

## National Institute for Environmental Studies

### Certificate of Analysis

#### NIES CRM No. 32 Bluegill

This environmental certified reference material (CRM) was developed and certified by the National Institute for Environmental Studies (NIES) for the determination of perfluorooctanesulfonic acid (PFOS) in the freshwater fish bluegill (*Lepomis macrochirus*) and in materials of similar matrix.

#### Certified Values

Compound	Mass fraction			Analytical method *
	Unit	Certified value	Uncertainty	
PFOS	ng/g	43.9	5.6	LC-MS, LC-MS/MS

\* LC-MS, liquid chromatography-mass spectrometry

LC-MS/MS, liquid chromatography-tandem mass spectrometry

#### Characterization of Certified Value

The property value of the material was statistically determined based on chemical analyses by 9 organizations using a wide range of methods. The property value satisfying the following conditions was accepted as a certified value:

- 1) the relative standard deviation associated with the mean of the laboratory means was under 10 %,
- 2) the number of laboratories contributing to the mean of the laboratory means was at least 8, and
- 3) the number of analytical methods contributing to the mean of the laboratory means was at least two.

The uncertainty attached to the certified value is the expanded uncertainty using a coverage factor  $k = 2$ , corresponding to the half-width of a confidence interval of approximately 95 %. The certified value was determined based on dry mass.

#### Description of the Material

The CRM is supplied as fine powder in a glass bottle. The CRM is ocher in color.

#### Preparation of the CRM

Bluegills used for the preparation of this CRM were caught in Biwa Lake in Shiga Prefecture, Japan, from November 2011 to February 2012. A total of 200 kg of fish yielded 46 kg of fillets, which were minced, freeze-dried, pulverized, sieved (106  $\mu\text{m}$ ), and homogenized. The final material (3.8 kg) was placed in glass bottles (5 g in each bottle, 597 bottles), and sterilized by  $^{60}\text{Co}$  irradiation (20 kGy). All procedures complied with ISO Guide 34 with particular care to avoid contamination by PFOS.

## Homogeneity

The homogeneity of the CRM was determined by analysing 10 bottles selected from the total 597 bottles by stratified random sampling. PFOS was determined by the LC-MS/MS method. The between-bottle variation evaluated by a one-way analysis of variance (ANOVA) showed the relative standard deviations between bottles for the analytes to be less than 1 %. The material, therefore, is sufficiently homogeneous for its intended use as a reference material.

## Reference Values

Element	Mass fraction		Analytical method *
	Unit	Reference value	
Calcium (Ca)	%	0.106	ICP-OES
Magnesium (Mg)	%	0.139	AAS, ICP-OES
Phosphorus (P)	%	0.875	ICP-OES, Molybdenum blue-FIA
Arsenic (As)	mg/kg	0.563	ICP-MS
Copper (Cu)	mg/kg	1.06	ICP-MS, ICP-OES
Iron (Fe)	mg/kg	9.69	ICP-MS, ICP-OES
Manganese (Mn)	mg/kg	0.881	ICP-MS, ICP-OES
Mercury (Hg)	mg/kg	0.161	CVAAS, Pyrolysis-gold amalgamation-AAS
Selenium (Se)	mg/kg	1.79	ICP-MS
Zinc (Zn)	mg/kg	29.3	ICP-MS, ICP-OES

\* AAS, atomic absorption spectroscopy

CVAAS, cold vapor atomic absorption spectrometry

ICP-MS, inductively coupled plasma-mass spectrometry

ICP-OES, inductively coupled plasma-optical emission spectrometry

Molybdenum blue-FIA, molybdenum blue-flow injection analysis

Pyrolysis-gold amalgamation-AAS, pyrolysis-gold amalgamation- atomic absorption spectroscopy

## Characterization of Reference Values

Of the measured values reported by 7 organizations, those with z-scores of 2 or more, obtained by use of robust statistics, were rejected. Reference values are means of reported values after those rejected were removed. These values were determined based on dry mass.

## Instructions for Use

1. Care should be taken to avoid contamination when opening the bottles. It is desirable to use up the contents as quickly as possible after opening.
2. This CRM should be kept tightly closed in its original bottle and stored in a freezer at under  $-20^{\circ}\text{C}$ .
3. Prior to weighing portions for analysis, the bottle should be allowed to come to room temperature and the contents of the bottle should be shaken gently.
4. It is recommended that a sample intake of 0.2 g is the minimum for convenient handling.
5. Precautions should be taken to avoid inhalation of the material.

6. This CRM should not be used for purposes other than research. When disposing of the material, local laws concerning processing and disposal of waste materials should be strictly adhered to.
7. The mass fractions of certified and reference values in this CRM are reported on a dry mass basis. This CRM has 4 % water as measured (n=20) in NIES by drying for 4 h at 85 °C. Correction to dry mass should be determined by drying a separate sub-sample.

#### **Expiry Date of Certification**

The expiry date for the certified values of this CRM is March 2026 assuming that the recommended storage conditions are adhered to. NIES will notify via its website if any changes in the contents are recognized within the term of validity.

#### **Collaborating Laboratories in Analysis**

The certified and reference values for this CRM were based on analytical values from the following 11 participating organizations:

National Institute for Environmental Studies; Environmental Control Center Co., Ltd.; Hyogo Prefectural Institute of Environmental Sciences; IDEA Consultants, Inc.; Kaneka Techno Research Corporation; Japan Food Research Laboratories; Murata Keisokuki Service Co., Ltd.; Nippon Steel & Sumikin Technology Co., Ltd.; Seikan Kensa Center Inc. ; Shimadzu Techno-Research Inc.; The University of Tokyo

#### **Collaborating Organizations in Preparation**

Shiga Prefecture Federation of Fishermen's Co-operative Association; Kasumigaura Fishermen's Co-operative Association

#### **Technical Information**

Technical information and the latest reports regarding this material can be obtained from the website.

<http://www.nies.go.jp/labo/crm-e/index.html>

March 24, 2016

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Original certificate date: March 24, 2016

Certificate revision date: April 1, 2021 (Editorial changes)

## Appendix

Information that could be useful for handling this material is provided, though the values are non-certified.

Table A1: Information values of Mass fractions of Perfluoro compounds (PFCs) in NIES CRM No. 32

Compound	Mass fraction		Analytical method *
	Unit	Content	
PFNA	ng/g	1.19 - 2.04	LC-MS/MS
PFDA	ng/g	12.3 - 15.3	LC-MS/MS
PFuDA	ng/g	16.9 - 21.7	LC-MS/MS
PFdDA	ng/g	7.1 - 12.6	LC-MS/MS

These values were determined based on dry mass.

\* LC-MS/MS, liquid chromatography-tandem mass spectrometry

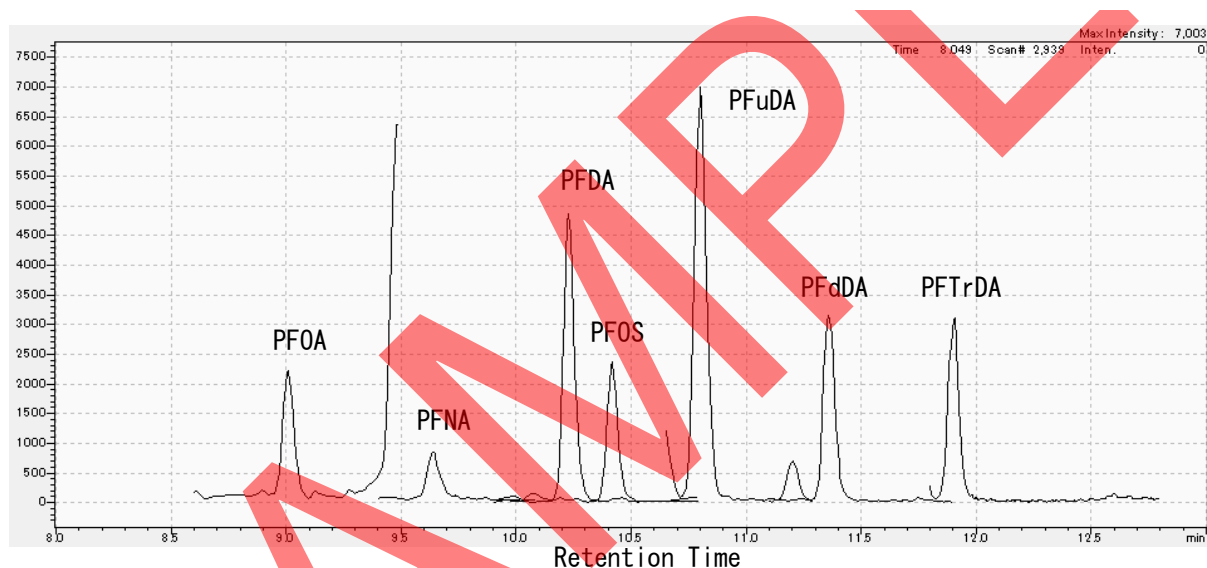


Fig. A1: Example of LC-MS/MS chromatograms of PFCs in NIES CRM No. 32

Column: Ascentis Express C18, 2.1 x 150 mm, 5  $\mu$ m, Solvent: 10-95 % CH<sub>3</sub>CN in 10 mM ammonium acetate, Flow rate: 0.3 ml/min, Temp.: 50 °C

PFOA: Perfluorooctanoic acid

PFNA: Perfluorononanoic acid

PFDA: Perfluorodecanoic acid

PFuDA: Perfluoroundecanoic acid

PFdDA: Perfluorododecanoic acid

PFTrDA: Perfluorotridecanoic acid