

# New preparation of fish material for inter-laboratory study on PFCs

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Report number C076/07

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Publication Date: 20.7.2007

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## Introduction

Institute for Environmental Studies, Vrije Universiteit (IVM) has requested Wageningen IMARES to prepare a new fish material for use in the inter-laboratory performance study on analysis of perfluorinated compounds (PFCs) due to the low amount of contaminants in the previously prepared material. The new material was requested to be prepared from fillet of flounder and spiked with artificial mixture prepared from material provided by IVM. Homogenized material should be then packed in glass jars, sterilized and delivered to IVM. This document provides report on the preparation of material.

## Assignment

In accordance with the order 07.071, the following activities were performed by Wageningen IMARES:

- Purchase of flounder
- Filleting of fish
- Mincing of fillets
- Preparation of artificial mixture of PFCs in methanol for spiking of the material using standards of PFCs provided by IVM.
- Spiking of the minced material with the prepared mixture to obtain PFC nominal concentration specified in Table 1.
- Homogenization of the material for at least 1 hour.
- Packing of ca. 100-grams of homogenized sub-sample into glass bottle, freezing it at -20°C and delivering it to IVM
- Packing the rest of homogenized material into glass jars leaving minimal amount of air inside the jars
- Sterilization of the material in the glass jars
- Delivery of the material to IVM
- Reporting on material preparation

Table 1. Targeted nominal concentrations in the material.

Full name	Abbreviation	Concentration* (ng/g ww)
Perfluorooctanoic acid	PFOA	25
Perfluorononanoic acid	PFNA	20
Perfluorodecanoic acid	PFDA	20
Perfluoroundecanoic acid	PFUnA	20
Perfluorododecanoic acid	PFDoA	20
Perfluorooctane sulfonate	PFOS	150
Perfluorosulfonamide	PFOSA	20

\* Targeted nominal concentrations +/- 10%

## Quality Assurance

IMARES utilises an ISO 9001:2000 certified quality management system (certificate number: 08602-2004-AQ-ROT-RvA). This certificate is valid until 15 December 2009. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. The last certification inspection was held the 16-22 of May 2007. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2000 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2009 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation, with the last inspection being held on the 12<sup>th</sup> of June 2007.

## Materials and Methods

### *Purchase of flounder*

About 120 kg of flounder originating from the North Sea (52°55'N - 03°30'E) was purchased (ship TX43) on June 15, 2007. After delivery to Wageningen IMARES, the fish was stored frozen at -20°C till the preparation.

### *Spiking solution*

Spiking solution of PFCs was prepared in methanol. The total volume was ca. 25 mL and the absolute amount of PFCs in this solution are listed in Table 2.

Table 2. Absolute amount of PFCs in the stock solution.

Full name	Abbreviation	Absolute amount (mg) in ca. 25 mL of methanol
Perfluorooctanoic acid	PFOA	0.5639
Perfluorononanoic acid	PFNA	0.4294
Perfluorodecanoic acid	PFDA	0.5470
Perfluoroundecanoic acid	PFUnA	0.4440
Perfluorododecanoic acid	PFDoA	0.5027
Perfluorooctane sulfonate	PFOS	3.8896
Perfluorosulfonamide	PFOSA	0.0810

### *Material preparation*

Filleting of the fish was performed June 19, 2007. After the filleting the material was minced using a mincer (Finis Machinefabriek, Ulfth) in combination with a Fryma mill equipped with toothed rotary knives (Fryma Maschinen AG, Rheinfelden, Switzerland) to a final size of 3.5 mm<sup>2</sup>. Subsequently, ca 25 kg of fillets was placed in a water-cooled Stephan cutter (Stephan Machines, Almelo, The Netherlands, type UMM/SK25, made in 1979), 0.02% butylhydroxytoluene (BHT) was added and sample was homogenized for 1 hour. At the beginning of the homogenization, 5 mL of spiking solution and flask-rinsing methanol was added every 3 minutes until all PFCs were quantitatively transferred to the material. After homogenization, ca. 100 g of homogenized material was placed into the glass bottle and frozen at -20°C without sterilization. The rest of material was filled into the glass jars and closed with the lid (ca. 60 g of material per jar). Special attention was paid to fill the glass jars to the top to minimize head space area. Starting with jar number 10, every thirty jar was marked to be used for the homogeneity test. The jars were then sterilised in a Muvero-Mat sterilizer (type 90E) for 45 minutes at 122 °C (pressure 1.4 bar, heating-time: 90 minutes, cooling time: 20 minutes). Each glass jar was then packed into the non-transparent (black) plastic back, placed into the paper box and dispatched to IVM.

## Results and discussion

One glass bottle containing ca. 100 g of homogenized, not sterilised and frozen material was prepared and delivered to IVM.

250 glass jars of homogenized and sterilized material including marked jars for homogeneity testing were prepared and delivered to IVM.

The final nominal concentration of PFCs in the material after spiking (not taking into account natural level of PFCs present in fish) is listed in Table 3. All PFCs except PFOSA has concentration as requested. PFOSA has lower concentration, because amount of delivered standard for spiking was not sufficient to achieve the required concentration. This was communicated with IVM and was agreed to achieve concentration as high as possible from amount of standard delivered.

Table 3. Final nominal concentration of PFCs in the material after spiking (not taking into account natural level of PFCs in fish tissue)

Full name	Abbreviation	Concentration (ng/g ww)
Perfluorooctanoic acid	PFOA	22.56
Perfluorononanoic acid	PFNA	17.18
Perfluorodecanoic acid	PFDA	21.88
Perfluoroundecanoic acid	PFUnA	17.76
Perfluorododecanoic acid	PFDoA	20.11
Perfluorooctane sulfonate	PFOS	155.58
Perfluorosulfonamide	PFOSA	3.24

## Conclusions

250 glass jars of homogenized and sterilized spiked flounder from the North Sea and 100 g of homogenized and not sterilized spiked flounder have been prepared for and delivered to the Institute for Environmental Studies (IVM) Free University, Amsterdam to be used in inter-laboratory performance study on analysis of perfluorinated compounds (PFCs).

# Referees and Authors

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Project Number: 439.51008.02

This report has been professionally prepared by Wageningen IMARES. The scientific validity of this report has been internally tested and verified by another researcher and evaluated by the Scientific Team at Wageningen IMARES.

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Number of copies: 8  
Number of pages: 7  
Number of tables: 4  
Number of graphs: -  
Number of appendix attachments: ~-