Wageningen IMARES BV
Institute for Marine Resources and Ecosystem Studies

Postbus 68
1970 AB IJmuiden
Tel.: 0255 564646
Fax.: 0255 564644
E-mail:visserijonderzoek.asg@wur.nl
Internet: www.rivo.wageningen-ur.nl

Vestiging Yerseke
Centrum voor Schelpdier Onderzoek
Postbus 77
4400 AB Yerseke
Tel.: 0113 672300
Fax.:0113 573477

Vestiging Texel
Postbus 167
1790 AD Den Burg
TEXEL
Tel: 0222 369700
Fax: 0222 319235

Report

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Survey on PFOS and other perfluorinated compounds in Dutch fish and shellfish

S.P.J. van Leeuwen and J. de Boer

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Postbus 20401
2500 EK 's-Gravenhage

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Approved by: drs. S.P.J. van Leeuwen
Hoofd cluster Milieu en Voedselveiligheid

Signature: _____

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Mailing list

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Dutch Fish Product Board (mr. E.M. Mens)

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Summary

Perfluorinated compounds (PFCs) are used in a wide variety of applications as a surfactant because of their 'wetting' properties. Other applications include moisture, stain and fat repellency for consumer products (e.g. leather and carpets) and as polymerization aid for the production of fluorinated polymers (e.g. Teflon). PFCs have been produced since the 1960s. Production of perfluoroctanesulfonate (PFOS) has been terminated by some producers on a voluntary basis but other PFCs (e.g. perfluorinated octanoic acid, PFOA) remain in production for a range of applications.
PFCs have entered the environment (and continue to do so) during production, use of treated products or after disposal of the treated products. Furthermore, volatile fluorotelomer alcohols, present in the atmosphere, may react leading to perfluoroalkyl acids (e.g. PFOA).
IMARES (formally known as RIVO) has developed an analytical method based on ion pair extraction and detection of the target compounds by liquid chromatography and mass spectrometric detection. The method was optimised for a range of PFCs including PFOS and PFOA.
PFOS was detected in 35 of the 45 analysed samples (78%) of shellfish, freshwater, marine and farmed fish at levels ranging from 2-730 ng/g ww. Highest levels were detected in flounder livers from the Western Scheldt and second highest levels were detected in freshwater fish. PFOA was found in the 10 out of the 45 samples analysed, although at lower levels (2-53 ng/g ww). In 10 out of the 45 analysed samples also longer chain PFCs (perfluoroundecanoic and –dodecanoic acid) were found. PFCs were more abundant in fish livers compared to the muscle tissue.
The results show that PFCs are ubiquitous in Dutch fish. Whether or not PFCs pose a risk to the consumer should be evaluated in the light of their toxicity and human intake.

This project is carried out on request of the Ministry of Agriculture, Nature Management and Food Quality (LNV) as part of the Program nr. 438, 'Guarding the Quality of Dutch Agricultural, Horticultural and Fishery Products'. This program is coordinated by RIKILT-Institute of Food Safety.

1. Introduction

Perfluorinated compounds (PFCs) are used in a wide variety of applications as a surfactant because of their 'wetting' properties. Other applications include moisture, stain and fat repellency for consumer products (e.g. leather and carpets) and as polymerization aid for the production of fluorinated polymers (e.g. Teflon). A range of different compounds have been produced since the 1960s. A selection is shown in Table 1.
Because of the wide distribution in wildlife and human samples the production of perfluorinated octane sulfonate (PFOS) some producers (e.g. 3M) decided to terminate the production PFOS and related compounds on a voluntary basis (EPA, 2000). Other PFCs (e.g. perfluorinated octanoic acid (PFOA)) remain in production by various producers for a range of applications. Dupont voluntarily plans to reduce PFOA residuals in consumer products with 98% by 2007 (Renner, 2006).

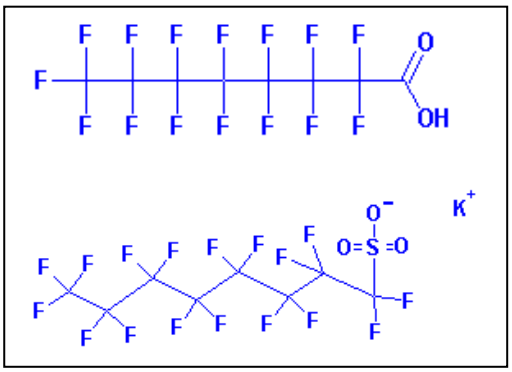

Figure 1. Chemical structures of PFOA (top) and the potassium salt of PFOS (bottom).

Perfluorinated telomer alcohols (FTOHs), named after the telomerisation production method, is another class of PFCs that are applied in a range of products such as paints, adhesives, polymers, and electronic materials (Hurley *et al.*, 2004). FTOHs are volatile and are therefore easily introduced into the environment during application, use and after disposal. FTOHs are found to be ubiquitous in the Northern American environment (Martin *et al.*, 2002). Scientists have proposed several abiotic and biotic mechanisms through which perfluorinated acids such as PFOA are formed from FTOHs (Dinglasan *et al.*, 2004; Ellis *et al.*, 2004), although abiotic formation and the formation kinetics should be confirmed through field studies.
PFCs have entered the environment (and continue to do so) during production, use of treated products or after disposal of the treated products. The other postulated route is through atmospheric and biotic transformation of FTOHs, as described above.
Research on PFCs in the environment has taken of since the first reports on their presence in the environment (Giesy and Kannan, 2001). Research groups in Canada, USA, Japan and Europe are active. The European Commission has funded the PERFORCE research project (www.science.uva.nl/perforce), which focuses on development of robust analytical techniques, environmental behavior and monitoring the geographical distribution of PFCs in Europe in fish, water, sediment and sewage sludge.
Perfluorinated compounds have been found in a wide variety of environmental matrices including water (Caliebe *et al.*, 2004; Yamashita *et al.*, 2005), fish (de Voogt et al., 2003; Giesy and Kannan, 2001; Kallenborn et al., 2004; Martin et al., 2004b; van de Vijver et al., 2002), marine mammals, polar bears (Smithwick et al., 2005a; Smithwick et al., 2005b; Smithwick et al., 2006), bird (eggs) (Hoff et al., 2005b; Kannan et al., 2002a; Kannan et al., 2001; Verreault et al., 2005) and sewage sludge (Kallenborn *et al.*, 2004; Wang *et al.*, 2005). PFOS and PFOA accumulate in various food chains. Apart from PFOS and PFOA, perfluorinated acids and sulfonates with different chain lengths (Kallenborn et al., 2004; Martin et al., 2004a) have been detected in various environmental samples.

A recent study in Polish consumers showed that people with a high fish consumption have PFC concentrations in their blood up to 4 ng/ml for PFOS (Falandysz *et al.*, 2006). There is considerable data available on PFOS and PFOA contamination of fish livers (see Table 3). Very limited data is available on the contamination of edible parts of fish (meat, fillet) which hampers the evaluation of intake of PFCs through human consumption of fish. The aim of this study was to determine the contamination of Dutch fish and shellfish with PFCs. The study was designed such that information was acquired on the following:
- geographical distribution and concentration levels of PFOS and PFOA;
- contamination hot spots (Western Scheldt and main rivers);
- distribution in organs (liver vs. fillet);
- presence of other PFCs (other than PFOS and PFOA).

In order to perform this work, IMARES has developed an analytical method based on ion pair extraction and detection of the target compounds by liquid chromatography and mass spectrometric detection. The method was optimised for a range of PFCs including PFOS and PFOA.

Table 1. Chemical structure and abbreviatios of selected perfluorinated compounds

Full name of determinand	Abbreviation	Chemical structure
Perfluorinated acids	**PFCAs**	
Perfluorobutanoic acid	PFBA	$CF_3(CF_2)_2COOH$
Perfluorohexanoic acid	PFHxA	$CF_3(CF_2)_4COOH$
Perfluoroheptanoic acid	PFHpA	$CF_3(CF_2)_5COOH$
Perfluorooctanoic acid	PFOA	$CF_3(CF_2)_6COOH$
Perfluorononanoic acid	PFNA	$CF_3(CF_2)_7COOH$
Perfluorodecanoic acid	PFDA	$CF_3(CF_2)_8COOH$
Perfluoroundecanoic acid	PFUnA	$CF_3(CF_2)_9COOH$
Perfluorododecanoic acid	PFDoA	$CF_3(CF_2)_{10}COOH$
Perfluorotridecanoic acid	PFTrA	$CF_3(CF_2)_{11}COOH$
Perfluorotetradecanoic acid	PFTA	$CF_3(CF_2)_{12}COOH$
Perfluoropentadecanoic acid	PFPA	$CF_3(CF_2)_{13}COOH$
Perfluorinated sulfonates	**PFSA**	
Perfluorobutane sulfonate	PFBS	$CF_3(CF_2)_3SO_3^-$
Perfluorohexane sulfonate	PFHxS	$CF_3(CF_2)_5SO_3^-$
Perfluorooctane sulfonate	PFOS	$CF_3(CF_2)_7SO_3^-$
Perfluorodecane sulfonate	PFDS	$CF_3(CF_2)_9SO_3^-$
Perfluorotelomer alcohols	**(FTOHs)**	
6:2 fluorotelomer alcohol	6:2 FTOH	$CF_3(CF_2)_5CH_2CH_2OH$
8:2 fluorotelomer alcohol	8:2 FTOH	$CF_3(CF_2)_7CH_2CH_2OH$
Other		
Perfluorosulfonamide	PFOSA	$CF_3(CF_2)_7SO_2NH_2$

2. Materials and methods

2.1 Sampling and sample preparation

The sample scheme (Table 2) is comparable with that of the survey on brominated flame retardants (van Leeuwen *et al.*, 2006). The selected species originated from Dutch waters or are regularly consumed by the Dutch population. Two popular farmed fish species, eel and salmon, have been included. Also, two samples of flounder were obtained from the Western Scheldt to monitor the effects of the (historical) fluorochemical production and application by industries en users in the Antwerp region and Scheldt river basin.

Table 2. Sample overview of the investigated samples

Species	Origin	Number
Cod	Central North Sea	1
Eel	Nieuwe Merwede, Meuse, Keizersveer, Haringvliet West, Rhine, Lobith, IJssel Lake Medemblik, Ketel Lake	5
Eel, farmed	Italy*, Netherlands*	2
Flounder	Western Scheldt*, Western Scheldt, Saeftinge*	3
Haddock	Central North Sea	1
Herring	English Channel, Central North Sea, Southern North Sea*, Skagerak, Shetland Islands	5
Mackerel	North Sea, Shetland Islands	2
Mussel	Eastern Scheldt, Western Wadden Sea, Eastern Wadden Sea	3
Oysters	Eastern Scheldt, Yerseke	1
Pike perch	Hollands Diep*, IJssel Lake	2
Plaice	Southern North Sea*	1
Salmon	Norway, Scotland	2
Sole	Southern North Sea*, North Sea (Mouth of Western Scheldt, West of Egmond, West of Texel, West of Hoek van Holland, West of IJmuiden)	5
Shrimp	Wadden Sea, North Sea, Rijnmond	2
Tuna	Mediterranean	1
	Total	36 (8)**

* Liver and fillet analysed
** Between brackets: number of liver samples analysed

The majority of locations were sampled between April and October 2004. Marine fish was mostly sampled during surveys of the research vessel Tridens. Remaining samples were obtained directly from fishermen, from the auction or from wholesale traders. Eel (mostly 30 – 40 cm) was caught by electric fishery within the framework of the monitoring programme for Dutch game fisheries (Pieters *et al.*, 2004). Farmed eels were only available in greater lengths. Sampling data are given in Annex 1.

After transportation to IMARES, lengths and weights of the individual fishes were measured (except for tuna, mussels and shrimps). Subsequently, fishes were filleted and equal amounts of filet per fish were pooled. The pooled samples were homogenized in a Waring Blender. The samples for mussels were obtained by taking the meat out of the shells of a pooled sample of 3 kg mussels (100 g mussel meat was pooled). Pooled shrimp samples were prepared from ca 500 g unpeeled and uncooked whole organisms.

2.2 Analytical determination

The seven compounds analysed in this study are PFOS, PFOA, PFHxS, PFNA, PFDcA, PFUnA and PFDoA. The concentration of PFCs were determined according an adapted method published by Hansen et al. (Hansen *et al.*, 2001). The principle of the method is as follows: 10 g of homogenised sample was extracted 3 times with methyl-*tert*-butylether (MTBE) in the presence of the ion pairing agent tetrabutylamine (TBA). The extracts are pooled and MTBE was evaporated to a final volume of 1 ml. A clean-up step for removal of co-extracted lipids was added to the Hansen et al. method as in the method development phase it was found that lipids disturbed the analytical determination. The lipids were removed from the sample by silica column chromatography (elution by 15 ml dichloromethane). The target compounds were subsequently eluted with 30 ml acetone. The acetone was removed by evaporation and replaced by 0.7 ml methanol, after which the extracts were ready for injection.
The extracts were injected on a Thermo Electron Surveyer high pressure liquid chromatography (HPLC) system, coupled with an LCQ-Advantage ion trap mass spectrometric system (MS) and electrospray ionisation interface (ESI). The compounds were separated in a methanol/water gradient. PFOA and other perfluorinated acids were detected by MS/MS, whereas PFOS and other perfluorinated sulfonates were detected by MS only (due to a limitation in the design of the ion trap MS system). In PFOS determination, collision energy was applied to reduce interferences originating from the sample matrix. Details on the LC-ESI-MS(/MS) settings can be found in Annex 2.
For PFOS and other perfluorinated sulfonates, 7H-perfluorinated heptanoic acid (7H-PFHpA) has been used as internal standard, whereas for PFOA and other acids $^{13}C_2$ labelled PFOA has been used. The sensitivity of 7H-PFHpA was limited at the MS conditions used for PFOS. Therefore, in some cases, $^{13}C_2$ labelled PFOA was used as internal standard for the calculation of PFOS.

Quality assurance

The method has been validated for all compounds analysed. Quality of analysis was controlled by the recovery determination, blank and duplicate analysis. The relative standard deviation (rsd) for a duplicate determination varied: 12-26% for PFOS and 95% for PFOA. In the latter case, the high rsd was due to the very low (near limit of quantification (LOQ)) level of PFOA. The recoveries of spiked herring oils varied as follows: PFOA 134-145%, PFUnA 99-132% and PFOS 91-552%.
The high recovery for PFOS in the latter case is due to a very low response for the internal standard 7H-PFHpA.
Recently, the first international interlaboratory study on perfluorinated compounds in environmental matrices has been organised by IMARES (van Leeuwen *et al.*, 2005). This study showed that the analytical techniques in this rapidly evolving field are not fully mastered yet by most participants. The results for a fish tissue and fish liver extract were highly variable. The main contributors to the inaccuracy are the general lack of selective extraction and clean-up methods resulting in a final matrix-effect free extract. Other contributors to the error in the final results of the participants were e.g. the lack of (suitable) internal standards. Given the ongoing developments in this field (e.g. availability of isotope labelled standards, development of new analytical techniques), it is expected that the quality of the analytical results will improve in the near future.

3. Results and discussion

3.1 PFOS and PFOA

PFOS and PFOA concentrations are given in Annex 3. Data on the origin of the samples is provided in Annex 1. Figure 2 to Figure 5 show the concentrations of PFOS and PFOA in fish and shellfish from various origins. Values below limit of quantification (<LOQ) are not shown in the graph but can be found in Annex 3.

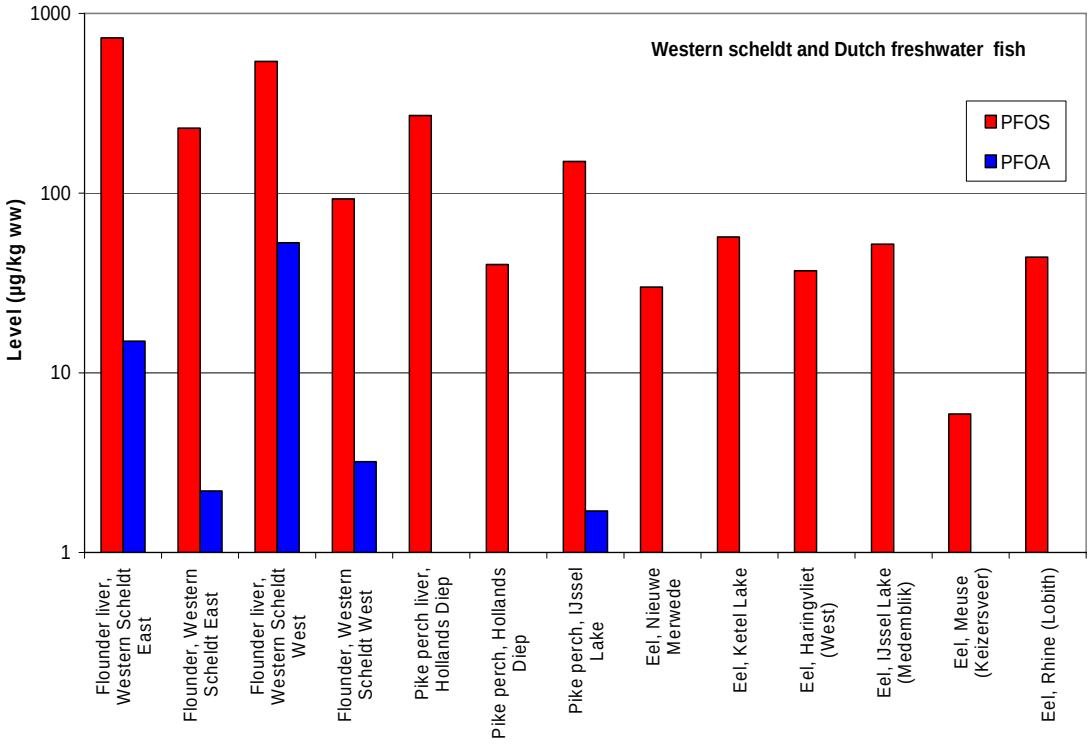

Figure 2. PFOS and PFOA concentrations in Dutch freshwater and Western Scheldt fish. In a number of cases both liver and muscle tissue were analysed.

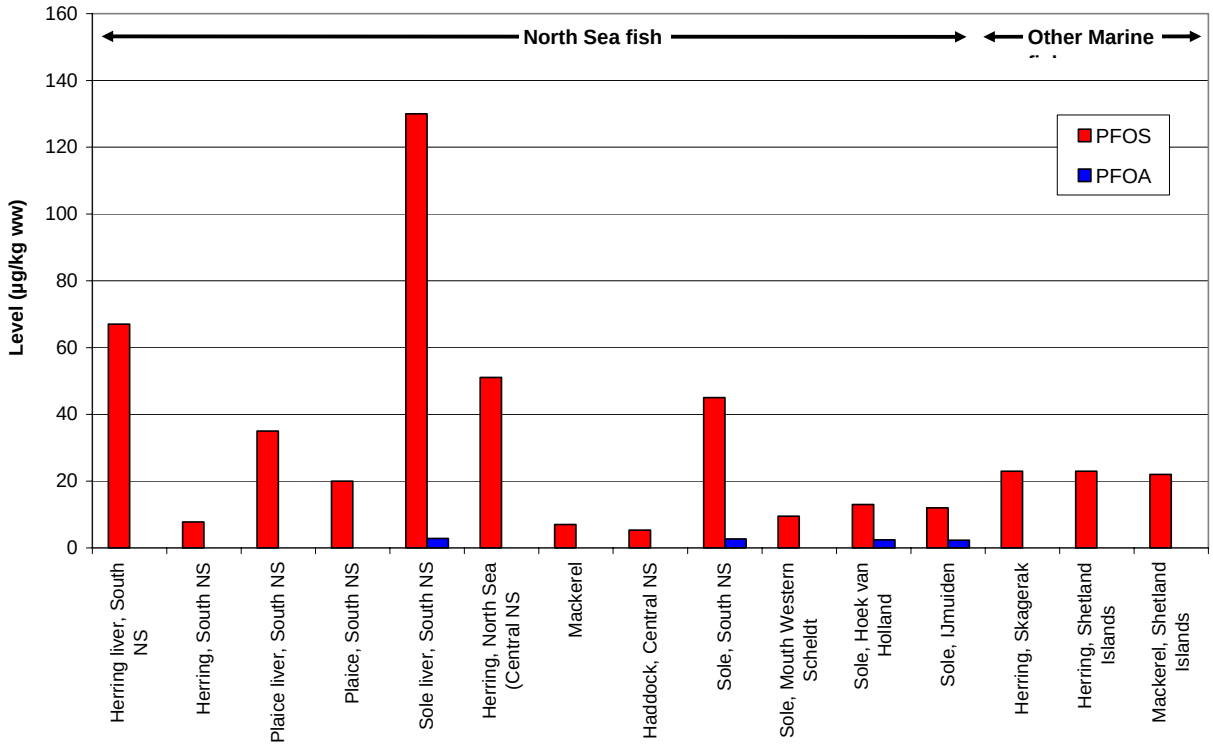

Figure 3. PFOS and PFOA concentrations in North Sea and other marine fish. In a number of cases both liver and muscle tissue were analysed.

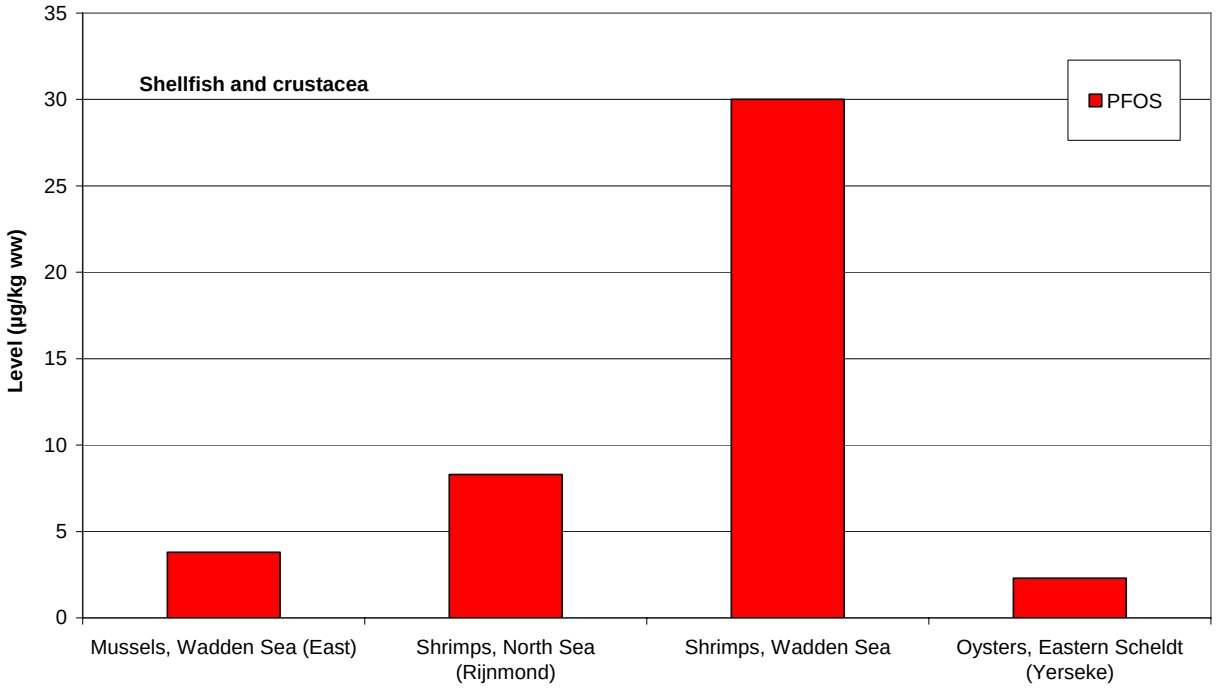

Figure 4. PFOS concentrations in shellfish and crustaceans. No PFOA was detected in any sample.

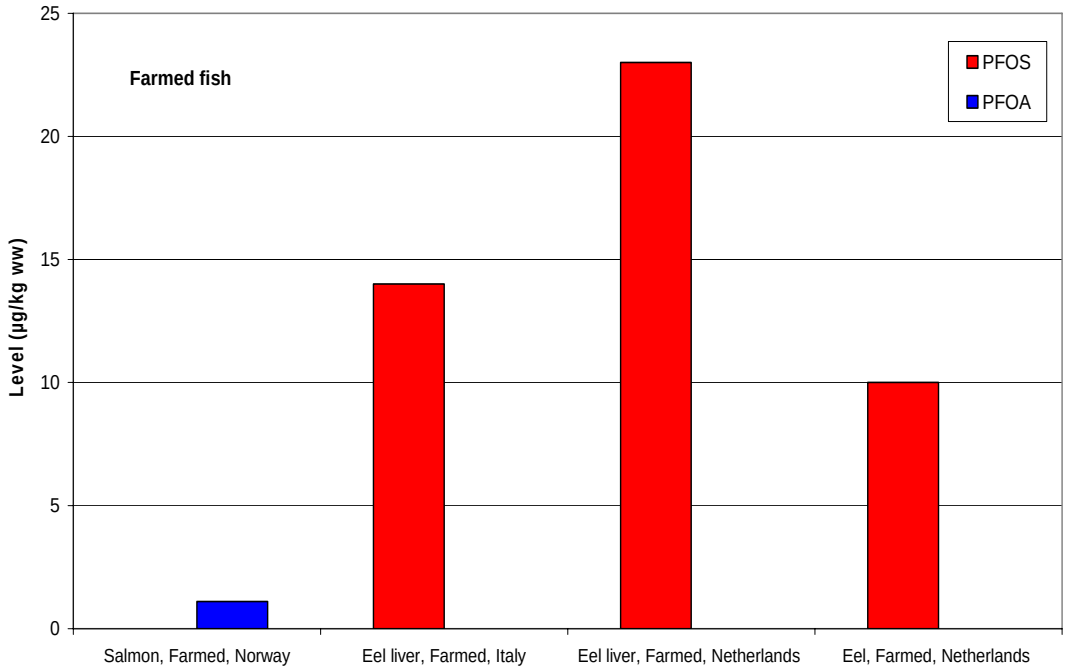

Figure 5. PFOS and PFOA concentrations in farmed fish.

Figure 2 to Figure 5 show that fish from all origins are contaminated with PFOS. PFOA is also found in fish from different origins, although at lower concentrations and in fewer samples. Generally, concentrations increase in the following order: open sea and ocean locations ≈ coastal water locations < freshwater locations < Western Scheldt. Highest concentrations are found in the Western Scheldt fish, which is related to the (historic) industrial production of PFCs in Antwerp and by industrial and domestic use of PFCs in the Scheldt basin. Freshwater fish (eel and pike perch) show the next highest concentrations as compared to the other origins. Concentrations in bivalves are below 5 ng/g ww but shrimps show somewhat higher concentrations (up to 30 ng/g ww).
PFCs preferentially accumulate in fish liver as compared to the muscle tissue. The different physicochemical properties of PFCs as compared to the classical contaminants (e.g. PCBs and dioxins) result in a different accumulation behaviour. Unlike the classical contaminants, the accumulation of PFCs is not related to the fat content. For example, fillets from eel from IJssel Lake are – although with higher lipid content (19.5%) – less contaminated with PFOS compared to the leaner fillets of the pike perch (1% fat). Jones *et al.* (2003) found that PFOS is associated to serum proteins (e.g. bovine serum albumine), which leads to different transportation mechanisms compared to the classical persistent organic pollutants (POPs).

3.2 Comparison with other data

The PFOS concentrations in Western Scheldt flounder livers are among the highest observed in fish reported from various locations worldwide and compare well to those reported by de Vijver *et al.* and Hoff *et al.* for Western Scheldt fish livers (see Table 3). The PFOS concentrations in muscle tissue compare also well to those reported earlier for plaice and bib (Hoff *et al.*, 2003). PFOS levels in eel liver samples from the leperlee canal at Boezinge (Belgium) were very high (up to 9 µg/g ww) and although no explanation for the high levels was found, the authors suggested that nearby industrial and household discharges may have caused these high levels (Hoff et al., 2005a).

The PFOS levels for sole and plaice from the Southern North Sea in our study compare well to those reported for plaice and bib caught near the Belgian North Sea coast (Hoff *et al.*, 2003). The other marine fish PFOS results are in the same order of magnitude as those reported by Kallenborn *et al.* (2004).
There are no data available on background contamination levels for PFCs in shellfish. The PFOS shrimp levels in our study (North Sea, Wadden Sea) were comparable to one of the Belgian coast samples, but lower compared to the other two locations that were not influenced by the Western Scheldt river plume (Van de Vijver *et al.*, 2003). PFOS levels in oysters from Gulf of Mexico and Chesapeake Bay (USA) showed levels of <45-1225 ng/g dry weight (ca 10-90 ng/g ww) (Kannan et al., 2002b).

Table 3. Selection of PFOS and PFOA literature data in biota from different locations. Values reported in muscle tissue or other edible tissues (e.g. meat of shellfish), unless otherwise specified.

Country	Location	Species	PFOS (ng/g ww)	PFOA (ng/g ww)	Reference
Netherlands	Various freshwater	Eel	<8-123	<2.6	(de Voogt *et al.*, 2003)
	Western Scheldt	Flounder	23	<2.6	
Netherlands	Western Scheldt	Shrimp	19-520	n.a.	(Van de Vijver *et al.*, 2003)
		Crab	24-877	n.a.	
		Starfish	9-176	n.a.	
Belgium	Various freshwater	Eel, liver	17-9031	n.a.	(Hoff et al., 2005a)
		(gibel) carp, liver	11-1822	n.a.	
Netherlands	Western Scheldt	Plaice, bib	<10-111	n.a.	(Hoff *et al.*, 2003)
	Belgian North Sea	Plaice, bib	<10-39	n.a.	
Finland		Pike, livers	204-551	<LOD-1.42	(Kallenborn *et al.*, 2004)
Sweden		Cod, livers	6.4-62	<LOD	
Denmark		Flounder, liver	18-21	<LOD	
		Herring, liver	15	5.4	
Iceland/Faeroe Isl.		Dab, liver	1.3-17	<LOD	
Japan	Various, mostly marine	Various, liver	3-558	n.a.	(Taniyasu *et al.*, 2003)
USA	New York state, freshwater	Various, livers	9-431	<1.5-71	(Sinclair *et al.*, 2006)
USA	Great Lakes, freshwater	Various, livers and muscle	<2-297	<2	(Kannan *et al.*, 2005)
Canada	Arctic and sub-artic, freshwater	Various, liver	6-50	<2	(Martin et al., 2004a)

*Norway Sweden, Iceland, Denmark

3.3 Other PFCs

In ten out of forty-five samples, other PFCs then PFOS and PFOA have been found (see Figure 6). The highest concentrations of these were determined for PFDcA and PFUnA (up to 60 ng/g ww) in flounder livers from the Western Scheldt. PFDcA and PFUnA have been detected in eel from the Rhine and Ketel Lake, but were not found further in the IJssel lake. These compounds were neither detected in the pike perch sample from that location. PFNA was found in four samples and PFDoA was found only in a flounder liver from the Western Scheldt and PFHxS was detected in one Western Scheldt flounder liver.
PFCs with longer chain lengths than PFOS and PFOA have been detected in other studies. In fish livers from Lac Minto and Kuujjaurapik (Canada), PFOA was below LOQ, whereas PFNA (C9) to PFTA (C14) were detected at levels from <0.5 to 8.5 ng/g ww (Martin et al., 2004a). Highest concentrations were found for the odd chain length compounds (viz. PFNA, PFUnA and PFTrA). Even chain numbered PFCs were somewhat less abundant. The levels of PFDcA and PFUnA in our study (flounder liver from the Western Scheldt, pike perch liver from Hollands Diep and eel fillet from the Ketel Lake) far exceed the levels reported in the Canadian study. Furthermore, the profile in our study is different from what they have observed. This is likely related to the high industrial activity in the (Western) Scheldt basin, although a mechanistic reason for this specific pattern is not yet known.
A study on PFCs in a variety of matrices from the Nordic countries (Kallenborn *et al.*, 2004) showed that PFOS was the predominant compound in nearly all freshwater fish livers (pike, perch, trout, burbot, arctic char), followed by PFOSA. PFOA was only detected in a few samples and at low levels. Apart from these compounds, low levels of PFHxS, PFDS, PFHpA and PFNA (<10 ng/g ww) were found in these samples. Total PFC levels (sum of PFOSA, PFOS, PFHxS, PFDS, PFHxA, PFHpA, PFOA and PFNA) ranged from 24-707 ng/g ww. Concerning marine fish (including cod, herring flounder and dab) PFOS again was the predominant PFC except for sculpins and cod from the Faeroe Islands where PFOSA was predominant. Icelandic samples showed surprisingly high PFDS levels. Total PFC levels in marine fish ranged from ca 2 to 83 ng/g (Kallenborn *et al.*, 2004). Perfluorinated acids with chains longer than C9 (PFNA) were not analysed in this study. For PFNA, the freshwater fish liver levels are comparable to those we have found in the liver samples and eel sample of the Ketel Lake. The PFHxS level found in our study (1 sample, flounder liver Western Scheldt) was higher than the levels detected in the Nordic study.

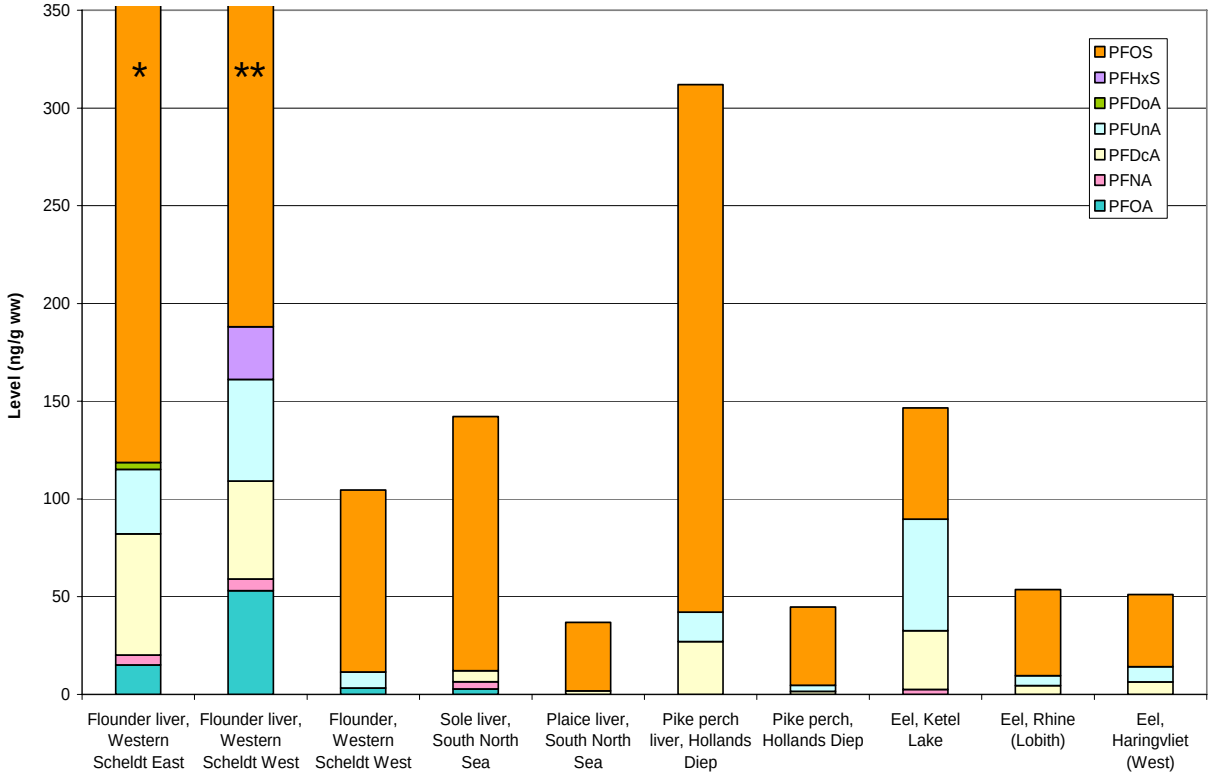

*Figure 6. PFCs in fish from various origins. PFOS and PFOA concentrations have been included for comparison only. *: PFOS 730 ng/g ww and sum of PFCs 849 ng/g ww. **: PFOS 540 ng/g ww and sum of PFCs 728 ng/g ww.*

4. Conclusions

Although PFCs predominantly accumulate in the fish liver, PFOS has also been found in substantial concentrations in edible fish tissues. Typical PFOS concentrations were around 10 μg/kg in marine fish with some exceptions up to 50 μg/kg. In fish livers the PFOS concentrations were 5-10 times higher. The highest concentrations were found in flounder (liver) from the Western Scheldt (93 to 730 ng/g ww). Lower PFOS levels were found in marine fish livers (95-130 ng/g ww). PFOS levels in farmed fish and shellfish/crustaceans were 2-30 ng/g ww. The high PFOS concentrations in fish from the Western Scheldt relate to the high industrial activity in the Scheldt river basin, including a fluorochemical production plant in Antwerp. PFOA was only detected in ten out of forty-five samples at levels below 5 ng/g ww (except for two Western Scheldt flounder liver samples).
The samples were also analysed for other PFCs (other than PFOS and PFOA). These were detected in ten out of forty-five samples at low concentrations (PFNA, PFHxS). Two long chain PFCs (PFUnA and PFDoA) were predominant (apart from PFOS) with levels from 2 to 62 ng/g ww in fish from various origins.

5. Recommendations

Together with available information from the literature for other European countries, these data ask for further studies on PFOS, PFOA and possibly other PFCs, both on occurrence and toxicology, in order to establish a proper risk assessment of these compounds.

To determine if PFCs pose a risk to consumer health it's recommended to carry out an intake estimation. A study from Poland showed that high levels of a range of PFCs (up to 84 ng/ml for PFOS) were determined in whole blood samples of non-occupationally exposed humans (Falandysz *et al.*, 2006). A relation with fish consumption was found. In addition, exposure via inhalation and dermal contact (from consumer products) requires further investigation.

Finally, the human and aquatic toxic modes of action of PFCs are still largely unknown. A European Food Safety Authority (EFSA) working group is currently evaluating available data on PFOS in food. Their report (draft opinion) is expected this summer (personal communication Jacob de Boer).

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Annex 1. Sample data

LIMS code 2004/								Weight (g)			Size (cm)		
Whole fish	Muscle/meat tissue	Liver tissue	Species	Catching area	Coordinates	Sample date	Number	Min	Mean	Max	Min	Mean	Max
0871	N.A.	N.A.	Shrimp	Wadden Sea	?	?	500 g	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
0798	N.A.	N.A.	Shrimp	North Sea, Rijnmond	52°05'NB-04°10'OL	09/04/2004	500 g	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
0853	0854	1095	Flounder	Western Scheldt, Saeftinge	N.A.	28/10/2004	25	205	242.7	268	26.0	27.4	29.0
0849	0850	N.A.	Herring	English Chanel	50°05'NB-00°53'OL	30/03/2004	25	63	97.7	167	21.0	24.4	29.3
0857	0858	N.A.	Herring	Central North Sea	56"54'NB - 02"15'OL	24/05/2004	25	76	93.6	128	21.0	22.4	26.0
0859	0860	1843	Herring	Southern North Sea	51"25'NB - 02"30'OL	27/10/2004	25	123	165.8	250	24.0	26.6	30.0
0861	0862	N.A.	Herring	Skagerak	Not available	08/06/2004	25	97	126.7	192	22.0	23.8	27.0
0863	0864	N.A.	Herring	Shetland Islands	60"26'NB 02"16'WL	20/08/2004	25	163	228.0	290	26.6	28.5	31.2
0869	0870	N.A.	Mackerel	North Sea	56"50'NB - 00"14'OL	24/05/2004	25	173	363.8	598	31.0	35.0	41.0
0872	0873	N.A.	Mackerel	Shetland Islands	59°24'NB - 06°34'WL	19/05/2004	25	141	414.3	615	27.0	38.9	45.0
0851	0852	N.A.	Mackerel	Atlantic Ocean, South West of Ireland	48°06'NB-08°06'WL	05/04/2004	25	90	240.1	672	23.0	31.1	44.2
0874	N.A.	N.A.	Mussel	Eastern Scheldt	N.A.	10/09/2004	3 kg	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
0875	N.A.	N.A.	Mussel	Western Wadden Sea	N.A.	02/09/2004	3 kg	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
0876	N.A.	N.A.	Mussel	Eastern Wadden Sea	N.A.	15/10/2004	3 kg	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
0877	0878	1845	Eel, farmed	Palinghandel Italie	N.A.	01/09/2004	22	160	344.0	426	44.0	57.8	63.0
0879	0880	1846	Eel, farmed	Palinghandel Nederland	N.A.	01/09/2004	20	207	260.2	376	44.0	48.1	58.0
0881	0882	N.A.	Eel	Nieuwe Merwede	N.A.	10/06/2004	25	65	94.8	162	32.0	35.5	40.0
0883	0884	N.A.	Eel	Meuse, Keizersveer	N.A.	22/06/2004	25	68	112.6	154	33.0	37.4	40.0
0885	0886	N.A.	Eel	Haringvliet West	Stellendam	08/06/2004	25	69	95.7	131	32.0	35.6	40.0
0887	0888	N.A.	Eel	Rhine, Lobith	Millingen aan de Rijn	14/06/2004	22	60	111.9	162	33.0	38.2	40.0
0889	0890	N.A.	Eel	IJssel Lake Medemblik	N.A.	14/05/2004	25	61	95.0	147	31.0	35.7	40.0
0893	0894	N.A.	Haddock	Central North Sea	54"10'NB - 02"40'OL	03/09/2004	25	367	546.1	701	32.2	38.7	42.4
0899	0900	1849	Plaice	Southern North Sea	52"24'NB - 04"18'OL	28/10/2004	25	315	495.9	704	31.0	34.2	39.0
0901	0902	1850	Pike perch	Hollands Diep	N.A.	02/11/2004	25	697	868.8	1249	43.2	46.8	52.0
0903	0904	N.A.	Pike perch	IJssel Lake	N.A.	18/10/2004	17	615	886.9	1398	40.0	44.9	52.0
0907	0908	1853	Sole	Southern North Sea	52"24'NB - 04"18'OL	28/10/2004	25	230	362.0	567	29.0	32.4	37.0
0909	0910	N.A.	Salmon	Norway	N.A.	26/08/2004	5	3832	3917.2	3997	70.4	72.9	74.4
0911	0912	N.A.	Salmon	Schotland	N.A.	26/08/2004	7	2931	3234.1	3457	67.5	69.9	72.7
1093	1094	1097	Flounder	Western Scheldt	51"24'NB - 03"48'OL	15/09/2004	25	35	85.0	136	14.4	19.1	23.0

LIMS code 2004/								Weight (g)			Size (cm)		
Whole fish	Muscle/meat tissue	Liver tissue	Species	Catching area	Coordinates	Sample date	Number	Min	Mean	Max	Min	Mean	Max
1098	1099	N.A.	Cod	Central North Sea	54"10'NB - 02"40'OL	03/09/2004	24	1053	1498.3	1999	45.0	51.3	59.0
1383	1384	N.A.	Sole	North Sea, Mouth Western Scheldt	N.A.	03/05/2004	22	134	185.1	246	26.0	28.2	29.0
1385	1386	N.A.	Sole	North Sea, Egmond	52"30'NB - 04"30'OL	14/05/2004	22	190	236.8	300	28.0	30.3	32.0
1414	1415	N.A.	Sole	North Sea, Texel	53°12'NB - 04°30'OL	29/09/2004	20	190	242.4	303	29.0	31.1	32.0
1416	1417	N.A.	Sole	North Sea, Hoek van Holland	51°50'NB - 03°50'OL	01/07/2004	21	198	245.2	286	29.0	30.6	33.0
1752	1753	N.A.	Eel	Ketel Lake	N.A.	09/06/2004	25	78	114.0	147	33.0	37.6	40.0
1801	1802	N.A.	Sole	North Sea, West of IJmuiden	N.A.	01/09/2004	25	146	291.6	484	24.0	29.8	37.0
1872	1873	N.A.	Oysters	Eastern Scheldt, Yerseke	N.A.	07/09/2004	25	54	110.6	207	7.4	10.0	11.8
N.A.	1800	N.A.	Tuna	Mediterannean	N.A.	02/09/2004	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

Annex 2. Analytical settings

HPLC-method

Injection volume: 20 µl
Flow: 300 µl/min

Table: LC-gradient

Time	MeOH (%)	Ammonium acetate 2 mM (%)
0	10	90
0.5	10	90
12	100	0
18	100	0
18.1	10	90
30	10	90
30	10	90

Ion trap MS(/MS) settings

Probe setting: 3 A
Sheat gas: 27
Auxillary gas: 17
Spray voltage: 4.5 kV
Capillary temperature: 200 °C (PFOA, PFNA, PFDA, PFUnA, PFDoA), 300°C (PFHxS, PFOS)
Capillary voltage: - 40 V
Tube lens offset voltage: - 57 V
Multipole 1 offset: 3 V
Lens voltage: 18 V
Multipole 2 offset: 83 V
Multipole RF amplitude: 560 V(p-p)
Number of microscans: 3
Ion injecton time: 200

Table: Purity of calibrants, MS(/MS) detection settings

Compound	Purity of calibrant	Molecular weight	MS method name	Parent ion (m/z)	Daughter ion (m/z)	Width	Collision energy	Retention time (estimate)
PFOA	96%	414	200-1	412.9	369	2	19	15.99
PFNA	97%	464	200-1	462.9	419	3	19	16.44
PFDcA	98%	514	200-1	512.9	469	3	19	16.88
PFUnA	95%	564	200-2	562.9	518.9	3	18	16.98
PFDoA	95%	614	200-2	612.8	568.8	3.5	20	17.48
PFHxS	>98%	399	300	399.1	na	2	25	15.46
PFOS	>98%	499	300	499.2	na	1	25	16.41

Annex 3. Levels of PFCs in Dutch fish samples

Table. Levels of PFCs in Dutch fish samples (μg/kg wet weight)

LIMS code 2004/		Species and origin	PFOA	PFNA	PFDcA	PFUnA	PFDoA	PFHxS	PFOS
		Shellfish and crustaceans							
0874		Mussels Eastern Scheldt	< 2	< 2	< 2	< 2	< 4	< 3	< 2
0876		Mussels, Wadden Sea (East)	< 2	< 2	< 2	< 2	< 4	< 4	4
1875		Mussels, Wadden Sea (West)	< 2	< 2	< 2	< 2	< 3	< 3	< 2
0798		Shrimps, North Sea (Rijnmond)	< 2	< 2	< 2	< 2	< 3	< 6	8
0871		Shrimps, Wadden Sea	< 2	< 2	< 2	< 2	< 3	< 6	30
1873		Oysters, Eastern Scheldt (Yerseke)	< 2	< 2	< 2	< 2	< 4	< 4	2
		Western Scheldt and freshwater							
1095		Flounder A liver, Wadden Sea	15	5	62	33	4	< 3	730
0854	1095	Flounder A, Western Scheldt	2	< 2	< 2	< 2	< 3	< 6	230
1097		Flounder B liver, Wadden Sea	53	6	50	52	< 3	27	540
1094		Flounder B, Wadden Sea	3	< 2	< 2	8	< 3	< 6	93
1850		Pike perch liver, Hollands Diep	< 2	< 2	27	15	< 3	< 3	270
0902		Pike perch, Hollands Diep	< 2	< 2	2	3	< 4	< 3	40
0904		Pike perch, IJssel Lake	2	< 2	< 2	< 2	< 4	< 4	150
0103	0882	Eel, Nieuwe Merwede	< 2	< 2	< 2	< 2	< 3	< 3	30
0144	1753	Eel, Ketel Lake	< 2	3	30	57	< 3	< 3	57
0091	0886	Eel, Haringvliet (West)	< 2	< 2	6	8	< 3	< 3	37
0136	0890	Eel, IJssel Lake (Medemblik)	< 2	< 2	< 2	< 2	< 3	< 3	52
0158	0884	Eel, Meuse (Keizersveer)	< 2	< 2	< 2	< 2	< 3	< 3	5.9
0066	0888	Eel, Rhine (Lobith)	< 2	< 2	4	5	< 3	< 3	44
		Marine fish							
1843		Herring liver, South NS	< 2	< 2	< 2	< 2	< 3	< 3	67
0860		Herring, South NS	< 1	< 1	< 2	< 1	< 3	< 5	8
1849		Plaice liver, South NS	< 2	< 2	2	< 2	< 3	< 3	35
0900		Plaice, South NS	< 2	< 2	< 2	< 2	< 4	< 4	20
1853		Sole liver, South NS	3	4	6	< 2	< 3	< 3	130
0850		Herring, English Channel	< 2	< 2	< 2	< 2	< 3	< 6	< 1
0858		Herring, North Sea (Central NS	< 2	< 2	< 2	< 2	< 3	< 6	51
0870		Mackerel	< 2	< 2	< 2	< 2	< 3	< 5	7
1099		Cod, Central NS	< 1	< 1	< 2	< 1	< 3	< 5	< 1
0894		Haddock, Central NS	< 1	< 2	< 2	< 1	< 3	< 5	5
0908		Sole, South NS	3	< 2	< 2	< 2	< 4	< 4	45
1384		Sole, Mouth Western Scheldt	< 2	< 2	< 2	< 2	< 4	< 4	10
1417		Sole, Hoek van Holland	2	< 2	< 3	< 2	< 4	< 4	13
1802		Sole, IJmuiden	2	< 2	< 2	< 2	< 4	< 4	12
1386		Sole, Egmond	< 2	< 2	< 2	< 2	< 4	< 4	< 2
1415		Sole, Texel	< 2	< 2	< 2	< 2	< 4	< 4	< 2
0862		Herring, Skagerak	< 2	< 2	< 2	< 2	< 3	< 6	23

LIMS code 2004/	Species and origin	PFOA	PFNA	PFDcA	PFUnA	PFDoA	PFHxS	PFOS
0864	Herring, Shetland Islands	< 2	< 2	< 2	< 2	< 3	< 6	23
0873	Mackerel, Shetland Islands	< 2	< 2	< 2	< 1	< 3	< 5	22
1800	Tuna, Mediterranean	< 2	< 2	< 2	< 2	< 4	< 3	< 2
	Farmed fish							
0912	Salmon, Farmed, Scotland	< 2	< 2	< 2	< 2	< 4	< 4	< 2
0910	Salmon, Farmed, Norway	1	< 2	< 2	< 2	< 4	< 4	< 2
1845	Eel liver, Farmed, Italy	< 2	< 2	< 2	< 2	< 3	< 3	14
0878	Eel, Farmed, Italy	< 2	< 2	< 2	< 2	< 3	< 6	< 1
1846	Eel liver, Farmed, Netherlands	< 2	< 2	< 2	< 2	< 3	< 3	23
0880	Eel, Farmed, Netherlands	< 2	< 2	< 2	< 2	< 3	< 5	10