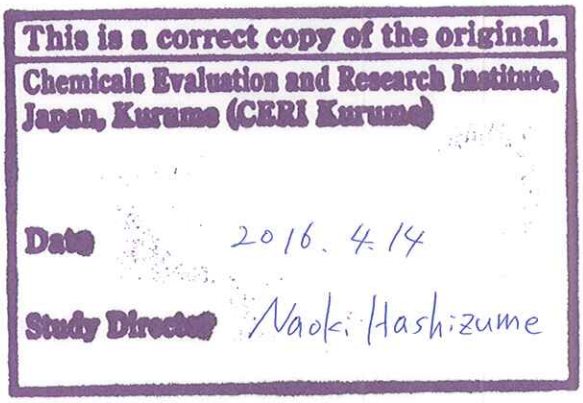
CERI

Receipt number	682-15-N-6100
Study number	46100

FINAL REPORT

Bioconcentration study of ANO in common carp



April, 2016

Chemicals Evaluation and Research Institute, Japan, Kurume

GLP STATEMENT

Chemicals Evaluation and
Research Institute, Japan, Kurume

Sponsor CBMM - Companhia Brasileira de Metalurgia e Mineracao

Title Bioconcentration study of ANO in common carp

Study number 46100

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

a) "Standard Concerning Testing Facility Relating to New Chemical Substances" (March 31, 2011; No. 0331-8, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; March 29, 2011, No. 6, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 110331010, Environmental Policy Bureau, Ministry of the Environment, Japan.)
b) OECD Principles of Good Laboratory Practice, November 26, 1997, ENV/MC/CHEM (98)17

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

Date April 12, 2016

Study Director Naoki Hashizume

Naoki Hashizume

QUALITY ASSURANCE STATEMENT

Chemicals Evaluation and Research Institute, Japan, Kurume

Sponsor: CBMM - Companhia Brasileira de Metalurgia e Mineracao

Title: Bioconcentration study of ANO in common carp

Study number: 46100

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

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

Item of inspection	Date of inspection	Date of report
Draft study plan	December 18, 2015	December 18, 2015
Re-inspection of draft study plan	December 21, 2015	December 21, 2015
Study plan	December 22, 2015	December 22, 2015
Acute toxicity test	January 5, 2016	January 5, 2016
Recovery test for analysis of test water	January 6, 2016	January 6, 2016
Recovery test for analysis of test fish	January 6, 2016	January 6, 2016
Preparation of stock solutions	January 12, 2016	January 12, 2016
Measurement of lipid content in test fish	January 13, 2016 January 14, 2016	January 14, 2016
Study plan amendment No.1	January 15, 2016	January 15, 2016
Start of exposure	January 15, 2016	January 15, 2016
Analysis of test water	January 20, 2016	January 20, 2016
Analysis of test fish	January 20, 2016	January 20, 2016
Study plan amendment No.2	March 8, 2016	March 8, 2016
Raw data and draft final report	March 8, 2016	March 8, 2016
Re-inspection of raw data and draft final report	March 14, 2016	March 14, 2016
Final report	April 12, 2016	April 12, 2016

Date April 12, 2016

Personnel of Quality Assurance Unit: Keiji Shiraishi

CONTENTS

Tables

Figures

1. Title

   Bioconcentration study of ANO in common carp

2. Sponsor

   Name CBMM - Companhia Brasileira de Metalurgia e Mineracao
   Address Córrego de Mata s/n, 38183-903 Araxá - MG, Brasil

3. Test facility

   Name Chemicals Evaluation and Research Institute, Japan, Kurume (CERI Kurume)
   Address 3-2-7 Miyanojin, Kurume-shi, Fukuoka 839-0801, Japan

4. Objective

   This study was performed to evaluate the bioconcentration potential of ANO in common carp.

5. Test method

   a) "Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body" stipulated in the "Testing Methods for New Chemical Substances" (March 31, 2011, No.0331-7, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; March 29, 2011, No.5, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 110331009, Environmental Policy Bureau, Ministry of the Environment, Japan; partially revised, April 2, 2012, No. 0402-1, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; March 28, 2012, No. 2, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, No. 120402001, Environmental Policy Bureau, Ministry of the Environment, Japan)
   b) "305-I : Aqueous Exposure Bioconcentration Fish Test" stipulated in the OECD Guidelines for Testing of Chemicals, No.305, October 2, 2012, "Bioaccumulation in Fish : Aqueous and Dietary Exposure"

6. GLP principles

   a) "Standard Concerning Testing Facility Relating to New Chemical Substances" (March 31, 2011; No. 0331-8, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; March 29, 2011, No. 6, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 110331010, Environmental Policy Bureau, Ministry of the Environment, Japan.
   b) OECD Principles of Good Laboratory Practice, November 26, 1997, ENV/MC/CHEM (98)17

7. Dates

Study initiation date	December 22, 2015
Experimental starting date	January 15, 2016
Experimental completion date	February 12, 2016
Study completion date	April 12, 2016

8. Storage of test sample and documents

The study plan (original), the final report (original), the raw data, documents concerning the study presented by the sponsor, other reports, and the test sample are stored in the archives of this laboratory.

The storage period is 10 years after the study completion date. In case of receipt of the notice specified under Clause 1 or Clause 2 of Article 4; Clause 2, Clause 3, or Clause 8 of Article 5; Clause 3 of Article 10; or Clause 2 of Article 14 of the "Law Concerning Examination and Regulation of Manufacture etc. of Chemical Substances", these items are stored for 10 years from that date. After 10 years from the study completion date, the storage period is discussed with the sponsor. The stability of the test sample is not confirmed during the storage period.

The management of the test sample and raw data, etc. after the storage period (continue, reject, or return) is discussed with the sponsor.

9. Personnel

Study Director	Naoki Hashizume (Section 3)
Study personnel (Operation of bioconcentration test)	Kaori Nishio
Study personnel (Operation of acute toxicity test)	Takeshi Ishibashi

10. Approval of final report

Date April 12, 2016

Study Director Naoki Hashizume

Naoki Hashizume

11. Summary

Test item

ANO

Objective

This study was performed to evaluate the bioconcentration potential of ANO in common carp.

Test method

a) "Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body" stipulated in the "Testing Methods for New Chemical Substances" (March 31, 2011, No.0331-7, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; March 29, 2011, No.5, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry; No. 110331009, Environmental Policy Bureau, Ministry of the Environment, Japan; partially revised, April 2, 2012, No. 0402-1, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare; March 28, 2012, No. 2, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, No. 120402001, Environmental Policy Bureau, Ministry of the Environment, Japan)

b) "305-I : Aqueous Exposure Bioconcentration Fish Test" stipulated in the OECD Guidelines for Testing of Chemicals, No.305, October 2, 2012, "Bioaccumulation in Fish : Aqueous and Dietary Exposure"

Test conditions

a) Acute toxicity test

Test fish	Common carp (*Cyprinus carpio*)
Duration of exposure	96 hours
Exposure method	Semi static system (Renewal of test water at every 24 hours)

b) Bioconcentration test

Test fish	Common carp (*Cyprinus carpio*)
Nominal concentrations of test item	
	High exposure level (Level 1) 700 μg/L
	Low exposure level (Level 2) 70 μg/L
Duration of exposure	28 days
Exposure method	Flow-through system
Analytical method	Inductively-coupled plasma atomic emission spectrometry

Results

a) Acute toxicity test

96-hour LC_{50} value 99.0 mg/L

b) Bioconcentration test

	Bioconcentration factor
Level 1	<2.3-12
Level 2	<23

The values are substantially lower than the threshold values for bioaccumulating (or very bioaccumulating) substances, according to Japan's Chemical Substances Control Law and Regulation (EC) No 1907/2006 (REACh). Therefore, the substance can be regarded as not bioaccumulative.

12. Materials

12.1 Test item

In the biodegradation study, ANO was completely biodegraded and Niobium hydroxide (V) remained after 28 days of incubation period. In general, bioconcentration study of Niobium hydroxide (V) is required in accordance with Japan's Chemical Substance Control Law. However, conducting the bioconcentration study of Niobium hydroxide (V) is expected to be difficult due to its low water solubility. After consultation with Japan's regulatory authority (Ministry of Economy, Trade and Industry, Japan), it was decided to conduct the bioconcentration study using ANO as test item and quantify the niobium ion to evaluate the bioconcentration potential of Niobium hydroxide (V). All concentration in this study was described as the test item, ANO.

a) Chemical name etc.

Chemical name	Mixture of ammonium oxobis(ethanedioato) bisaquo niobate(V) hydrates and ammonium hydrogen ethanedioate ethandioic acid dehydrate
Synonym (anhydrous form)	Reaction mass of ammonium diaqua[bis(oxalate)]oxoniobate(1-) and ammonium hydrogen oxalate oxalic acid (1:1:1)
Abbreviation	ANO

b) Chemical structure etc.

Molecular formula	Constituent 1 : $NH_4[NbO(C_2O_4)_2 \cdot 2H_2O] \cdot 3H_2O$
	Constituent 2 : $(NH_4C_2HO_4)_2 \cdot (C_2O_4H_2 \cdot 2H_2O)_2$
Molecular Weight	Constituent 1 : 393.06
	Constituent 2 : 466.26

c) Test sample

Purity of test item	97% (constituent 1 : 70%, constituent 2 : 27%)
Impurity	Water 2.5%
	Inorganic impurities 0.5%
Supplier	CBMM - Companhia Brasileira de Metalurgia e Mineracao
Lot number	AD/5275

The test item was treated as 100% in purity.

d) Physicochemical properties

Water solubility	10 g/L (Visual, confirmed in CERI Kurume)
Appearance	White solid
Stability	Stable

e) Storage conditions

The test sample was stored in a dark storage place at room temperature.

f) Confirmation of the identity for test item and the stability under storage condition

The infrared (IR) spectrum of the test item measured in our laboratory was confirmed to be identical to that of the sponsor (see Fig. 6, Reference 2).

The stability of the test item in the storage condition was confirmed by comparing the IR spectrum of the test item before the experimental start and after the experimental completion (see Fig. 6).

g) Stability under testing conditions

As a result of biodegradation study, ANO was confirmed to be hydrolyzed and generate the niobium ion immediately after contact with water. Stability of generated niobium ion under the testing conditions was confirmed by a preliminary test.

h) Caution when handling

In order to avoid inhalation and contact with the skin and eyes, chemically resistant gloves, mask, safety glasses, and white coats were worn when handling test item.

12.2 Test fish

a) Acute toxicity test

Species	Common carp (*Cyprinus carpio*) Reason for selection: The same fish species for the bioconcentration test.
Supplier	CERI Kurume
Lot No.	TFC-151118
Length	3.4-3.7 cm

b) Bioconcentration test

Species	Common carp (*Cyprinus carpio*) Reason for selection: The previous data conducted with this species can be compared and the size of this species is adequate for handling.
Supplier	CERI Kurume
Conditions for acclimatization	The external disinfection was carried out in an aqueous solution containing OTC for fisheries (Oxytetracycline hydrochloride, Kyoritsu Seiyaku) and sodium chloride (The Salt Industry Center of Japan). Thereafter fish were acclimatized in the following conditions. Period: After rearing in the underground water for 16 days, the fish were acclimatized in the dechlorinated water for 21 days. Temperature: 25±2°C Mortality during acclimatization was less than 5%.
Lot No.	TFC-151126
Length	7.0-8.6 cm (at the beginning of exposure 7.0-7.4 cm)
Age	Yearling fish
Feeding	Feed : Feed for carp Composition : Proteins content ≥30.0% Lipid content ≥4.0% Manufacturer : Kyorin Food Industries Feeding amount and interval : Amount corresponding to approximately 3% of total body weight was fed twice a day in halves (once a day in all at holiday). The fish were fasted for 24 hours before sampling.

13. Performance of acute toxicity test

13.1 Test method

The test was performed in accordance with Japanese Industrial Standard (JIS K 0102-2013-71.).

13.2 Certification

The 48-hour LC_{50} value of the reference substance (Pentachlorophenol sodium salt, CAS number 131-52-2, Tokyo Kasei Kogyo Co., Ltd., Lot No. GE01) for the fish of the same lot was 0.286 mg/L.

13.3 Dilution water for test

a) Origin

Dechlorinated water [City water was treated by charcoal bed system (ORGANO Co., Ltd.)]

b) Water quality assessment

The result for the water quality assessment of the dilution water are shown in Reference 1. It was confirmed that the dilution water meets the criteria as stipulated in the standard operation procedure.

13.4 Preparation of stock solution

Test sample (15.0 g) was dissolved in 1.5 L of ion-exchange water to prepare 10.0 g/L stock solution. This procedure was conducted twice to prepare total 3 L stock solution.

13.5 Test conditions

Test concentrations	140 mg/L, 70.0 mg/L, 35.0 mg/L and Control	
Test tank	30 L glass tank	
Volume of test water	30 L / Level	
Number of fish	10 fish / Level	
Temperature of test water	At the initial of exposure	24.5-24.7℃
	Before the renewal of test water (At first renewal)	24.5-24.6℃
Concentration of dissolved oxygen in test water		
	At the initial of exposure	8.0-8.1 mg/L
	Before the renewal of test water (At first renewal)	7.2-7.9 mg/L
pH of test water	At the initial of exposure	4.2-7.7
	Before the renewal of test water (At first renewal)	4.1-7.8
Duration of exposure	96 hours	
Exposure method	Semi static system (Renewal of test water at every 24 hours)	
Aeration	No aeration	
Time of irradiation with light	16 hours light / 8 hours dark (artificial light of white fluorescent lamp)	

13.6 Performance of test

Place	Aquatron room B
Date	January 4, 2016 - January 8, 2016

13.7 Estimation of 96-hour LC_{50} value

The 96-hour LC_{50} value was estimated by the Doudoroff method.

13.8 Test result

96-hour LC_{50} value of the test item 99.0 mg/L (see Fig. 3)

No abnormalities were observed in control.

14. Performance of bioconcentration test

14.1 Dilution water for test

The same as described in Section 13.3

14.2 Conditions of test and circumstances

Supply of test water: Flow-through system assembled at this laboratory was used.

Test tank: 70 L glass tank

Flow rate of test water: 0.04 mL/min for stock solution and 800 mL/min for dilution water, 1152 L/day of test water, were supplied. Test water was supplied to the test tanks from January 12, 2016.

Stock solution bottle: 500 mL glass brown bottle
(Frequency of renewal Once a week)

Temperature of test water:
Before uptake phase
- Level 1 23.9-24.0°C
- Level 2 23.9-24.0°C
- Control 23.8-23.9°C

Uptake phase
- Level 1 23.9-24.2°C
- Level 2 23.9-24.2°C
- Control 23.8-24.1°C

Concentrations of dissolved oxygen in test water
Before uptake phase
- Level 1 7.9 mg/L
- Level 2 7.9 mg/L
- Control 7.5 mg/L

Uptake phase
- Level 1 7.7-8.0 mg/L
- Level 2 7.6-7.9 mg/L
- Control 7.6-8.0 mg/L

pH of test water: Before uptake phase
- Level 1 7.6
- Level 2 7.6
- Control 7.6

Uptake phase
- Level 1 7.4, 7.5
- Level 2 7.4, 7.5
- Control 7.6, 7.6

Total organic carbon: Before uptake phase (before 48 and 24 hours)
- Level 1 <1.00 mgC/L, <1.00 mgC/L
- Level 2 <1.00 mgC/L, <1.00 mgC/L
- Control <1.00 mgC/L, <1.00 mgC/L

Uptake phase
- Level 1 <1.00 mgC/L
- Level 2 <1.00 mgC/L
- Control <1.00 mgC/L

Total hardness	Level 1	41.4 mg$CaCO_3$/L
	Control	40.2 mg$CaCO_3$/L

Aeration: The test tanks were supplied with air during the exposure.

Time of irradiation with light: 14 hours light / 10 hours dark (artificial light of white fluorescent lamp)

Number of fish (at the beginning of exposure)

Level 1 and 2	28
Control	12

Duration of exposure: 28 days

Reason : A steady-state was reached after 28 days in accordance with test method 5 a).

Place: Aquatron room A

14.3 Preparation of stock solutions

a) Level 1

Test sample (7.00 g) was dissolved in ion-exchange water to prepare 14.0 g/L stock solution (500 mL).

b) Level 2

Test sample (0.700 g) was dissolved in ion-exchange water to prepare 1.40 g/L stock solution (500 mL).

14.4 Test concentrations

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

Level 1 700 µg/L

Level 2 70 µg/L

14.5 Observation, measurement and cleaning

Observation and measurement were conducted as following table. Excreta of carp, dirt on test tank, etc. were removed approximately once a day.

Items	Frequency
Observation of test fish	Twice a day (once a day at holiday) in experimental period.
Water temperature	Daily before addition of test fish and during experimental period.
Flow rate of test water	Daily before addition of test fish and during experimental period.
Dissolved oxygen	Once at the beginning and 1-2 times a week in experimental period.
pH	Once at the beginning and twice in experimental period.
Total organic carbon	Before addition of the test fish (24h and 48h before the uptake phase), and once a week during experimental period.
Total hardness	Once in the experimental period for Control and Level 1, respectively.

14.6 Analysis of test water and test fish

Analysis of the test item, as niobium ion, in test water and test fish was performed with inductively-coupled plasma atomic emission spectrometry (ICP-AES) analysis.

14.6.1 Frequency of analysis

a) Test water analysis

Test water of Level 1 and Level 2 were analyzed once before addition of the test fish and thereafter, analyzed at the same time as analysis of test fish during the uptake phase. Test water of Control was analyzed before addition of the test fish and after the experimental completion. One sample was analyzed at each sampling time.

b) Test fish analysis

Test fish of Level 1 and Level 2 were analyzed five times during the uptake phase. Four fish per treatment level were taken out at each sampling time and divided into two groups (two fish per group) because one fish was too small to take out the lipid content measurement sample. Test fish analysis was carried out at intervals of more than 48 hours, and the final analysis was conducted after 28 days.

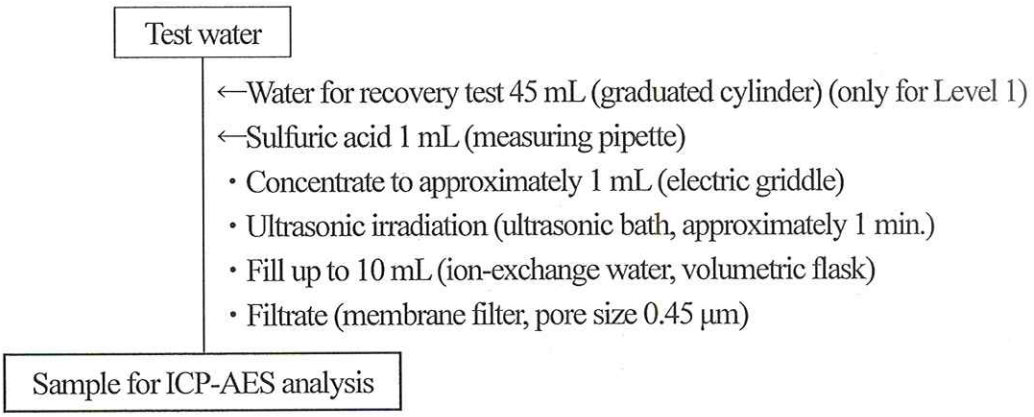
Test fish of Control was analyzed before the experimental start and after the experimental completion. Four fish were taken out at the sampling time and divided into two groups (two fish per group), and analyzed individually. Test fish before the experimental start was taken from the stock population.

14.6.2 Pretreatment for analysis

a) Test water

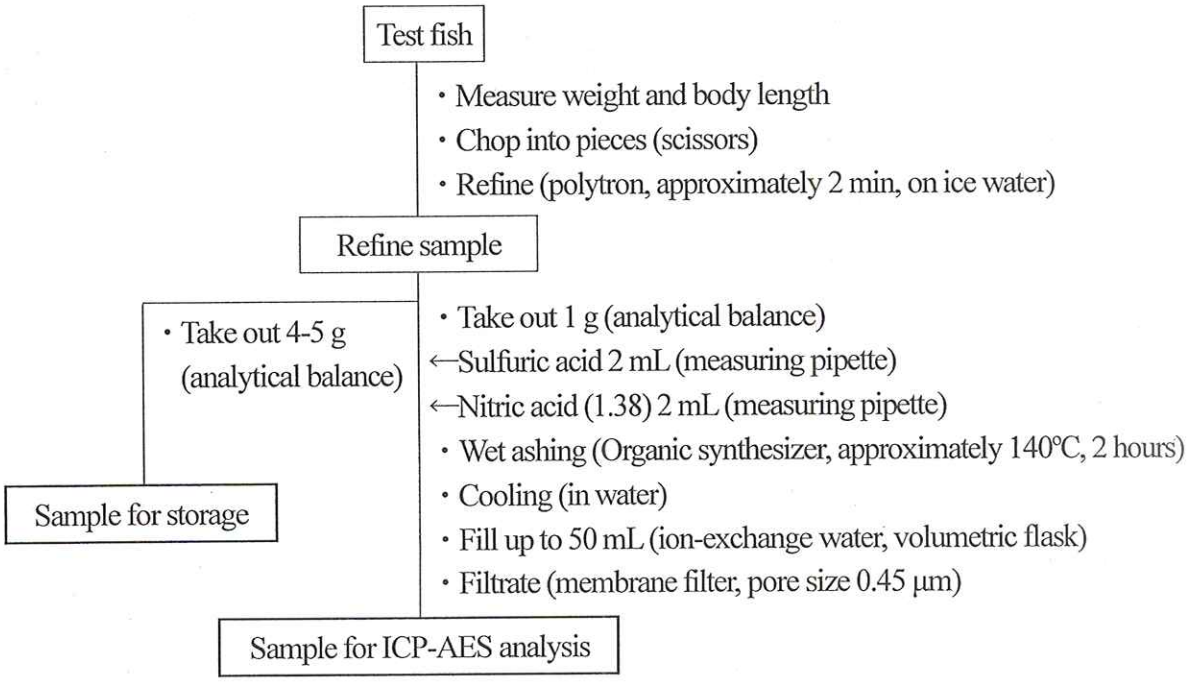
An aliquot of the test water was taken from test tank and pretreated for ICP-AES analysis as follows.

Level 1	5 mL (whole pipette)
Level 2	50 mL (whole pipette)
Control	50 mL (whole pipette)



b) Test fish

Test fish were taken from each test tank and pretreated for ICP-AES analysis as follows.



14.6.3 Quantitative analysis for test item

a) Quantitative method

Concentration of the test item was quantified with absolute calibration curve method using one concentration of standard solution.

In order to confirm the validity of this quantification method, the calibration curve was made using four concentrations of standard solution for test water analysis and test fish analysis, 35.0, 175, 350 and 700 μg/L (see Figs. 4, 5). As a result, the regression lines of the calibration curves were straight line from the origin, therefore the validity of the quantification method was confirmed.

b) Analytical conditions

Instrument	Inductively-coupled plasma atomic emission spectrometer Optima 5300 DV (PerkinElmer, Inc.)
Element	Niobium
Wavelength	269.706 nm
Plasma gas	Argon 15 L/min
Carrier gas	Argon 0.70 L/min
Auxiliary gas	Nitrogen 0.2 L/min
RF power	1300 W
View	Radial
View height	15.0 mm
Sample flow rate	1.00 mL/min

c) Preparation of standard solution and calculation of the test item concentration in sample

1) Test water analysis

Matrix-matched standard solution was used for test water analysis since matrix of test water influence the analytical sensitivity of the test item. Test sample (100 mg) was accurately weighed with an electronic analytical balance and dissolved in ion-exchange water to prepare 1000 mg/L solution (100 mL). This was diluted with ion-exchange water to prepare 35.0 mg/L solution. This solution was pretreated as follows to prepare 350 μg/L standard solution.

The concentration of the test item in the sample for ICP-AES was calculated proportionally by comparing the emission intensity of the sample for ICP-AES with that of 350 μg/L standard solution (see Tables-4, 5).

The lowest quantifiable concentration of the test item was regarded as 35.0 μg/L which is the lowest concentration of standard solution in the calibration curve (see Fig. 4).

50 mL of water described in Section 14.6.4

←Sulfuric acid 1 mL (measuring pipette)
- Concentrate to approximately 1 mL (electric griddle)
- Ultrasonic irradiation (ultrasonic bath, approximately 1 min.)

←Ion-exchange water approximately 7 mL (measuring pipette)
←35.0 mg/L solution of test item 100 μL (micro syringe)
- Fill up to 10 mL (ion-exchange water, volumetric flask)
- Filtrate (membrane filter, pore size 0.45 μm)

350 μg/L Standard solution
(Analysis for test water)

2) Test fish analysis

Test sample (100 mg) was accurately weighed with an electronic analytical balance and dissolved in ion exchange water to prepare 1000 mg/L solution (100 mL). This was diluted with ion-exchange water/sulfuric acid/nitric acid (1.38) (50/2/2 v/v/v) to prepare 350 μg/L standard solution.

The concentration of the test item in the sample for ICP-AES was calculated proportionally by comparing the emission intensity of the sample for ICP-AES with that of 350 μg/L standard solution (see Tables-7, 8, 9).

The lowest quantifiable concentration of the test item was regarded as 35.0 μg/L which is the lowest concentration of standard solution in the calibration curve (see Fig. 5).

14.6.4 Recovery test

a) Method

Recovery test were conducted using test water for recovery test and chopped fish sample (10 g) of test fish for recovery test. Specified amount of the test item were added to water and fish sample, and pretreated in the same manner as described in Section 14.6.2. The blank tests were also performed in the same manner but without adding the test item. The recovery and blank tests were performed in duplicate and average recovery rate were calculated.

b) Results of recovery test

In the blank tests, the emission intensity for both of test water and test fish analyses were below the lowest quantifiable concentration described in Section 14.6.3 c). The duplicate recovery rates and the average of them in the pretreatment are shown below (see Table-3,6). The average recovery rate was used as correction factors for the quantification of the test item concentrations in the analytical samples.

Recovery rate for each treatment

For analysis of test water

Amount of test item spiked (3.50 μg)

= Nominal concentration of Level 2 (70 μg/L) × Amount of sampling water (50 mL)

Addition method (100 μL of 35.0 mg/L stock solution of test item)

104%, 108% average 106%

For analysis of test fish

Amount of test item spiked (175 μg)

= Nominal concentration of Level 2 (70 μg/L)

× Assumed bioconcentration factor (250 L kg^{-1}) × Fish weight (10 g)

Addition method (250 μL of 700 mg/L stock solution of test item)

111%, 105% average 108%

14.6.5 Lipid content in test fish

Lipid contents of test fish were determined using control fish samples before and after the experiment to confirm whether the fish lipid after the experimental completion is within ±25% of the fish lipid before the experimental start. Lipid contents were analyzed using the storage sample of the same fish for the test item analysis. Additionally, two fish were taken out and total of three groups (two fish per group) were analyzed individually. Lipid contents were determined with gravimetric analysis after chloroform-methanol extraction.

14.6.6 Calculation of the test item concentration in sample and limit of quantification (LOQ)

a) Calculation of the test item concentration in test water

The equations in Tables-4 and 5 were used to obtain the concentrations, and they were rounded off to 3 figures.

b) LOQ of the test item in test water

LOQ*1 of the test item in test water was calculated on the basis of lowest quantifiable concentration of the test item described in Sections 14.6.3 c).

Level 1 66 μg/L

Level 2 6.6 μg/L

c) Calculation of the test item concentration in test fish

The equations in Tables-7, 8 and 9 were used to obtain the concentrations, and they were rounded off to 3 figures.

d) LOQ of the test item in test fish

Assuming the fine sample of test fish to be 1 g, the LOQ*1 of the test item in test fish was calculated to be 1600 ng/g on the basis of lowest quantifiable concentration of the test item described in Section 14.6.3 c).

*1 LOQ of the test item (μg/L or ng/g) $= \dfrac{A}{\dfrac{B}{100} \times \dfrac{C \times E}{D}}$

A : Lowest quantifiable concentration of the test item (μg/L)

B : Recovery rate (%)

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

D : Final volume of sample solution (mL)

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

Results were rounded off to 2 figures.

14.7 Calculation of results

14.7.1 Calculation of lipid content

Lipid contents were calculated according to following equation.

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

T_0 : Weight of vessel (g)

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

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

14.7.2 Calculation of average concentration of the test item in test water

$\overline{C_{wt}} = \{C_w(1) + \ldots\ldots + C_w(n)\} / n$

$\overline{C_{wt}}$: Average concentration of the test item in test water (μg/L)

n : Number of analysis for test water (measurement times)

$C_w(1)$: Concentration of the test item in 1st analysis of test water (μg/L)

$C_w(n)$: Concentration of the test item in *n*-th analysis of test water (μg/L)

14.7.3 Calculation of bioconcentration factor (BCF)

BCF was calculated as follows.

a) Calculation of bioconcentration factor

$BCF = C_f / \overline{C_w}$

BCF	:	Bioconcentration factor
C_f	:	Concentration of the test item in test fish (subtract FB) (ng/g)
$\overline{C_w}$	:	Average concentration of the test item in test water (μg/L)
FB	:	Arithmetical average concentration of the test item or the blank in the control test fish anayzed before and after experiment (ng/g)

b) Average bioconcentration factor in *m*-th analysis

$BCFm = (BCFa + BCFb) / n$

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

BCFm was not calculated when one or more concentrations of the test item in test fish at *m*-th analysis were not higher than the LOQ.

14.7.4 Definition of steady-state

It is evaluated that a steady-state has been reached when a variation of the concentration of the test item in test fish (C_f) for four successive analysis made on samples taken at intervals of at least 48 hours are within 20% of each other. When BCFs are less than 100, it is evaluated that a steady-state has been reached on the 28th day even if the variation of the C_fs are over 20% according to the test method of 5.a).

If one or more non-quantifiable data of C_f exist in the four successive analysis or the variation of C_fs is over 20%, BCFss cannot be calculated and the range of all BCF is reported.

Criterion of the steady-state was reached : V(m-3), V(m-2), V(m-1), V(m) ≤20 (%)

$$V(m\text{-}3) = \frac{|C_f(m\text{-}3) - \overline{C_f}|}{\overline{C_f}} \times 100$$

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

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

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

V(m-3)V(m-2), V(m-1), V(m)	:	Variation rate of concentration of the test item in test fish (%)
C_f(m-3),C_f(m-2),C_f(m-1),C_f(m)	:	Average of concentration of the test item in test fish for *m*-3, *m*-2, *m*-1, *m*-th analysis of group n
$\overline{C_f}$	:	$\{C_f(m\text{-}3) + C_f(m\text{-}2) + C_f(m\text{-}1) + C_f(m)\}/4$

14.7.5 Calculable BCF

On the basis of LOQ for the test item in Section 14.6.6 d), BCF can be obtained when BCF exceeds the following. The average concentration of the test item in test water obtained from all the analyzed sample was used to calculate the following calculable BCF.

Level 1 2.3

Level 2 23

14.7.6 Calculation of growth rate constant (k_g)

All individual test fish weight data were converted to natural logarithms and plotted against time (day). Then a linear least squares correlation was calculated and growth rate constant of test fish (k_g) was calculated as the slope of the linear regression line.

14.8 Treatment of numerical values

Values were rounded off in accordance with JIS Z 8401:1999 rule B. Furthermore, numerical values were used for calculation without being rounded.

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

15. Factors that affected reliability of test

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

16. Results and discussion

16.1 Lipid content in test fish

The measured lipid contents in the test fish are shown below. The change of lipid contents after the experimental completion versus those before the experimental starting was -7% which was within ±25%.

Before the experimental start	3.10%
After the experimental completion	2.87%

16.2 Concentration of the test item in test water

The measured concentrations of the test item in test water are shown in Table-1. Concentrations of the test item were maintained ≥ 94% of nominal concentrations and the variations were within ±20% of the average measured concentrations. Concentrations of the test item in test water before the uptake phase were 736 µg/L (Level 1) and 63.0 µg/L (Level 2). The measured concentration of the test item in test water of control before and after the uptake phase were less than the LOQ of the test item.

Table-1 Measured concentrations of test item in test water (Unit : µg/L)

Level	After 5 days	After 13 days	After 18 days	After 21 days	After 28 days	Average (Standard deviation)	Table
1	733	795	688	691	674	716 (49.3)	4
2	66.1	80.1	67.4	72.8	68.1	70.9 (5.73)	5

16.3 Bioconcentration factors

BCFs are shown in Table-2. These BCFs plotted against the duration of uptake phase are shown in Figs. 1 and 2. BCFs of the test item were as follows.

Level 1 <2.3-12

Level 2 <23

Table-2 BCFs Values in the parenthesis shows the average.

Level	After 5 days	After 13 days	After 18 days	After 21 days	After 28 days	Table
1	<2.3 2.5	4.4 4.9 (4.6)	12 8.4 (10)	8.0 8.7 (8.4)	5.8 7.0 (6.4)	7
2	<23 <23	<23 <23	<23 <23	<23 <23	<23 <23	8

16.4 BCFs at a steady-state (BCFss)

The BCFss for Level 1 was not calculated because the variation of the concentration of the test item in test fish for four successive analysis were over ±20%. The BCFss for Level 2 was not calculated because the test item in all test fish for four successive analyses were less than the LOQ. However, all BCFs were less than 100 and it was evaluated that a steady-state was reached after 28 days in accordance with test method 5. a).

16.5 Growth rate constant (k_g)

The k_g calculated from the all individual test fish weight data were 0.0195 (Level 1), 0.0204 (Level 2) and 0.0155 (Control).

To compare the growth rate constant of the test group (Level 1 and 2) and the control group, growth rate constant were calculated using the test fish data before the experimental start and after the experimental completion. As a result, k_g value for Level 1, 2 and control was 0.0209, 0.0212 and 0.0155 and there were statistically significant differences between Level 1 and Control ($P<0.05$), and Level 2 and Control ($P<0.01$). However, it was considered that statistically significant differences of growth rate constant between the test group and the control group was not due to the toxicity of the test item since the difference of k_g were not concentration dependent, and no abnormalities were observed in test fish of Level 1 and 2. Therefore, we concluded that this difference did not affect the evaluation of bioconcentration potential of the test item.

16.6 Results of test fish observation

No abnormality in behavior or appearance was noted.

16.7 Discussion

The validity criteria of test method

In this test, the validity criteria of test method were applied as following conditions. Therefore we concluded that it was reasonable test conditions to estimate bioconcentration potential of the test item.

a) The temperature variation is less than ±2°C of set water temperature 25°C.

b) The concentration of dissolved oxygen does not fall below 60% of saturated concentration 8.1 mg/L at 25°C.

c) The concentration of the test item in the test tank is maintained within ±20% of the mean of the measured values during the test period.
d) The mortality or other adverse effects/disease in both control and test group is not observed.

17. Conclusion

The values are substantially lower than the threshold values for bioaccumulating (or very bioaccumulating) substances, according to Japan's Chemical Substances Control Law and Regulation (EC) No 1907/2006 (REACh). Therefore, the substance can be regarded as not bioaccumulative.

Table-3 Calculation table for recovery and blank test (analysis of test water)

Study No. 46100

Sample description	A	B	C	D	E	F
Standard 350µg/L	450.6					
Recovery a	469.5	1/1	10	-	3650	104
Recovery b	488.3	1/1	10	-	3790	108
						Average
Standard 350µg/L	450.6					106
Blank a	n.d.	1/1	10	-	-	
Blank b	n.d.	1/1	10	-	-	
				Average		
				-		

(a, b : individual sample)

A : Emission intensity (cps)

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

B : Ratio of portion used for analysis

C : Final volume (mL)

D : Amount of blank in test water (ng)

E : Amount of test item recovered (ng)

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

F : Recovery rate (%)

$F = E / Q \times 100$

P : Concentration of test item in standard solution 350µg/L

Q : Amount of test item added (3500ng)

February 18, 2016 Name K. Nishio

Table-4 Calculation table for analysis of test water (Level 1)

Study No. 46100

Sample description	A	I
Standard 350µg/L	404.6	
Before uptake phase	452.4	736
Standard 350µg/L	434.1	
Test water after 5 days	483.4	733
Standard 350µg/L	426.2	
Test water after 13 days	514.5	795
Standard 350µg/L	481.9	
Test water after 18 days	503.5	688
Standard 350µg/L	434.9	
Test water after 21 days	456.2	691
Standard 350µg/L	483.7	
Test water after 28 days	494.9	674

Average concentration of test item in test water after exposure 716 (S.D. 49.3)

A: Emission intensity (cps)

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

B: Ratio of portion used for analysis 1/1

C: Final volume 10mL

F: Recovery rate 106%

H: Volume of test water taken out 5mL

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

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

J: Average concentration of test item in test water after exposure (µg/L)

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

n : Number of test water analyses (n = 5)

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

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

P: Concentration of test item in standard solution 350µg/L

February 18, 2016 Name K. Nishio

Table-5 Calculation table for analysis of test water (Level 2)

Study No. 46100

Sample description	A	I
Standard 350µg/L	404.6	
Before uptake phase	386.8	63.0
Standard 350µg/L	434.1	
Test water after 5 days	435.5	66.1
Standard 350µg/L	426.2	
Test water after 13 days	518.2	80.1
Standard 350µg/L	481.9	
Test water after 18 days	493.3	67.4
Standard 350µg/L	434.9	
Test water after 21 days	480.7	72.8
Standard 350µg/L	483.7	
Test water after 28 days	500.1	68.1

Average concentration of test item in test water after exposure 70.9 (S.D. 5.73)

A: Emission intensity (cps)

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

B: Ratio of portion used for analysis 1/1

C: Final volume 10mL

F: Recovery rate 106%

H: Volume of test water taken out 50mL

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

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

J: Average concentration of test item in test water after exposure (µg/L)

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

n : Number of test water analyses (n = 5)

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

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

P: Concentration of test item in standard solution 350µg/L

February 18, 2016 Name K. Nishio

Table-6 Calculation table for recovery and blank test (analysis of test fish)

Study No. 46100

Sample description	A	C	D	E	F	G
Standard 350µg/L	539.6					
Recovery a	599.2	1/1	50	-	194000	111
Recovery b	566.6	1/1	50	-	184000	105
						Average
						108
Standard 350µg/L	539.6					
Blank a	n.d.	1/1	50	-	-	
Blank b	n.d.	1/1	50	-	-	
				Average		
				-		

(a, b : individual sample)

A: Emission intensity (cps)

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

B: Ratio of portion used for analysis (fine sample) 1/10

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

D: Final volume (mL)

E: Amount of blank in test fish (ng)

F: Amount of test item recovered (ng)

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

G: Recovery rate (%)

$G = F / Q \times 100$

P: Concentration of test item in standard solution 350µg/L

Q: Amount of test item added (175000ng)

February 18, 2016 Name K. Nishio

Table-7 Calculation table for analysis of test fish (Level 1)

Study No. 46100

Sample description	A	D	G	K	J	M
Standard 350μg/L	551.6					
Test fish after 5 days a	n.d.	1	1.00	-	-	-
Test fish after 5 days b	61.6	1	1.00	1810	2.5	
Standard 350μg/L	570.0					
Test fish after 13 days a	109.9	1	1.00	3120	4.4	4.6
Test fish after 13 days b	123.5	1	1.00	3510	4.9	
Standard 350μg/L	560.7					
Test fish after 18 days a	286.8	1	1.00	8290	12	10
Test fish after 18 days b	207.3	1	1.00	5990	8.4	
Standard 350μg/L	604.7					
Test fish after 21 days a	213.8	1	1.00	5730	8.0	8.4
Test fish after 21 days b	233.5	1	1.00	6260	8.7	
Standard 350μg/L	595.0					
Test fish after 28 days a	153.5	1	1.00	4180	5.8	6.4
Test fish after 28 days b	185.0	1	1.00	5040	7.0	

(a, b : individual sample)

A: Emission intensity (cps)
A(std) : Standard solution A(t) : Sample
B: Ratio of portion used for analysis 1/1
C: Final volume 50mL
D: Dilution factor
E: Average concentration of blank in analysis of control 0ng/g
F: Recovery rate 108%
G: Weight of fine sample (g)
K: Concentration of test item in test fish (ng/g)
$K = \{ P \times (A(t) / A(std)) / B \times D \times C / G - E \} / F \times 100$
H: Average concentration of test item in test water after exposure (μg/L) 716
J: BCF
$J = K / H$
M: Average value of BCF(a) and BCF(b)
$M = \{ BCF(a) + BCF(b) \} / 2$
P: Concentration of test item in standard solution 350μg/L

February 18, 2016 Name K. Nishio

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

Study No. 46100

Sample description	A	D	G	K	J	M
Standard 350µg/L	551.6					
Test fish after 5 days a	n.d.	1	1.00	-	-	-
Test fish after 5 days b	n.d.	1	1.00	-	-	
Standard 350µg/L	570.0					
Test fish after 13 days a	n.d.	1	1.00	-	-	-
Test fish after 13 days b	n.d.	1	1.00	-	-	
Standard 350µg/L	560.7					
Test fish after 18 days a	n.d.	1	1.00	-	-	-
Test fish after 18 days b	n.d.	1	1.00	-	-	
Standard 350µg/L	604.7					
Test fish after 21 days a	n.d.	1	1.00	-	-	-
Test fish after 21 days b	n.d.	1	1.00	-	-	
Standard 350µg/L	595.0					
Test fish after 28 days a	n.d.	1	1.00	-	-	-
Test fish after 28 days b	n.d.	1	1.00	-	-	

(a, b : individual sample)

A: Emission intensity (cps)
A(std) : Standard solution A(t) : Sample
B: Ratio of portion used for analysis 1/1
C: Final volume 50mL
D: Dilution factor
E: Average concentration of blank in analysis of control 0ng/g
F: Recovery rate 108%
G: Weight of fine sample (g)
K: Concentration of test item in test fish (ng/g)
$K = \{ P \times (A(t) / A(std)) / B \times D \times C / G - E \} / F \times 100$
H: Average concentration of test item in test water after exposure (µg/L) 70.9
J: BCF
$J = K / H$
M: Average value of BCF(a) and BCF(b)
$M = \{ BCF(a) + BCF(b) \} / 2$
P: Concentration of test item in standard solution 350µg/L

February 18, 2016 Name K. Nishio

Table-9 Calculation table for analysis of test fish (Control)

Study No. 46100

Sample description	A	E	G	I
Standard 350µg/L	519.5			
Before the experimental start a	n.d.	-	1.00	-
Before the experimental start b	n.d.	-	1.00	-
Standard 350µg/L	536.5			
After the experimental completion a	n.d.	-	1.00	-
After the experimental completion b	n.d.	-	1.00	-
				Average
				-

(a, b : individual sample)

A: Emission intensity (cps)

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

B: Ratio of portion used for analysis 1/1

C: Final volume 50mL

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

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

G: Weight of fine sample (g)

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

$I = E / G$

P: Concentration of test item in standard solution 350µg/L

February 18, 2016 Name K. Nishio

Reference 1 Analytical results of dilution water (Dechlorinated water)

Sampling date January 5, 2016

Item	Unit	Measured value	Determination limit
Total hardness (Ca, Mg)	mg/L	35	1
Suspended solid	mg/L	< 1	1
pH	-	7.7	-
Total organic carbon	mg/L	< 0.5	0.5
Chemical oxygen demand	mg/L	< 1	1
Residual chlorine	mg/L	< 0.02	0.02
Ammonium ion	mg/L	< 0.1	0.1
Total cyanide	mg/L	< 0.05	0.05
Alkalinity	mg/L	38	1
Electric conductivity	μS/cm	150	1
Total mercury	mg/L	< 0.0005	0.0005
Cadmium	mg/L	< 0.001	0.001
Chromium (VI)	mg/L	< 0.01	0.01
Lead	mg/L	< 0.001	0.001
Arsenic	mg/L	< 0.005	0.005
Iron	mg/L	< 0.01	0.01
Copper	mg/L	0.001	0.001
Cobalt	mg/L	< 0.001	0.001
Manganese	mg/L	< 0.005	0.005
Aluminium	mg/L	< 0.02	0.02
Zinc	mg/L	< 0.1	0.1
Nickel	mg/L	< 0.001	0.001
Silver	mg/L	< 0.0001	0.0001
Organochlorine pesticides			
1,2-Dichloropropane	mg/L	< 0.002	0.002
Chlorothalonil	mg/L	< 0.001	0.001
Propyzamide	mg/L	< 0.0008	0.0008
Chlornitrofen	mg/L	< 0.0001	0.0001
Simazine	mg/L	< 0.0003	0.0003
Thiobencarb	mg/L	< 0.001	0.001
Organophosphorous pesticides			
Diazinon	mg/L	< 0.0005	0.0005
Isoxathion	mg/L	< 0.0008	0.0008
Fenitrothion	mg/L	< 0.0003	0.0003
EPN	mg/L	< 0.0006	0.0006
Dichlorvos	mg/L	< 0.001	0.001
Iprobenfos	mg/L	< 0.0008	0.0008
PCB	mg/L	< 0.0005	0.0005
Coliform bacteria count	-	n.d.	-
Fluorine	mg/L	0.4	0.1
Anionic surfactant	mg/L	< 0.02	0.02

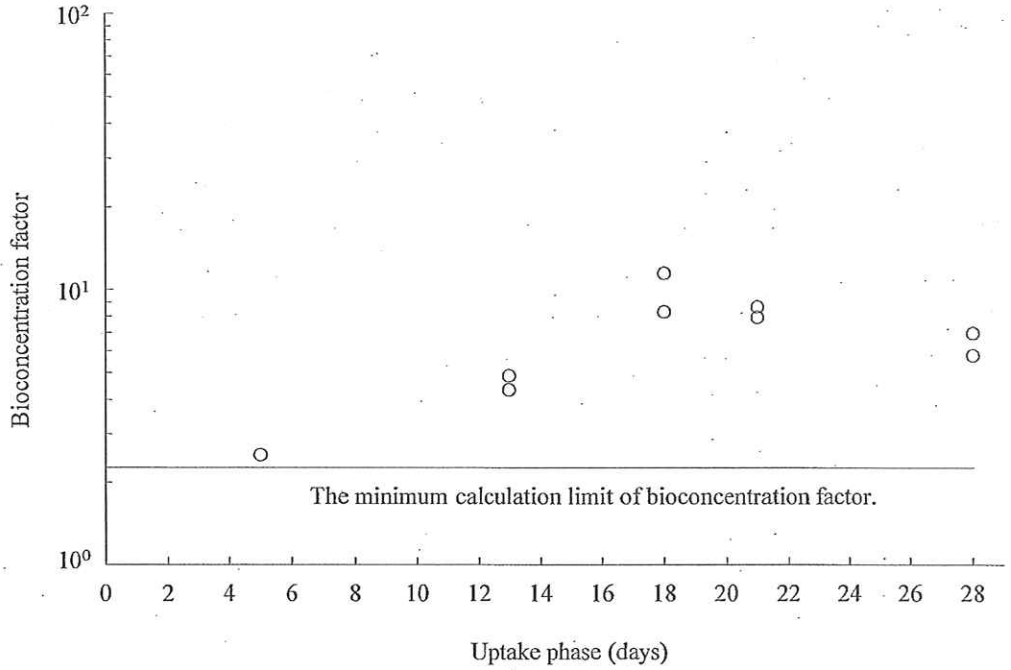


Fig.1 Correlation between uptake phase and bioconcentration factor (Level 1).

One data after 5 days was lower than detection limit.

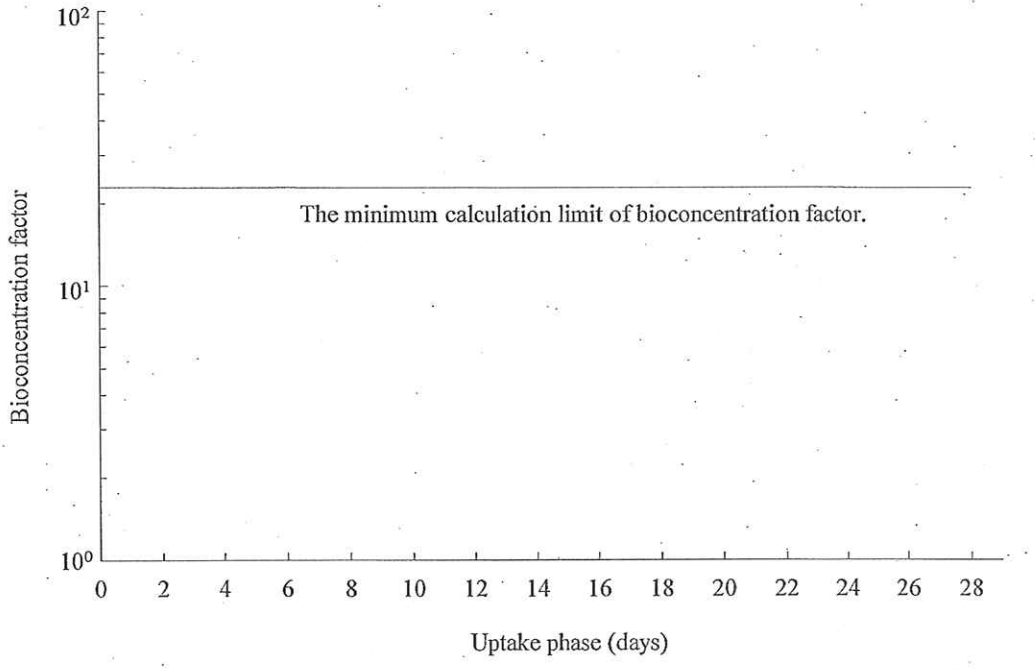
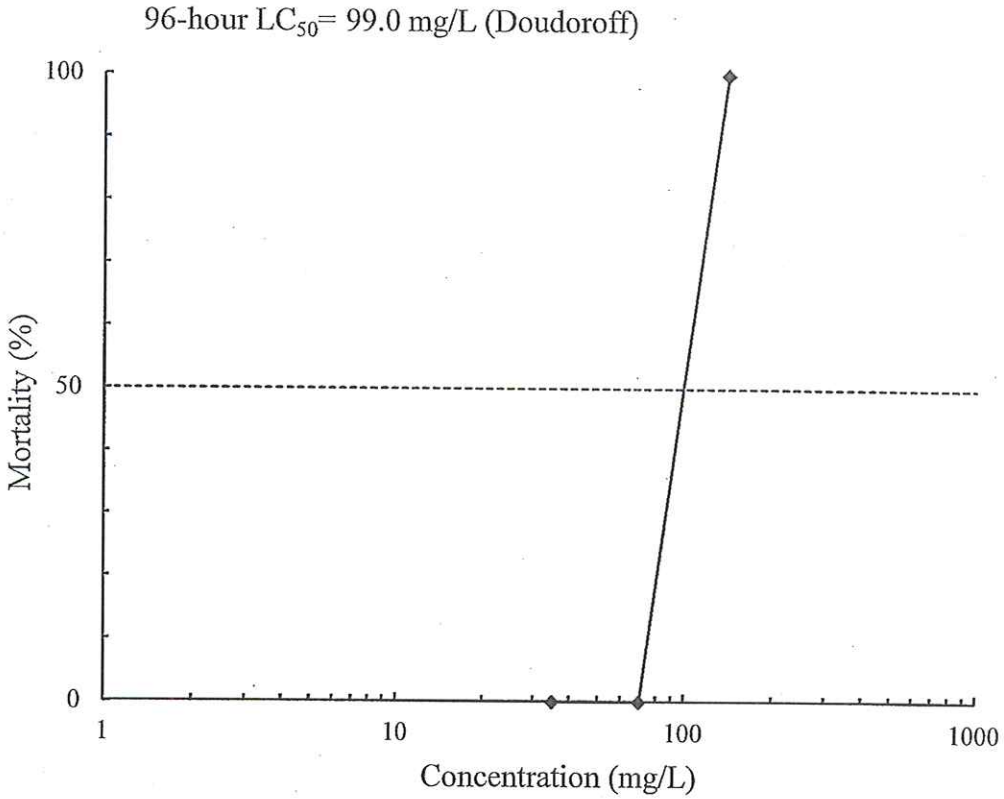


Fig.2 Correlation between uptake phase and bioconcentration factor (Level 2).

Ten data after 5, 13, 18, 21 and 28 days were lower than detection limit.

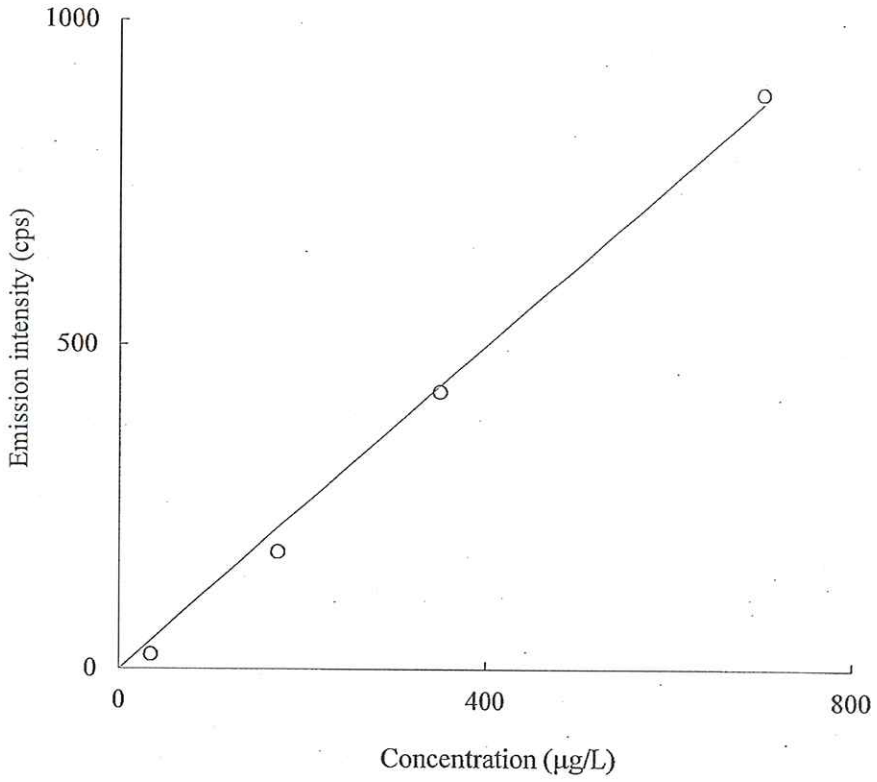
February 23, 2016 Name K. Nishio



Concentration (mg/L)	Cumulative Mortality (%)			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
35.0	0	0	0	0
70.0	0	0	0	0
140	100	100	100	100

Fig. 3 Concentration - mortality curve.

Date : January 11, 2016 Name Takeshi Ishibashi

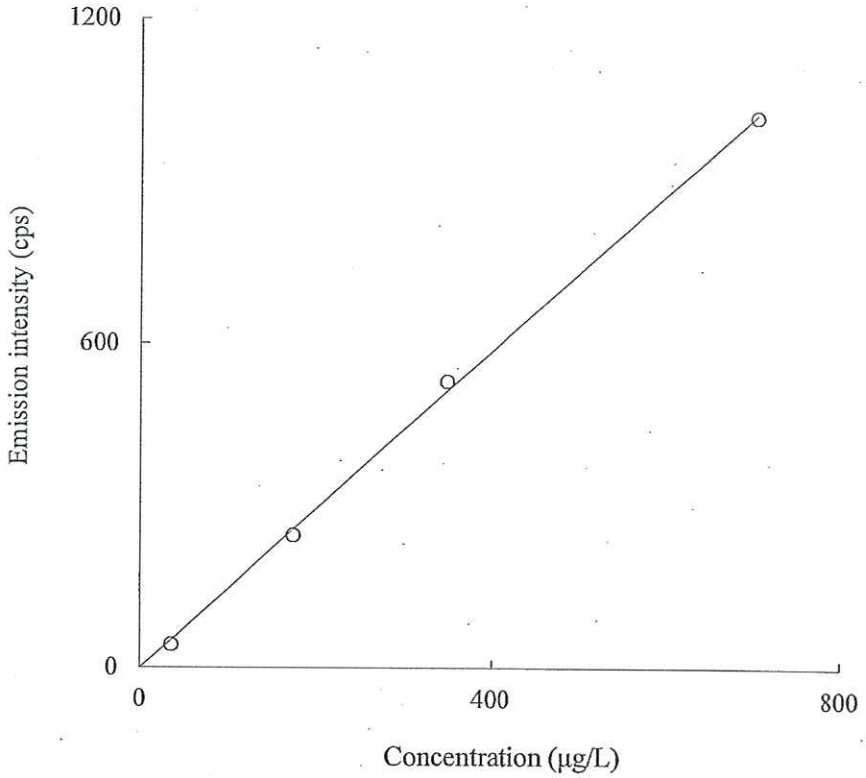


$y = 1.25x$
$r = 0.997$

Concentration (μg/L)	Emission intensity (cps)
35.0	22.5
175	181.3
350	428.7
700	891.4

Fig. 4 Calibration curve of test item (analysis of test water).

February 12, 2016 Name K. Nishio

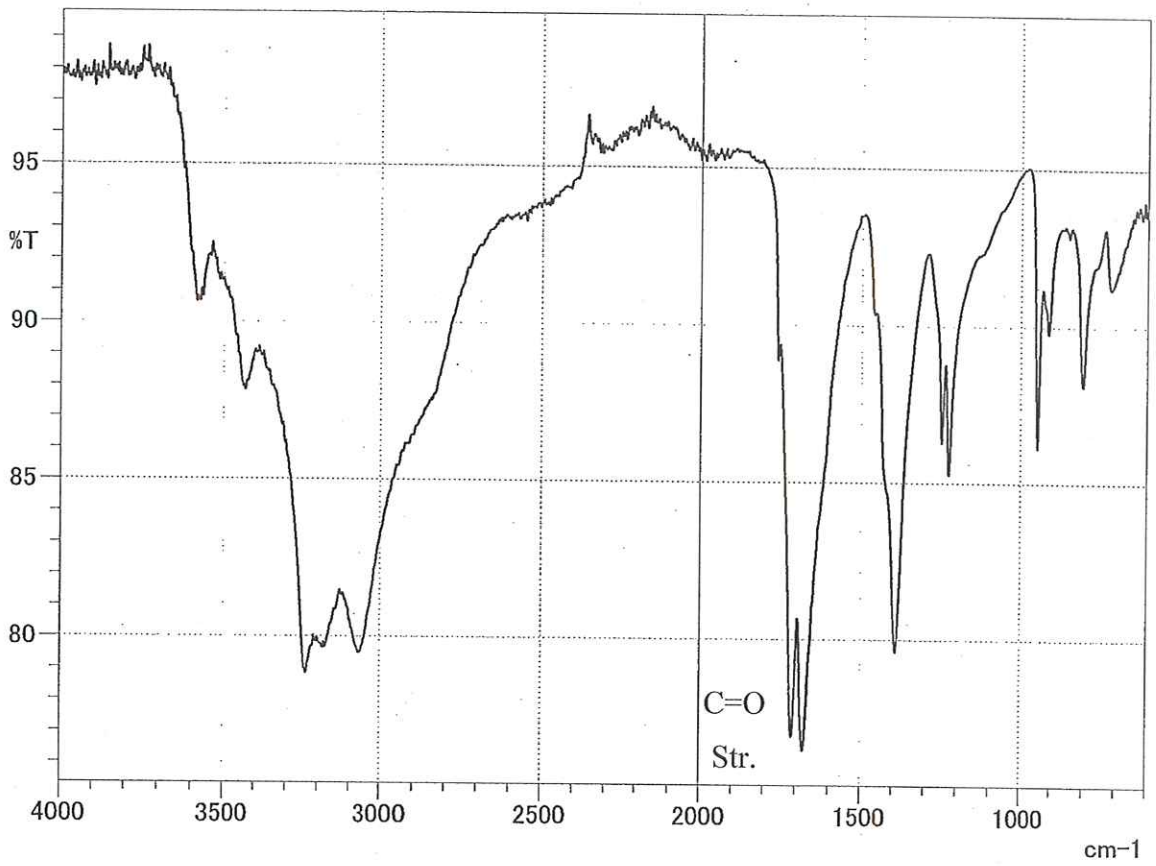


y = 1.47x
r = 1.000

Concentration (µg/L)	Emission intensity (cps)
35.0	43.5
175	245.5
350	531.0
700	1023.8

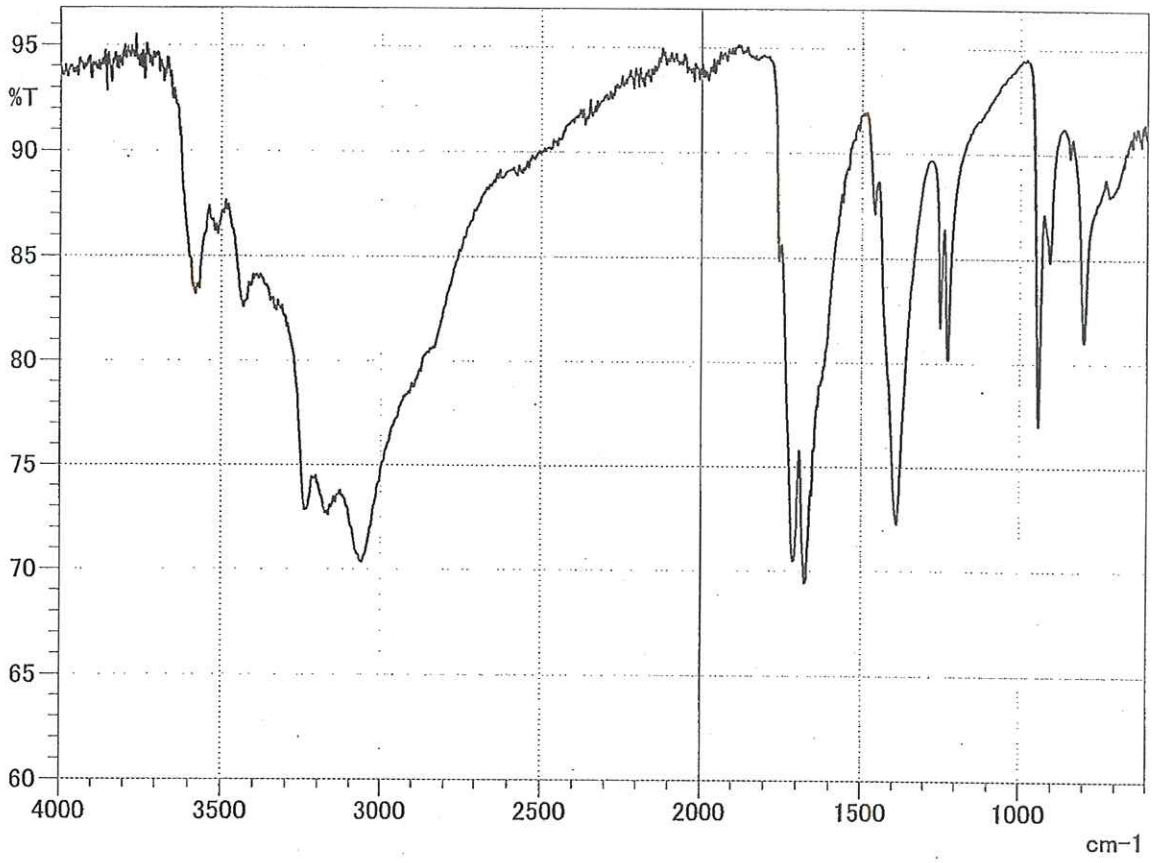
Fig. 5 Calibration curve of test item (analysis of test fish).

February 12, 2016 Name K. Nishio



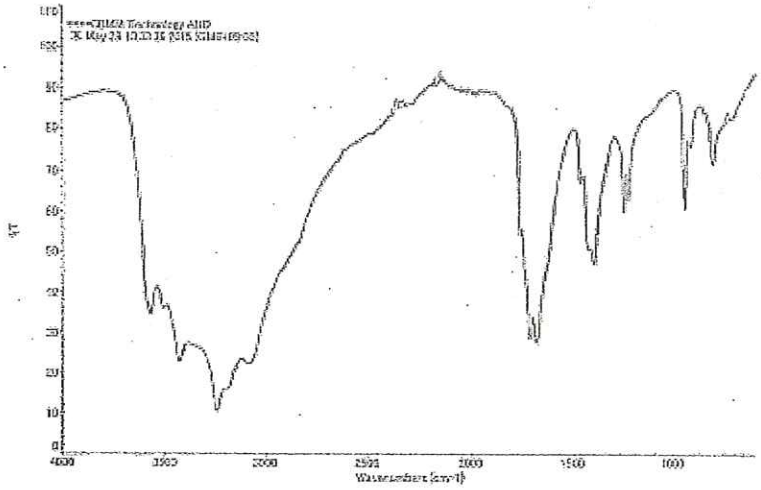
Instrument	: Shimadzu IRAffinity-1S
Study No.	: 46100
Sample	: Test item
Method	: ATR
Date	: December 17, 2015
Name	: K. Nishio

Fig.6-1 IR spectrum of test item measured before experimental start.



Instrument	: Shimadzu IRAffinity-1S
Study No.	: 46100
Sample	: Test item
Method	: ATR
Date	: February 16, 2016
Name	: K. Nishio

Fig.6-2 IR spectrum of test item measured after experimental completion.



Reference 2 IR spectrum supplied by sponsor.

STUDY PLAN AMENDMENT No. 2

Chemicals Evaluation and
Research Institute, Japan, Kurume

1. Title (Study number)
   Bioconcentration study of ANO in common carp (46100)

2. Content
   12.1 Test item (Page 5) (See the attached sheet for reason and detail.)

3. Approval
   Study Director Date March 8, 2016

   Name Naoki Hashizume
   Naoki Hashizume

Attached sheet

Content 12.1 Test item (Page 5)

Reason for amendment:

Information provided from the sponsor has changed.

Amendments detail

Before amendments	a) Chemical name Chemical name: Reaction mass of ammonium oxobis (ethanedioato) bisaquo niobate (V) hydrates and ammonium hydrogen ethanedioate ethanedioic acis dihydrate Another name: ANO
After amendments	a) Chemical name etc. Chemical name: Mixture of ammonium oxobis(ethanedioato) bisaquo niobate(V) hydrates and ammonium hydrogen ethanedioate ethandioic acid dehydrate Synonym (anhydrous form) Reaction mass of ammonium diaqua[bis(oxalate)] oxoniobate(1-) and ammonium hydrogen oxalate oxalic acid (1:1:1) Abbreviation: ANO

ATTN: CBMM Technology Suisse SA

April 18, 2016

Reference No.	8479092

SCAS Sumika Chemical Analysis Service

Sumika Chemical Analysis Service, Ltd.
Chemicals Compliance Division
Sumitomo Fudosan Hongo Bldg,
Hongo 3-22-5, Bunkyo-ku,
Tokyo, 113-0033, JAPAN

Analysis Report

Sampling and testing was conducted by the client order dated 21 May, 2015.

1. Title of the test: Bioconcentration study
2. Test item: ANO
3. Study number: 46100
4. Methods and results: Presented in the attached documents
5. Reports: Final report (English); 1 copy
   Test report (Japanese); 1 copy

If you have any questions about this report, please make inquiries to the person in charge.

General supervisor: *Koichi Matsumoto*
K. Matsumoto

Person in charge: *Kazumi Kawahara*
K. KAWAHARA

TEL +81-3-5689-1216
FAX +81-3-5689-1221

濃縮度試験結果報告書

1．一般的事項

新規化学物質の名称（IUPAC 命名法による）	Mixture of ammonium oxobis(ethanedioato) bisaquo niobate(V) hydrates and ammonium hydrogen ethanedioate ethandioic acid dehydrate		
別名	ANO		
CAS 番号	-		
構造式又は示性式（いずれも不明の場合は、その製法の概要）	Constituent 1 : $NH_4[NbO(C_2O_4)_2 \cdot 2H_2O] \cdot 3H_2O$ Constituent 2 : $(NH_4C_2HO_4)_2 \cdot (C_2O_4H_2 \cdot 2H_2O)_2$		
分子量	Constituent 1 : 393.06 Constituent 2 : 466.26		
試験に供した新規化学物質の純度（%）	97%（Constituent 1 : 70%, Constituent 2 : 27%）		
試験に供した新規化学物質のロット番号	AD/5275		
不純物の名称及び含有率	Water 2.5% Inorganic impurities 0.5%		
蒸気圧	-		
対水溶解度	10 g/L（目視，当試験施設測定データ）		
1-ｵｸﾀﾉｰﾙ／水分配係数	-		
融点	-		
沸点	-		
常温における性状	白色固体		
安定性	安定		
溶媒に対する溶解度等	溶媒	溶解度	溶媒中の安定性
	-	-	-

分解度試験の結果、被験物質（ANO）は完全に生分解され、28 日間の培養終了時における残留物として水酸化ニオブ（V）が認められた。化審法においては、水酸化ニオブ（V）の濃縮度試験が求められるが、水酸化ニオブは水に不溶であることから、水酸化ニオブ（V）の濃縮度試験の実施は困難であると考えられた。経済産業省との相談の結果、ANO を被験物質として、ニオブイオンを定量することにより濃縮度試験を実施し、水酸化ニオブ（V）の濃縮性を評価することとなった。本試験における濃度は、被験物質である ANO の濃度で示した。

2. 急性毒性試験

供試魚（学名）	コイ（*Cyprinus carpio*）	
LC_{50}（96hr）	99.0 mg/L	
助剤の使用	無	
助剤を使用した場合の名称及び濃度	名称	濃度
	-	-

3. 試験方法

試験方法	a) 「新規化学物質等に係る試験の方法について」（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、環保企発第110331009号；一部改正 平成24年4月2日、薬食発0402第1号、平成24･03･28製局第2号、環保企発第120402001号）に定める「魚介類の体内における化学物質の濃縮度試験」 b) "305-I : Aqueous Exposure Bioconcentration Fish Test" stipulated in the OECD Guidelines for Testing of Chemicals, No.305, October 2, 2012, "Bioaccumulation in Fish : Aqueous and Dietary Exposure"	
供試魚（学名）	コイ（*Cyprinus carpio*）	
脂質含量（%）	開始時：3.10	終了時：2.87
被験物質設定濃度（μg/L）	第一濃度区	700
	第二濃度区	70
助剤の使用	無	
助剤を使用した場合の名称及び濃度	名称	濃度
	-	第一濃度区： -
		第二濃度区： -

4. 試験結果

(1) 濃縮度試験の結果表

( ) 内は平均値

	取込期間	5日後	13日後	18日後	21日後	28日後
第一濃度区	水中の被験物質濃度(μg/L)	733	795	688	691	674
	濃縮倍率	<2.3 2.5	4.4 4.9 (4.6)	12 8.4 (10)	8.0 8.7 (8.4)	5.8 7.0 (6.4)
第二濃度区	水中の被験物質濃度(μg/L)	66.1	80.1	67.4	72.8	68.1
	濃縮倍率	<23 <23	<23 <23	<23 <23	<23 <23	<23 <23

(2) 定常状態における濃縮倍率又は濃縮倍率の上下限

濃度区	濃縮倍率	
第一濃度区	BCF	2.3倍未満～12倍
第二濃度区	BCF	23倍未満

5. 試験水及び魚体分析方法

試験水及び供試魚中の被験物質分析（ニオブイオン）は誘導結合プラズマ発光分光分析法（ICP-AES）により行った。

(1) 試験水及び魚体分析フロー

①試験水分析

採水 → 加熱（硫酸添加）→ 分析

②供試魚分析

酸分解 → 分析

(2) 使用した分析機器の種類とその条件

機　器	誘導結合プラズマ発光分光分析計 Optima 5300 DV（パーキンエルマー）
測定元素	ニオブ
測定波長	269.706 nm
プラズマガス	アルゴン 15 L/min
キャリアガス	アルゴン 0.70 L/min
補助ガス	窒素 0.2 L/min
RF 出力	1300 W
観測方向	ラジアル
観測点	15.0 mm
試料導入速度	1.00 mL/min

6. 回　収　率（平均値）

水からの回収率	(%)	106
魚体からの回収率	(%)	108

7. 考　　察

試験の有効性

本試験において、試験法に規定された試験の有効性を下記のとおり満たしており、被験物質の濃縮性を妥当に評価できると考えられる。

a) 試験温度の変動は、設定値 25℃の±2℃未満であった。
b) 溶存酸素濃度は、25℃の飽和濃度 8.1 mg/L の 60%以上であった。
c) 試験水中の被験物質濃度の変動は、実験期間中の測定値の平均に対して±20%以内であった。
d) 対照区及び試験区の供試魚において、死亡又は病気などの異常はみられなかった。

8. そ の 他

試験実施施設	名　　称	一般財団法人化学物質評価研究機構　久留米事業所
	所 在 地	〒839-0801　福岡県久留米市宮ノ陣三丁目２番７号 電話：0942-34-1500 FAX：0942-39-6804
試験責任者	職 氏 名	橋 爪 直 樹
	経験年数	7年
試 験 番 号	46100	
試 験 期 間	2015 年 12 月 22 日　から　2016 年 4 月 12 日　まで	

本様式の作成責任者	所　　属	一般財団法人化学物質評価研究機構　久留米事業所
	氏　　名	橋 爪 直 樹