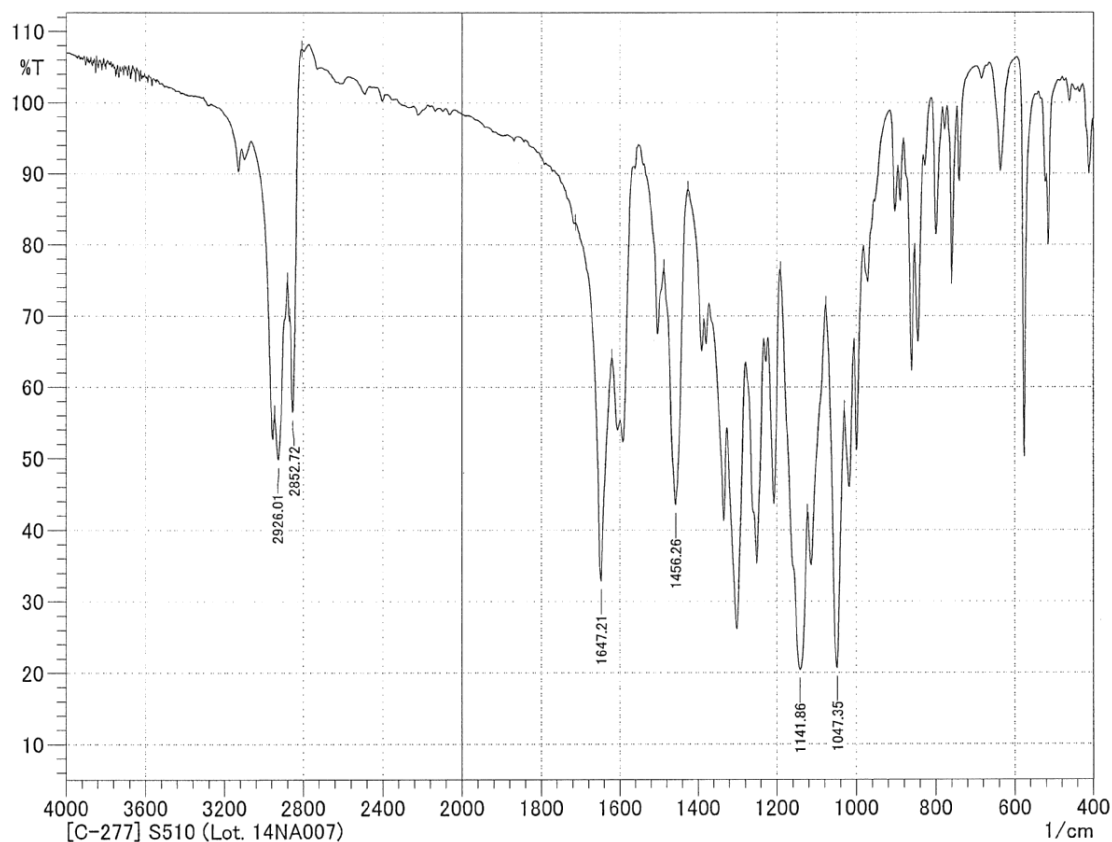
Date/time: 2015/04/16 11:23 (Standard time in Tokyo)

Sample: S510 (3HHXFFCF3)

Lot: 14NA007

Operator: Ichiro Sakabe

IR spectrum obtained at our laboratory before the test



File Name: C277S1

Sample: [C-277] S510 (Lot. 14NA007)

Date/Time: 2015/04/24 16:12:31

Operator: T. Sato

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]

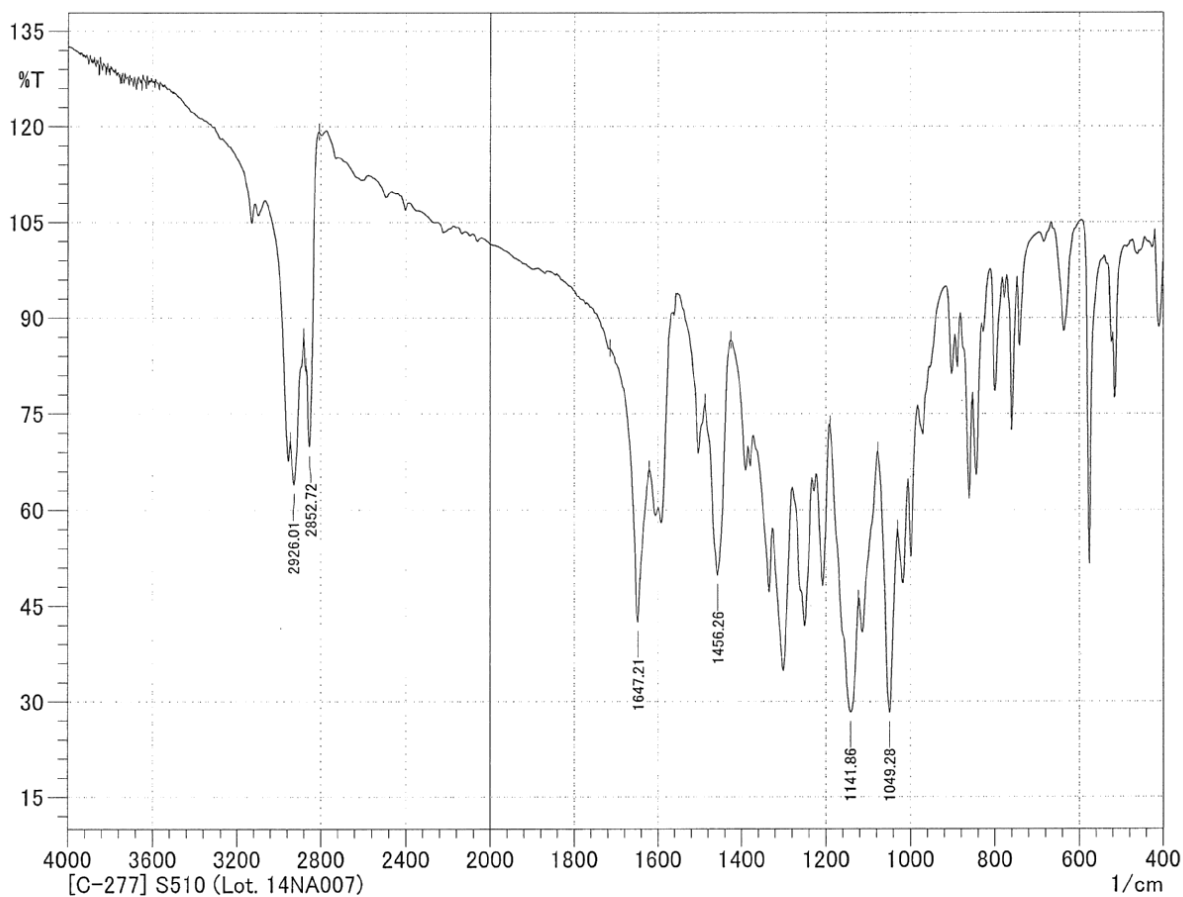
Gain: auto

Aperture: auto

SpeedStd: 2.8

	Peak	Intensity	
1	1047.35	20.72	C-O stretch
2	1141.86	20.474	C-F stretch
3	1456.26	43.532	C=C ring stretch
4	1647.21	32.902	
5	2852.72	56.508	C-H stretch
6	2926.01	49.873	

IR spectrum obtained at our laboratory after the test



File Name: C277E1

Sample: [C-277] S510 (Lot. 14NA007)

Date/Time: 2015/07/08 15:09:59

Operator: T. Sato

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

	Peak	Intensity	
1	1049.28	28.23	C-O stretch
2	1141.86	28.28	C-F stretch
3	1456.26	49.81	C≡C ring stretch
4	1647.21	42.5	
5	2852.72	69.85	C-H stretch
6	2926.01	63.99	

On the following pages, the original (Japanese version) of the final report, excluding reference materials, is attached.

最 終 報 告 書

S510のコイ *Cyprinus carpio* における濃縮度試験

報告書番号 E 4 - 1 5 0 2 2 ・ C 2 7 7 ・ C H

2015 年 7 月 15 日

株式会社エコトキシコロジー研究所

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陳 述 書

株式会社エコトキシコロジー研究所

試験委託者 JNC株式会社

試験の表題 S510 のコイ *Cyprinus carpio* における濃縮度試験

試験番号 C-277

上記試験は、「新規化学物質等に係る試験を実施する試験施設に関する基準について」（平成23年3月31日薬食発0331第8号 厚生労働省医薬食品局長，平成23・03・29製局第6号 経済産業省製造産業局長及び環境企発第 110331010号 環境省総合環境政策局長連名通知）および「OECD Principles of Good Laboratory Practice」（as revised in 1997）に従って実施したものです。

2015 年 7 月 15 日

試験責任者

佐藤 崇



信 頼 性 保 証 書

表 題 S510 のコイ *Cyprinus carpio* における濃縮度試験

試験委託者 JNC株式会社

試験責任者 佐藤 崇

試験担当者 佐藤 崇

試験実施施設 株式会社エコトキシコロジー研究所

本最終報告書は、試験の方法が正確に記載されており、内容が試験計画書および標準操作手順書に従って行われ、かつ生データを正確に反映していることを保証します。

なお、査察および監査を行った日付は下記の通りです。

		記	
		実 施 日	運営管理者および試験責任者への報告日
試験実施状況査察	暴露開始 7日目水・魚分析	2015 年 6 月 17 日	2015 年 6 月 17 日
	暴露開始14日目水・魚分析	2015 年 6 月	日
	暴露開始21日目水・魚分析	2015 年 7 月 1 日	2015 年 7 月 1 日
試験計画書監査	計 画 書 監 査	2015 年 5 月 11 日	2015 年 5 月 11 日
	計画書(改訂)監査	2015 年 6 月 8 日	2015 年 6 月 8 日
	計画書(改訂)(変更)監査	2015 年 7 月 13 日	2015 年 7 月 13 日
最終報告書監査	報 告 書 監 査	2015 年 7 月 9 日	2015 年 7 月 9 日
		2015 年 7 月 15 日	2015 年 7 月 15 日

株式会社エコトキシコロジー研究所信頼性保証部門

信頼性保証業務責任者：

後藤 幹保



署名日： 2015 年 7 月 15 日

(試験実施状況査察および試験計画書・最終報告書監査)

信頼性保証業務担当者：

高松 みつ子



署名日： 2015 年 7 月 15 日

(試験実施状況査察および試験計画書・最終報告書監査)

信頼性保証業務担当者：

栗原 みゆき



署名日： 2015 年 7 月 15 日

(試験計画書および最終報告書の監査)

最 終 報 告 書

前 文

1. 表 題 S510 のコイ *Cyprinus carpio* における濃縮度試験
2. 試験委託者 社名 JNC株式会社
住所 〒100-8105 東京都千代田区大手町2-2-1
3. 試験施設 株式会社エコトキシコロジー研究所
住 所 〒331-0811 埼玉県さいたま市北区吉野町2丁目28番地1
電 話 XXXXXXXXXX
(代 表 者) 後藤幹保
(運営管理者) 菅 忠義
4. 試験目的 被験物質の魚における濃縮性の程度についての知見を得る。
5. 採用した試験法 「新規化学物質等に係る試験の方法について<魚介類の体内における化学物質の濃縮度試験>」(平成23年3月31日薬食発0331第7号 厚生労働省医薬食品局長, 平成23・03・29製局第5号 経済産業省製造産業局長及び環境企発第110331009号 環境省総合環境政策局長連名通知)および
OECD化学品テストガイドライン, “No. 305, Bioaccumulation in Fish: Aqueous and Dietary Exposure”(2012年10月2日採択)による。
6. 適用GLP 化学物質 GLP: 「新規化学物質等に係る試験を実施する試験施設に関する基準について」(平成23年3月31日薬食発0331第8号 厚生労働省医薬食品局長, 平成23・03・29製局第6号 経済産業省製造産業局長及び環境企発第110331010号 環境省総合環境政策局長連名通知) による。
OECD-GLP: 「OECD Principles of Good Laboratory Practice」(as revised in 1997)」による。

7. 被験物質の名称 *trans*-4- { [3, 5-ジフルオロ-4- (トリフルオロメチル) フェノキシ] (ジフルオロ) メチル} -4'- *trans*-プロピル-1, 1'-ビスクロヘキシル
略 称: S510 (Lot No. 14NA007)
CAS番号: 208338-86-7

8. 試験期間 (1) 試験開始日 2015 年 5 月 11 日
(2) 試験実施期間
供試魚受入日 2015 年 5 月 18 日
馴化終了日 2015 年 6 月 10 日
実験開始日 2015 年 6 月 10 日
実験終了日 2015 年 7 月 8 日
(3) 試験終了日 2015 年 7 月 15 日

9. 試験結果 被験物質 S510 のコイ *Cyprinus carpio* における濃縮度試験を行った。
28日間の暴露期間における濃縮倍率の結果を下表にまとめた。

S510 の濃縮倍率 (BCF)	第 1 濃度区 (0.02 mgL ⁻¹)	第 2 濃度区 (0.002 mgL ⁻¹)
濃縮倍率 (BCF)	12~30	10~29

10. 試験結果の評価と考察および結論 被験物質のゼブラフィッシュ *Danio rerio* における96時間LC₅₀値は 25.6 mgL⁻¹ より大きかった。また対水溶解度は、 2.3×10^{-2} mgL⁻¹ であったことより、試験濃度は第1濃度区 0.02 mgL⁻¹、第2濃度区 0.002 mgL⁻¹ の2濃度に設定した。本濃縮度試験の結果、第1濃度区、第2濃度区ともに、濃縮倍率 (BCF) が28日間を通して50倍未満であったため、28日目で定常に達したとみなして暴露を終了した。被験物質S510の濃縮性は低いことが確認された。

11. 試資料の保管 試験に関する下記の記録および試料は、化学物質の審査及び製造等の規制に関する法律（昭和48年法律第117号。以下「化審法」という。）第4条第1項若しくは第2項、第5条第2項、第3項若しくは第8項、第10条第3項又は第14条第2項の規定による通知を受けた後10年間、被験物質、対照物質、その他の試料は、化審法第4条第1項若しくは第2項、第5条第2項、第3項若しくは第8項、第10条第3項又は第14条第2項の規定による通知を受けた後10年間又は品質低下を起こさないで安定に保存しうる期間のいずれか短い方の期間、株式会社エコトキシコロジー研究所に保管する。
その後の保管については別途協議の上決める。

- (1) 試験計画書、修正・変更記録
- (2) 被験物質等に関する記録
- (3) 実験ノート、生データ、チャート類

- (4) 被験物質, 対照物質, その他の試料 (約20℃, 暗所で保存)
- (5) 本試験に関する信頼性保証担当者の査察・監査記録
- (6) 最終報告書
- (7) その他必要なもの

12. データの信頼性に影響を及ぼしたと思われる環境要因
特に異常は認められなかった。

13. 試験関係者氏名および業務分担

試験責任者 佐 藤 崇

試験担当者 佐 藤 崇

- ・ 96時間LC₅₀試験
- ・ 対水溶解度の測定
- ・ 試験水分析法の確立
- ・ 魚体分析法の確立
- ・ 検量線の作成
- ・ 試験水の添加回収試験
- ・ 魚体の添加回収試験
- ・ 被験物質分散方法の検討
- ・ 試験期間およびサンプリングスケジュール設定のための予備試験
- ・ 実験期間中の試験水および魚体分析
- ・ 実験期間中の試験液の調製・保守
- ・ 実験期間中の水環境の測定
- ・ 脂質含量の測定
- ・ IRの測定
- ・ 全吸光係数の測定
- ・ 魚の状態観察
- ・ 溶存有機炭素濃度の測定

最終報告書作成者 佐 藤 崇

14. 最終報告書の報告に関与した専門家の氏名と所属
該当なし

15. 最終報告書の承認

試験責任者: 佐 藤 崇 

署名日: 2015 年 7 月 15 日

本 論

1. 被験物質

本報告書において被験物質は、次の名称、構造式を有するものとする(委託者提供資料による)。

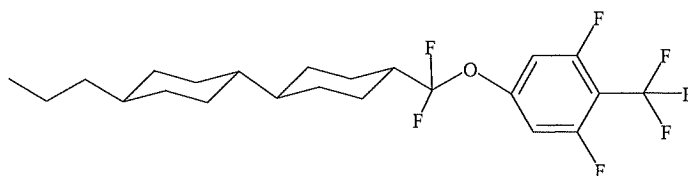
1.1 名 称

trans-4-{[3,5-ジフルオロ-4-(トリフルオロメチル)フェノキシ](ジフルオロ)メチル}-4'-*trans*-プロピル-1,1'-ビスクロヘキシル

略 称: S510

CAS番号: 208338-86-7

1.2 構造式



分子式: $C_{23}H_{29}F_7O$ (MW 454.46)

1.3 提供者およびロット番号

(1) 提 供 者 JNC株式会社

(2) ロット番号 14NA007

1.4 同 定

試験開始時に委託者提供の赤外吸収スペクトルと当所で測定した被験物質のスペクトルが一致することにより同一性を確認した。さらに試験終了時の赤外吸収スペクトルが、試験開始時のそれと一致したことにより、試験期間中の被験物質の安定性を確認した(参考資料 13 参照)。

1.5 物理化学的性状(*委託者提供データによる)

外 観 : 白色固体

純 度* : $\geq 99.9\%$

不純物 不明 $\leq 0.1\%$

融 点* : 83°C

溶 解 度 : [当所測定データ, 25°C]

水 $2.3 \times 10^{-2} \text{ mgL}^{-1}$ (フラスコ法)

n-ヘキサン $> 1.0 \times 10^5 \text{ mgL}^{-1}$

ジクロロメタン $> 1.0 \times 10^5 \text{ mgL}^{-1}$

アセトン $> 1.0 \times 10^5 \text{ mgL}^{-1}$

アセトニトリル $1.0 \times 10^4 \text{ mgL}^{-1}$

ジメチルスルホキシド $1.2 \times 10^3 \text{ mgL}^{-1}$

2. 試験方法および分析法

平成23年3月31日薬食発0331第7号, 平成23・03・29製局第5号, 環境企発第110331009号: 新規化学物質等に係る試験の方法について<魚介類の体内における化学物質の濃縮度試験>および「OECD Guidelines for Testing of Chemicals」に定める “No.305, Bioaccumulation in Fish: Aqueous and Dietary Exposure”(2012年10月2日採択)に従って行った。

2.1 被験物質の濃度設定のためのゼブラフィッシュによる急性毒性試験(96時間 LC_{50} 試験)(SOP 2.6)

予備試験の結果をもとに, 濃度設定を行い96時間 LC_{50} 値を求めた(OECDテストガイドライン 203の方法を参考にして行った)。

(1) 試験魚

ゼブラフィッシュ (*Danio rerio*)

Lot No. 150310

入 手 先 神畑養魚株式会社 (千葉県千葉市花見川区千種町 292-1)

入 手 日 2015 年 3 月 10 日

平均全長 $3.4 \pm 0.1 \text{ cm}$

平均体重 $0.25 \pm 0.02 \text{ g}$

馴化期間 2015 年 3 月 10 日 ~ 2015 年 5 月 12 日

入手・馴化は, SOP 2.1, SOP 2.5 に従って行った。

- (2) 被験物質原液調製法 分散剤 Polyoxyethylene Sorbitan Monooleate (Tween 80)
- 分散法 被験物質および被験物質の 4 倍量の Tween 80 をガラス製サンプル管に秤り込み、加温しながら混合した。三角フラスコ中で超純水を攪拌しながら沸騰させた後、先の混合物に加えた。攪拌しながら室温まで空冷した後、超純水を加えて 1000 mgL^{-1} 原液を調製した。原液を試験用水で希釈し試験水とした。
- (3) 試験温度 $25.1 \pm 0.1^{\circ}\text{C}$
- (4) 試験水槽 ガラス製，試験水量 4 L
- (5) 暴露方法 半止水式（48時間目に換水）
- (6) 試験用水 種 類 さいたま市上水の脱塩素水
- 水質検査 水質検査の結果，参考資料 10 に示す項目が「水産用水の水質基準」に適合していることを確認した。
- (7) 生物量 10尾／4 L水槽
- (8) 試験期間 2015年 5 月 12 日 ～ 2015年 5 月 16 日
- (9) 感受性試験 基準物質として硫酸銅（Ⅱ）五水和物を用いた感受性試験を実施し，当所の定める検定値（96時間 LC_{50} 値 $0.08 \sim 0.3 \text{ mgL}^{-1}$ ）に合格した同一のロットのものを供試魚とした。
- (10) 試験結果 被験物質は 4 倍量の分散助剤（Tween 80）を用いて分散を行った。分散助剤濃度が 100 mgL^{-1} を超えないように被験物質濃度を設定した場合，分散可能な被験物質濃度の上限は 25 mgL^{-1} である。予備試験の 96 時間 LC_{50} 値は 25 mgL^{-1} より大きかったことより，本試験は被験物質濃度 25 mgL^{-1} を最高濃度とする限度試験とした。その結果，96 時間 LC_{50} 値は 25.6 mgL^{-1} より大きいことを確認した。被験物質の濃度は，HPLC 測定により求めた初期実測値である（下表参照）。

試 験 水		死 亡 率 (%)			
化 合 物	濃 度 (mgL^{-1})	24 時間	48 時間	72 時間	96 時間
硫酸銅(Ⅱ)五水和物	0.5	50	80	100	100
	0.05	0	0	0	0
被験物質区	25.6	0	0	0	0
対照(助剤)区 Tween 80	100	0	0	0	0
対照(試験用水)区	—	0	0	0	0

2.2 濃縮度試験の実施

(1) 試験魚

濃縮度試験の試験魚として、日本の代表的魚種コイ *Cyprinus carpio* を選定(SOP 2.1②)し、濃縮性を確認した。

コイ *Cyprinus carpio* (Lot No. 150518 No. 3)
購入先 北村養鯉場 (熊本県八代市郡築12番町388)
購入日 2015年 5 月 18 日
蓄養および馴化 (株)エコトキシコロジー研究所にて受領後、全長 8.0 ± 4.0 cm程度のものを選別(SOP2.4)し、エルバーージュ(上野製薬(株))、岩塩にて薬浴後、馴化水槽No.1にて馴化を開始した(SOP2.3, SOP2.5)。最終的に $25 \pm 2.0^\circ\text{C}$ の水温で 7 日間慣らし、死亡率が 5% 以下であることを確認し、試験に使用した。

試験魚の状態

コイ	平均体重	4.45 ± 0.33 g	(SOP 2.8)
	平均全長	7.8 ± 0.1 cm	(SOP 2.8)
試験期間中の	第1濃度区	0%	(SOP 2.7)
魚の死亡率	第2濃度区	0%	
	対照区	0%	

脂質含有量 (クロロホルム-メタノール抽出法による)

試験開始前	4.1% (平均 n=2)	(SOP 2.9)
試験終了時	3.8% (平均 n=2)	(SOP 2.9)

(試験魚の状態観察は、参考資料 11 に示した。)

(2) 給餌

種類	まるは印配合飼料 コイ稚魚用 C-2
	粗蛋白質 42.0%
	粗脂肪 3.0%
製造元	林兼産業株式会社
給餌方法	供試魚体重の約 1.5%を目安に毎日給餌した。ただし、試験魚の採取日前日は給餌しなかった。

(3) 試験の実施

試験用水

2.1 (6)に同じ。

被験物質溶液

原液調製法 被験物質および被験物質の 4 倍量の Tween 80 をガラス製サンプル管に秤り込み, 加温しながら混合した。三角フラスコ中で超純水を攪拌しながら沸騰させた後, 先の混合物に加えた。攪拌しながら室温まで空冷した後, 超純水を加えて 1000 mgL^{-1} 原液を調製した。原液を試験用水で希釈し試験水とした。

第 1 濃度区 上記原液を試験用水で希釈し, 2 mgL^{-1} とした。これをさらに試験用水で希釈し, 連続的に試験水槽に流した。

第 2 濃度区 上記原液を試験用水で希釈し, 0.2 mgL^{-1} とした。これをさらに試験用水で希釈し, 連続的に試験水槽に流した。

対 照 区 Tween 80 の 8 mgL^{-1} 液を試験用水で希釈し, 連続的に試験水槽に流した。

濃縮度試験装置 参考資料 1 参照

試験水槽 “流水式”

ガラス製 容 量 100 L (試験水量 50 L)

流量 300 mL min^{-1} (432 L day^{-1})

第 1 濃度区は No.1 水槽, 第 2 濃度区は No.2 水槽, また対照区は No.3 水槽を使用した。

試験濃度 被験物質の対水溶解度は $2.3 \times 10^{-2} \text{ mgL}^{-1}$ であった。濃縮度試験では, 対水溶解度以下での設定濃度とした。

さらにゼブラフィッシュ *Danio rerio* による 96 時間 LC_{50} 値は 25.6 mgL^{-1} より大きかったことより, 安全係数を $1/1280$, $1/12800$ とし, 設定濃度を次のように定めた。

	第 1 濃度区	第 2 濃度区	対 照 区
被験物質濃度	0.02 mgL^{-1}	0.002 mgL^{-1}	—
助剤濃度 (Tween 80)	0.08 mgL^{-1}	0.008 mgL^{-1}	0.08 mgL^{-1}

試験水温度 $24.7 \pm 0.3 \text{ }^{\circ}\text{C}$

試験水 pH 7.6 ± 0.1

試験水 DO $7.36 \pm 0.22 \text{ mgL}^{-1}$

(各分析日の温度, 試験水 pH, 試験水 DO は, 参考資料 2-2 に示した。)

試験水の全有機炭素濃度の測定 (SOP 2.12)

第1濃度区 0.4886 mgL⁻¹

第2濃度区 0.1364 mgL⁻¹

対照区（助剤添加区） 0.2262 mgL⁻¹

10 mgL⁻¹以下であることを確認した。

照明の種類 紫外線カット蛍光灯

照光時間 約10時間

暴露期間 2015年6月10日～2015年7月8日

分析日程は2.8項に示した。

試験魚数 第1濃度区45尾，第2濃度区45尾，対照区15尾

分析魚数および繰り返し数

1回の分析は2尾1連とし， $n = 2$ で実施した。

対照区の分析は2尾1連とし， $n = 1$ で実施した。

2.3 魚体および試験水の分析

2.3.1 魚体分析法 (SOP 2.11)

前処理

第1濃度区、第2濃度区および対照区より分析日に試験魚を6尾（ただし対照区においては2尾）ずつ手網で採取し、氷水に入れ仮死させ、水道水で魚体表面を洗った後ガーゼで軽く拭き、上皿天秤（SOP/INS/4.2.2）で体重を0.01 g単位まで測定した。全長は口の先端から尾ひれの先端までの長さを0.1 cm単位まで測定した。うち2尾ずつを1連とし、各濃度区2連を分析に用い、残りの1連はアルミホイルに包み、1尾ずつポリ袋に入れた後、試験水槽ごとにポリ袋（チャック付き）に入れて密封し冷凍保存した（SOP 2.15）。

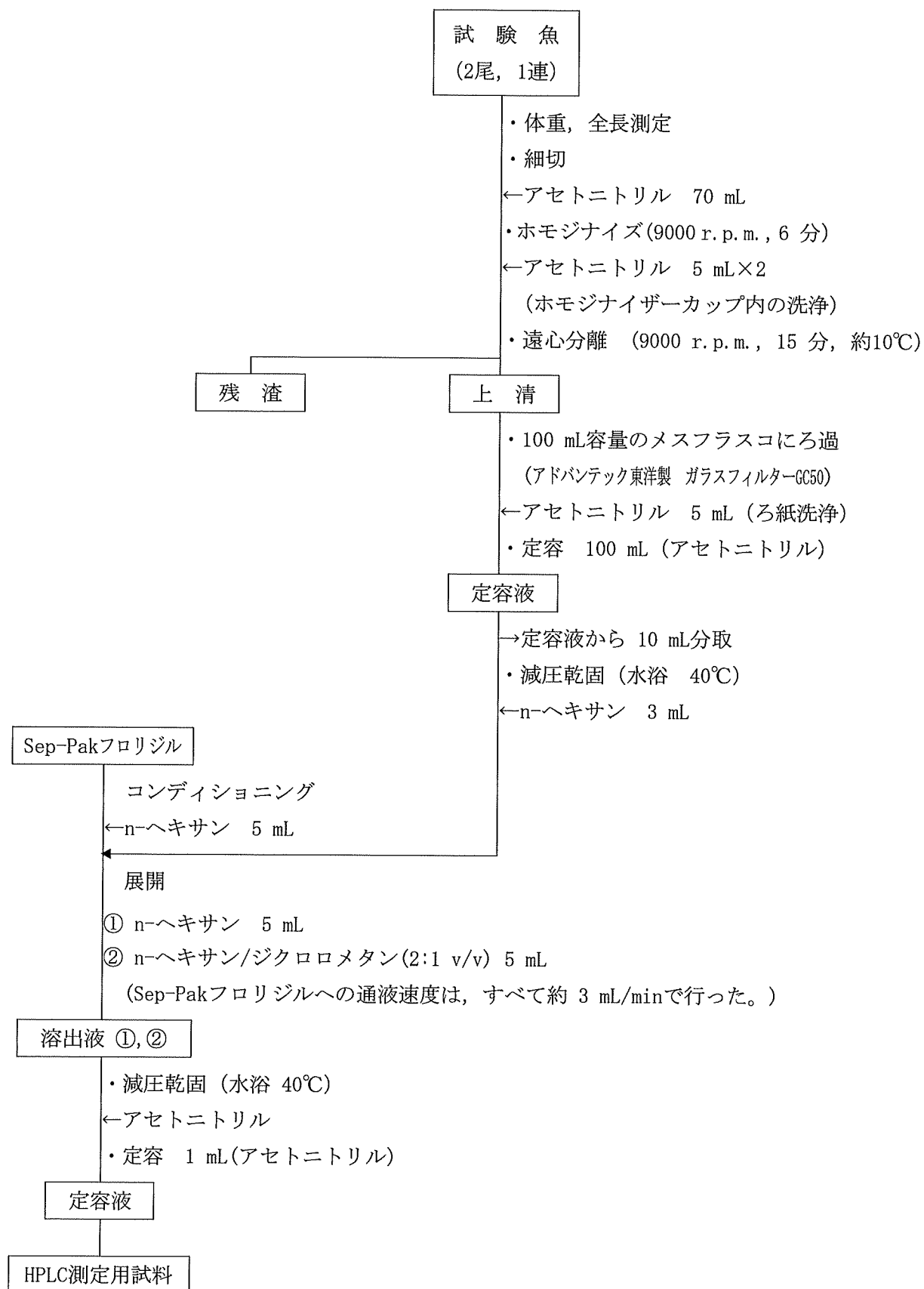
試験魚を細切れにし、ホモジナイザーカップに入れた。アセトニトリル 70 mLを加え、9000 r.p.m. で6分間、ホモジナイズ（SOP/INS/4.17.2）した。内容物を遠心管に移した。ホモジナイザーカップ内をアセトニトリル 5 mLで2回（5 mL×2）洗浄し、その洗液も遠心管に加えた。15分間遠心分離（SOP/INS/4.16.3）（9000 r.p.m., 約10℃）した後、100 mL容量のメスフラスコにろ過（アドバンテック東洋製 ガラスフィルターGC50）し、アセトニトリル 5 mLでろ紙を洗浄した後、アセトニトリルで100 mLに定容した。

この定容液の一部を10 mLホールピペットで分取し、50 mL容量のナスフラスコに移し、ロータリーエバポレーター（SOP/INS/4.14.3.2）で減圧乾固した（水浴 40℃）。ナスフラスコ内に空気を吹き付け、蒸気を追い出した。

一方、Sep-Pakフロリジルに、n-ヘキサン 5 mLを流してコンディショニングしておいた。ナスフラスコ内の魚体抽出物をn-ヘキサン3 mLで溶解し、Sep-Pakフロリジルに注入した。続いて

① n-ヘキサン 5 mLでナスフラスコ内を洗い、洗液を注入した。次に ② n-ヘキサン/ジクロロメタン（2:1 v/v）5 mLをSep-Pakに注入し、溶出液 ①, ②を50 mL容量のナスフラスコに受けた。

Sep-Pakフロリジルへの通液速度はすべて約 3 mL/minで行った。溶出液をロータリーエバポレーターで減圧乾固した（水浴 40℃）。少量のアセトニトリルで乾固物を溶解させ、1 mL容量のメスフラスコに洗い移し、洗液で1 mLに定容した。この定容液をHPLC測定用試料とした。これをフローシートで示すと次頁のようになる。



2.3.2 試験水分析法 (SOP 2.10)

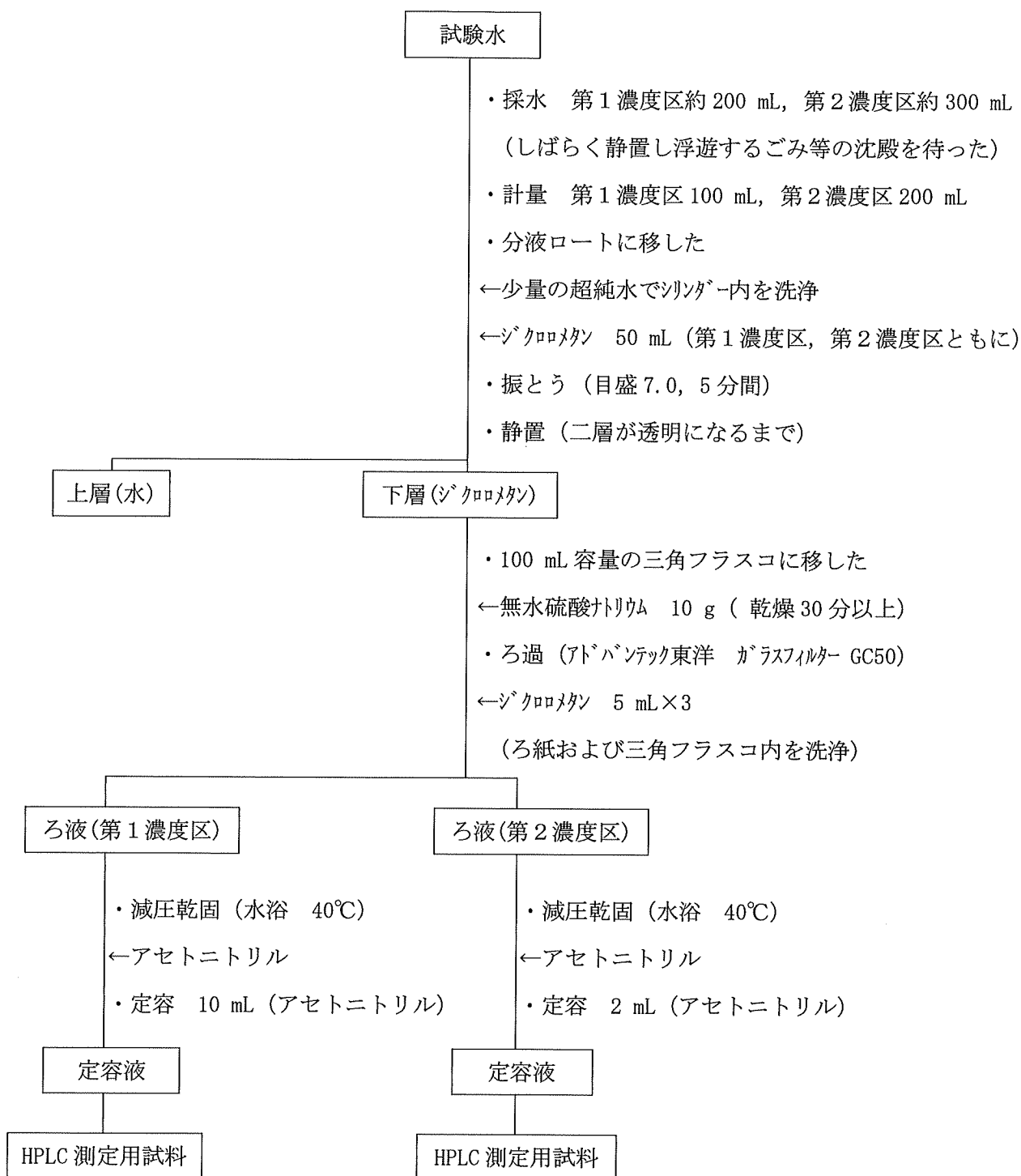
前処理

試験水槽の中央部より、三角フラスコで第1濃度区 約200 mL、第2濃度区 約300 mL を採水した。しばらく静置し浮遊するごみ等の沈殿を待った。上清をメスシリンダーで第1濃度区 100 mL、第2濃度区 200 mL計量した。

計量した試験水を第1濃度区は200 mL容量、第2濃度区は 300 mL 容量の分液ロートに入れた。各メスシリンダー内を少量の超純水で洗いこれも加えた。ジクロロメタンを第1濃度区、第2濃度区ともに 50 mL 加え、振とう機(SOP/INS/4.15)で、ダイヤルを 7.0 にセットし、5 分間振とうした。二層が透明になるまで静置し、下層（ジクロロメタン）を100 mL容量の三角フラスコに移した。抽出液に無水硫酸ナトリウムを各濃度区ともに 10 g 加え、30分以上放置した。乾燥後、抽出液を第1濃度区、第2濃度区ともに 100 mL 容量のナスフラスコにろ過（アドバンテック東洋製 ガラスフィルターGC50）し、硫酸ナトリウムを除いた。さらにろ紙および三角フラスコ内をジクロロメタン 5 mLで 3 回(5 mL×3)洗浄した。この洗液もろ過し、先のナスフラスコに加えた。ろ液をロータリーエバポレーター(SOP/INS/4.14.3.2)で減圧乾固（水浴 40℃）した。

第1濃度区においては、この乾固物にアセトニトリルを加えて溶解し、10 mL 容量のメスフラスコに洗い移し、洗液で 10 mL に定容した。第2濃度区においては、乾固物にアセトニトリルを加えて溶解し、2 mL容量のメスフラスコに洗い移し、洗液で 2 mL に定容した。この定容液をHPLC測定用試料とした。

これをフローシートで示すと次頁のようになる。



対照区の水分析 (試験開始時) の操作は, 第2濃度区の方法に従って行った。

2.3.3 測定方法

【魚体分析用】

<分析装置>

高速液体クロマトグラフ LC-10A (島津製作所製) No. 6 (SOP/INS/4.11.5)

<分析条件>

カラム	Asahipak C8-50 4E	4.6 × 250 mm (5 μm) (Lot No. P103321) 昭和電工(株)製
移動相	アセトニトリル/水 (85:15)	
流速	1.0 mL/min	
温度	40℃	
検出器	UV-VIS 214 nm	
注入量	20 μL	

<定量計算>

機器 LCsolution 島津製作所製
計算法 2点検量線法 (絶対検量線法)

得られたクロマトグラムは、標準溶液2点によりキャリブレーションを行い、測定試料の定量計算を行った。

【試験水分析用】

<分析装置>

高速液体クロマトグラフ LC-10A (島津製作所製) No. 6 (SOP/INS/4.11.5)

<分析条件>

カラム	InertSustain C18	4.6 × 250 mm (5 μm) (Lot No. 1BR98005) GL Sciences 製
移動相	アセトニトリル	
流速	1.0 mL/min	
温度	40℃	
検出器	UV-VIS 214 nm	
注入量	20 μL	

<定量計算>

機器 LCsolution 島津製作所製
計算法 2点検量線法 (絶対検量線法)

得られたクロマトグラムは、標準溶液2点によりキャリブレーションを行い、測定試料の定量計算を行った。

■ 被験物質の紫外可視吸収スペクトルは、参考資料 12 に示した。

2.4 高速液体クロマトグラフによる魚体分析用検量線

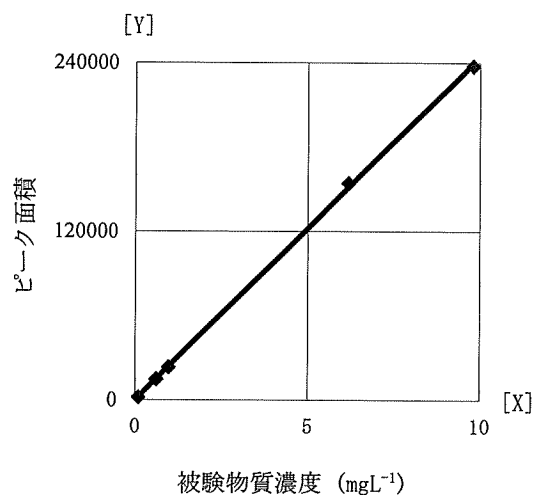
2.4.1 標準溶液の調製

Mettler Toledo XS105DU (SOP/INS/4.1.4)にて被験物質 4.90 mg, 3.10 mgを秤り取り, それぞれ50 mLメスフラスコにアセトニトリルで定容した。これらの溶液の濃度は純度を考慮して 97.9020 mgL^{-1} , 61.9380 mgL^{-1} となる。これをさらにアセトニトリルで希釈して, 検量線用の標準溶液を調製した。

2.4.2 検量線の作成

2.4.1 で調製した標準溶液について, 検量線を作成し, 直線性および定量性を確認した。

溶液 番号	被験物質濃度 (S510) (mgL^{-1}) [X]	ピーク面積 [Y]
1	0.0979	2151
2	0.6194	15037
3	0.9790	23500
4	6.1938	154396
5	9.7902	237859



検量線 $Y = 24459.1955 X + 99.4172$ (最小二乗法)

$$r = 1.000$$

■魚体分析用検量線作成時のクロマトグラムは参考資料 6-1 に示した。

2.5 高速液体クロマトグラフによる試験水分析用検量線

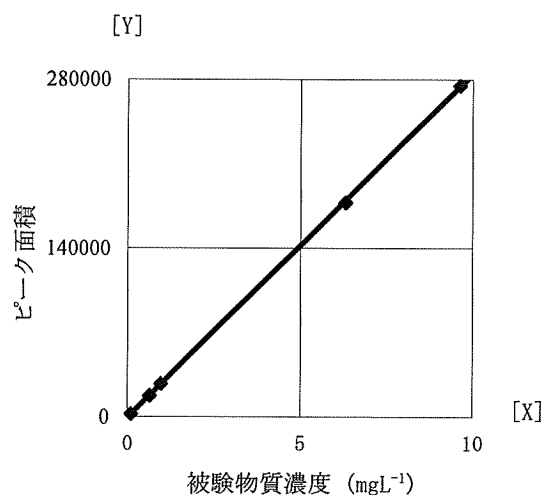
2.5.1 標準溶液の調製

Mettler Toledo XS105DU (SOP/INS/4.1.4)にて被験物質 4.82 mg, 3.16 mgを秤り取り, それぞれ50 mLメスフラスコにアセトニトリルで定容した。これらの溶液の濃度は純度を考慮して 96.3036 mgL^{-1} , 63.1368 mgL^{-1} となる。これをさらにアセトニトリルで希釈して, 検量線用の標準溶液を調製した。

2.5.2 検量線の作成

2.5.1 で調製した標準溶液について, 検量線を作成し, 直線性および定量性を確認した。

溶液 番号	被験物質濃度 (S510) (mgL^{-1}) [X]	ピーク面積 [Y]
1	0.0963	2745
2	0.6314	18011
3	0.9630	27867
4	6.3137	178107
5	9.6304	275278



検量線 $Y = 28482.0941 X - 53.6066$ (最小二乗法)

$$r = 1.000$$

■試験水分析用検量線作成時のクロマトグラムは参考資料 6-2 に示した。

2.6 被験物質の回収試験およびブランク試験

2.6.1 魚体からの被験物質の回収率 (SOP 2.11に準ずる)

魚体 (2尾を1連とした) をブレンダーカップで粉碎し, これに被験物質 (S510) のアセトニトリル溶液 (0.2 mL) を添加後, 「2.3.1 魚体分析法」に従って分析試料を調製し, 高速液体クロマトグラフで測定した。その結果, 被験物質の回収率は次のようになった。ここで, 被験物質の添加量 $1.964 \mu\text{g}$ は, 魚体 2.61 g ($5.22 \text{ g} / 2\text{尾}$) に対して $0.376 \mu\text{g g}^{-1}$ に相当し, 濃縮倍率は第2濃度区 (0.002 mg L^{-1}) で 188 倍程度である。

添加区

検体 番号	魚体量 (g)	定容量 (mL)	濃縮率	添 加 量		回収量 実測値 面 積	回収率 (%)
				実測値 面 積	算出濃度 (mg L^{-1})		
1	2.47 2.73	100	1/10	4594	0.1964	3936	85.7
2	2.54 2.55	100	1/10	4594	0.1964	3912	85.2
3	2.66 2.59	100	1/10	4594	0.1964	3613	78.6

平均回収率 $83.2 \pm 3.2\%$

<魚体のバックグラウンド>

魚体 (2尾を1連とした) の体重, 全長を測定後ブレンダーで粉碎し, これにアセトニトリルを 0.2 mL 添加後, 「2.3.1 魚体分析法」に従って分析試料を調製し, 高速液体クロマトグラフで測定した結果, 被験物質のピーク位置にはバックグラウンドは検出されなかった。

対照区

検体 番号	魚体量 (g)	定容量 (mL)	濃縮率	実測値面積
1	3.01 2.46	100	1/10	N. D.
2	2.40 2.73	100	1/10	N. D.
3	2.84 2.44	100	1/10	N. D.

■ 魚体からの被験物質の回収率を求めたクロマトグラムは参考資料 7-1 に示した。

2.6.2 試験水からの被験物質の回収率 (SOP 2.10に準ずる)

魚飼育水を採取し、これに被験物質分散溶液を添加後、「2.3.2 試験水分析法」に従って分析試料を調製し、高速液体クロマトグラフで測定した。その結果、次表のごとく、被験物質の回収率は第1濃度区で平均 $91.8 \pm 1.2\%$ 、第2濃度区では、平均 $97.8 \pm 1.4\%$ であった。

第1濃度区 添加区

検体 番号	採水量 (mL)	定容量 (mL)	添 加 量		回収量 実測値 面 積	回収率 (%)
			実測値 面 積	算出濃度 (mgL ⁻¹)		
1	100	10	5521	0.1932	5027	91.1
2	100	10	5521	0.1932	5005	90.7
3	100	10	5521	0.1932	5160	93.5

平均回収率 $91.8 \pm 1.2\%$

第2濃度区 添加区

検体 番号	採水量 (mL)	定容量 (mL)	添 加 量		回収量 実測値 面 積	回収率 (%)
			実測値 面 積	算出濃度 (mgL ⁻¹)		
1	200	2	5590	0.1975	5394	96.5
2	200	2	5590	0.1975	5433	97.2
3	200	2	5590	0.1975	5578	99.8

平均回収率 $97.8 \pm 1.4\%$

<試験水のバックグラウンド>

魚飼育水を採取し、分散剤 (Tween 80) を添加した後、「2.3.2 試験水分析法」に従って分析試料を調製し、高速液体クロマトグラフで測定した。その結果、被験物質のピーク位置には第1濃度区、第2濃度区ともにバックグラウンドは検出されなかった。

第1濃度区 対照区

検体 番号	採水量 (mL)	定容量 (mL)	実測値 面 積
1	100	10	N. D.
2	100	10	N. D.
3	100	10	N. D.

第2濃度区 対照区

検体 番号	採水量 (mL)	定容量 (mL)	実測値 面 積
1	200	2	N. D.
2	200	2	N. D.
3	200	2	N. D.

■ 試験水からの被験物質の回収率を求めたクロマトグラムは参考資料 7-2 に示した。

2.7 定量限界

2.7.1 魚体の定量限界

高速液体クロマトグラムにおいて、被験物質のピーク位置に魚体バックグラウンドのピークは認められなかった。いま、ピーク面積 300 counts を検出限界と設定したときの定量可能な濃縮倍率を次表に示した。

各濃度区の設定濃度	検出限界濃度 ¹⁾ (mgL ⁻¹)	定量限界値 ²⁾ (μ gg ⁻¹)	濃 縮 倍 率 ³⁾	
			第 1 濃度区 (0.02 mgL ⁻¹)	第 2 濃度区 (0.002 mgL ⁻¹)
第 1 濃度区 0.02 mgL ⁻¹ 第 2 濃度区 0.002 mgL ⁻¹	0.0082	0.0189	1倍以上	9倍以上

注) ¹⁾ 検出限界濃度は、2.4.2 の検量線により算出した。

²⁾ 魚体中における定量限界値は、次の計算式により算出した。

なお、魚体重は 5.22 g (1 尾あたり 2.61 g, 2 尾を 1 連とする), 回収率 83.2%, 定容量は 100 mL, 濃縮率を 1/10 として計算した。

$$\text{定量限界値}(\mu \text{gg}^{-1}) = \frac{\text{検出限界濃度}(\text{mgL}^{-1}) \times \text{定容量}(\text{mL}) \times \text{濃縮率}}{\text{回収率}(\%) \times \text{魚体重}(\text{g})} \times 100$$

$$\text{濃 縮 倍 率 (BCF)} = \frac{\text{定量限界値}(\mu \text{gg}^{-1})}{\text{試験濃度区における設定濃度(理論値)}(\text{mgL}^{-1})}$$

2.7.2 試験水の定量限界

高速液体クロマトグラムにおいて、被験物質のピーク位置に試験水のバックグラウンドのピークは認められなかった。いま、ピーク面積 300 counts を検出限界と設定したときの定量限界値を次表に示した。

各濃度区の設定濃度		検出限界濃度 ¹⁾ (mgL ⁻¹)	定量限界値 ²⁾ (mgL ⁻¹)	
第 1 濃度区	第 2 濃度区		第 1 濃度区 (0.02 mgL ⁻¹)	第 2 濃度区 (0.002 mgL ⁻¹)
0.02 mgL ⁻¹	0.002 mgL ⁻¹	0.0124	0.00135	0.000127

注) ¹⁾ 検出限界濃度は、2.5.2 の検量線より算出した。

²⁾ 試験水における定量限界値は、次の計算式により算出した。

なお、定容量は第 1 濃度区 10 mL, 第 2 濃度区 2 mL, 回収率は第 1 濃度区 91.8%, 第 2 濃度区 97.8%, 採水量は第 1 濃度区 100 mL, 第 2 濃度区 200 mL として計算した。

$$\text{定量限界値}(\text{mgL}^{-1}) = \frac{\text{検出限界濃度}(\text{mgL}^{-1}) \times \text{定容量}(\text{mL})}{\text{回収率}(\%) \times \text{採水量}(\text{mL})} \times 100$$

2.8 予備試験

暴露期間の第1濃度区の濃度条件(0.002 mgL⁻¹)で、試験魚(コイ *Cyprinus carpio*)を流水式で暴露した。7日目に試験水および魚体(2尾1連でn=2)を採取し、「2.3.1 魚体分析法」「2.3.2 試験水分析法」に従って分析した。その結果、暴露7日目における被験物質の濃縮倍率は、62倍、51倍であることが確認された。

2.9 試験水および試験魚分析日

次の日程により、試験を実施した。

	サンプリング日	経過日数	試験区	対照区
取り込み 期 間 (28日間)	2015年6月10日	0日目	水分析	水分析, 魚分析, 脂質含量
	2015年6月17日	7日目	水分析, 魚分析	—
	2015年6月24日	14日目	水分析, 魚分析	—
	2015年7月 1日	21日目	水分析, 魚分析	—
	2015年7月 6日	26日目	水分析, 魚分析	—
	2015年7月 8日	28日目	水分析, 魚分析	魚分析, 脂質含量

水分析日には、各水槽のpH, DO (7 mgL⁻¹程度となるようにした), 水温を測定し、記録した (pHメーター SOP/INS/4.3.3, DOメーター SOP/INS/4.4.5)。

水分析は、SOP 2.10 (試験水中の被験物質の濃度測定) に準じて、「2.3.2 試験水分析法」に従って行い、被験物質の水中濃度の変動は、取込み期間中の測定値の平均に対して±20%以内であることを確認した。

魚体分析は、SOP 2.11 (供試魚中の被験物質の濃度測定) に準じて、「2.3.1 魚体分析法」に従って行った。脂質含量の測定は、SOP 2.9 (供試魚の脂質含量の測定) に準じ、「クロロホルム-メタノール抽出法」に従って行った。実験開始時、実験終了時の変動は±25%以内であることを確認した。

供試魚の分析前の保存、供試魚の分析時の廃棄物の処理は、SOP 2.15 (供試魚の分析前の保存), SOP 2.16 (供試魚の分析時の廃棄物の廃棄) に従って行った。

3. 試験結果

3.1 試験水中の被験物質濃度

試験水中の各濃度区の被験物質濃度 (mgL⁻¹) を次表に示した。

試験区 \ 経過日数	0 日目	7 日目	14 日目	21 日目	26 日目	28 日目
第 1 濃度区	0.0179	0.0201	0.0203	0.0181	0.0202	0.0198
第 2 濃度区	0.00176	0.00200	0.00198	0.00175	0.00185	0.00185

単位 mgL⁻¹

■ 試験水分析時の分析データは参考資料 5 に示した。

以上の結果，試験期間中の平均濃度および標準偏差は次のようになった。

第 1 濃度区 : 0.0194 ± 0.00100 mgL⁻¹

第 2 濃度区 : 0.00186 ± 0.0000967 mgL⁻¹

計算式は次によった。

平均

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

標準偏差

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

■ 水中濃度，水温，pHおよびDOの経時変化は参考資料 2 に示した。また，暴露0，7，14，21，26，28日目の分析時のクロマトグラムは参考資料 9-1 に示した。また，対照区の水分析時のクロマトグラムは参考資料 9-2 に示した。

3.2 濃縮倍率(BCF)

本試験の結果、被験物質の濃縮倍率(BCF)は第1濃度区、第2濃度区ともに、28日で定常状態を確認して暴露を終了した。分析日毎の被験物質の濃縮倍率(BCF)を次表に示した。

経時 日数	第1濃度区		第2濃度区	
	検体番号(n=2)	濃縮倍率(BCF)	検体番号(n=2)	濃縮倍率(BCF)
7	No. 1	22	No. 1	20
	No. 2	16	No. 2	23
14	No. 1	14	No. 1	14
	No. 2	18	No. 2	14
21	No. 1	12	No. 1	14
	No. 2	18	No. 2	10
26	No. 1	15	No. 1	29
	No. 2	21	No. 2	20
28	No. 1	12	No. 1	22
	No. 2	30	No. 2	< 7

(注1) 第1濃度区および第2濃度区の検体番号は、蓄積量測定時に各濃度区より取り出した魚2尾を1連として付けた番号である。

(注2) 分析時の魚体重、分析データ等は参考資料 4、また 7、14、21、26、28日目の分析時のクロマトグラムは参考資料 8-1 に示した。また、対照区の魚分析時のクロマトグラムは参考資料 8-2 に示した。

(注3) 魚体中の濃縮倍率(BCF)の経時変化は参考資料 3 に示した。

(1) 濃縮倍率(BCF)の平均と標準偏差

試験期間中の被験物質の濃縮倍率(BCF)の平均および標準偏差を次に示した。

第1濃度区 BCF = 18 ± 5 (n=10)

第2濃度区 BCF = 18 ± 6 (n=9)

ここで、

$$\text{濃縮倍率(BCF)} = \frac{\text{被験物質の魚体中濃度} (\mu\text{g g}^{-1})}{\text{被験物質の水槽濃度} (\text{mg L}^{-1})}$$

平均

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

標準偏差

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

(2) 定常状態の確認

各濃度区の定常状態については、第1濃度区、第2濃度区ともに、濃縮倍率（BCF）が28日間を通して50倍未満であったため、28日目で定常に達したとみなして暴露を終了した。

第1濃度区での暴露48時間以上の間隔で3回の測定における濃縮倍率の変動（%）は、次のごとくであった。

$$\text{第1濃度区 変動(\%)} = \frac{6}{18} \times 100 = 33$$

（暴露21日目、26日目、28日目の濃縮倍率より算出した）

第2濃度区においては、暴露28日目で検出限界以下であった（n=1）ことより濃縮倍率の変動（%）を算出していない。

ここで、

$$\text{変動(\%)} = \frac{\text{連続3時点の濃縮倍率(BCF)の標準偏差}}{\text{連続3時点の濃縮倍率(BCF)の平均}} \times 100$$

平均

標準偏差

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

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

《参考事項》

暴露開始前0日目（n=1）、暴露終了時28日目（n=1）の対照区の魚を分析した結果、被験物質位置には、添加回収試験における対照区と同様、魚体バックグラウンドは検出されなかった。クロマトグラムは参考資料 8-2 に示した。

3.3 試験結果の評価と考察および結論

被験物質のゼブラフィッシュ *Danio rerio* における96時間LC₅₀値は 25.6 mgL⁻¹より大きかった。また対水溶解度は、2.3×10⁻² mgL⁻¹であったことより、試験濃度は第1濃度区 0.02 mgL⁻¹、第2濃度区 0.002 mgL⁻¹ の2濃度に設定した。本濃縮度試験の結果、第1濃度区、第2濃度区ともに、濃縮倍率（BCF）が28日間を通して50倍未満であったため、28日目で定常に達したとみなして暴露を終了した。被験物質S510の濃縮性は低いことが確認された。