UNIVERSITY OF LATVIA

FACULTY OF CHEMISTRY

DEVELOPMENT OF COMPLEX ANALYTICAL SCHEME FOR DETERMINATION OF PRIORITY PERSISTENT ORGANIC POLLUTANTS IN FISH USING HIGH RESOLUTION MASS SPECTROMETRY

DOCTORAL THESIS

PRIORITĀRO NOTURĪGO ORGANISKO PIESĀRŅOTĀJU KOMPLEKSĀS ANALĪZES SHĒMAS IZSTRĀDĀŠANA ZIVĪS AR AUGSTAS IZŠĶIRTSPĒJAS MASSPEKTROMETRIJAS METODI

PROMOCIJAS DARBS

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CONTENTS

ABBREVIATIONS

BFR	Brominated flame retardants
DL	Dioxin-like
EI	Electron ionization
ESI	Electrospray ionization
FWHM	Full width half maximum
GC	Gas chromatography
HBCD	Hexabromocyclododecane
HRMS	High resolution mass spectrometry
LB	Lowerbound
MS/MS	Tandem mass spectrometry
NDL	Non-dioxin-like
Orbitrap-MS	Orbitrap mass spectrometry
PBDD	Polybrominated dibenzo-*p*-dioxin
PBDE	Polybrominated diphenyl ether
PBDF	Polychlorinated dibenzofuran
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-*p*-dioxin
PCDF	Polychlorinated dibenzofuran
POP	Persistent organic pollutant
PXB	Mixed bromo/chloro- polyhalogenated biphenyl
PXDD	Mixed bromo/chloro-polyhalogenated dibenzo-*p*-dioxin
PXDF	Mixed bromo/chloro-polyhalogenated dibenzofuran
QqQ	Triple quadruprole
TCDD	2,3,7,8-tetrachloro dibenzo-*p*-dioxin
TEF	Toxic equivalency factor
TEQ	Toxic equivalent
TOF-MS	Time-of-flight mass spectrometry
UB	Upperbound
UHPLC	Ultra high performance liquid chromatography
WHO	World health organization

ABSTRACT

Development of complex analytical scheme for determination of priority persistent organic pollutants in fish using high resolution mass specrometry. Začs D., supervisors Dr. chem., prof. Vīksna A. and Dr. chem., asoc. prof. Bartkevičs V. Doctoral thesis in analytical chemistry, 129 pages, 23 figures, 5 tables, 190 literature references, 11 appendices. In English.

A new analytical scheme for efficient and reliable simultaneous analysis of seven groups of persistent organic pollutants (POPs) in fish from a single sample extraction procedure has been elaborated. Separation of components by gas chromatography (GC) or ultra high performance liquid chromatography (UHPLC) and detection by high resolution mass spectrometry (HRMS) was used to ensure selective determination of the analytes of interest at toxicologically significant levels. Intensive multi-stage column chromatography clean-up and fractionation procedure ensured the elimination of mass spectral interferants from the fraction containing poorly investigated toxicologically significant polyhalogenated (brominated and mixed bromo/chloro-substituted) dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs and PXDD/Fs). For the first time, the analytical capabilities of Orbitrap mass spectrometry were applied to the analysis of POPs. The application of $^{13}C_{12}$-labeled surrogates of the analyzed compounds allowed internal standardization and accurate measurement of selected contaminants. The developed procedure was robustly validated and used to measure the occurrence of selected POPs in Baltic wild salmon samples and eel samples collected from Latvian lakes. The universal presence of poorly studied PBDD/Fs and PXDD/Fs, as well as polyhalogenated mixed bromo/chloro-biphenyls (PXBs) was confirmed in the investigated samples. However, these compounds were found to be minor contaminants, compared to their chlorinated analogs.

PERSISTENT ORGANIC POLLUTANTS, FISH, HIGH RESOLUTION MASS SPECTROMETRY, BROMINATED FLAME RETARDANTS, POLYHALOGENATED AROMATIC COMPOUNDS, ORBITRAP

ANOTĀCIJA

Prioritāro noturīgo organisko piesārņotāju kompleksās analīzes shēmas izstrādāšana zivīs ar izšķirtspējas masspektrometrijas metodi. Začs D., zinātniskie vadītāji Dr. ķīm., asoc. prof. Bartkevičs V. un Dr. ķīm., asoc. prof. Vīksna A. Promocijas darbs, 129 lappuses, 23 attēli, 5 tabulas, 190 literatūras avoti, 11 pielikumi. Angļu valodā.

Tika izstādāta jauna analītiskā shēma efektīvai un drošai septiņu grupu noturīgo organisko piesārņotāju (NOP) vienlaicīgai analīzei zivīs no vienas ekstrakcijas procedūras. Komponentu atdalīšana ar gāzu hromatogrāfiju (GH) vai ultra augsti efektīvo šķidruma hromatogrāfiju (UAEŠH) un detektēšana ar augstas izšķirtspējas masspektrometriju (AIMS) nodrošina interesējošo analītu selektīvu noteikšanu toksikoloģiski nozīmīgākajos līmeņos. Intensīva multi-soļu kolonnas hromatogrāfijas attīrīšanas un frakcionēšanas procedūra nodrošina traucējošo vielu izslēgšanu no frakcijas, kas satur maz pētīto toksikoloģiski nozīmīgos polihalogenētos bromētos un jauktos bromo/hloro-aizvietoto dibenzo-*p*-dioksīnus un dibenzofurānus (PBDD/F un PXDD/F). Pirmo reizi tika izvērtētas Orbitrap masspektrometrijas analītiskās iespējas NOP analīzēs. $^{13}C_{12}$-iezīmēto analīzējamo savienojumu surogātu izmantošana ļauj lietot iekšējo standartizēšanu un izmeklējamo kontaminantu precīzu noteikšanu. Izstrādātā procedūra tika validēta un pielietota izmeklēto NOP sastopamības noteikšanai Baltijas jūras savvaļas laša paraugos un zušu paraugos, kas tiek ņemti no Latvijas ezeriem. Universāla PBDD/F un PXDD/F, kā arī polihalogenēto jaukto bromo/hloro-bifenīlu (PXB) klātbūtne tiek apstiprināta izmeklētajos paraugos, taču tika konstatēts, ka šo savienojumi koncentrācija salīdzinot ar hlorētiem analogiem ir neliela.

NOTURĪGIE ORGANISKIE PIESĀRŅOTĀJI, ZIVIS, AUGSTAS IZŠKIRTSPĒJAS MASSPEKTROMETRIJA, BROMĒTIE LIESMAS SLĀPĒTĀJI, POLIHALOGENĒTI AROMĀTISKIE SAVIENOJUMI, ORBITRAP

INTRODUCTION

The 20th century was associated with rapid technological progress, which led to increased welfare in many parts of the world. A large number of halogenated aromatic chemical substances were developed and put to use in numerous applications, such as PCBs for insulating fluids in electrical equipment, halogenated pesticides to protect crops from insects and fungi, and polybrominated diphenyl ethers (PBDEs) as flame retardants incorporated into polymers to reduce the risk of fires. The majority of these chemicals were designed to be stable with regard to long lasting use in their applications. However, the chemical stability is also a significant drawback of these compounds, causing them to be persistent in the environment. The stability of these substances resulted in increasing environmental presence, and they were soon shown to cause adverse effects in wildlife, in particular at high trophic levels. Due to the unfavorable properties, production of these POPs was gradually phased out and the unintentional release was decreased by optimization of the industrial processes and stricter legislative control. Although production of most POPs has been phased out for over 20 years, we are still facing considerable POP levels in the environment [1-3].

The practical relevance of the problem. According to the sources of POPs in the environment, they can be sub-divided as (i) chlorinated pesticides, (ii) industrial chemicals (PCBs, brominated flame retardants (BFRs) (e.g., PBDEs), and (iii) unintentionally produced compounds (such as PCDD/Fs) [4]. The majority of these compounds are toxic and due to the persistency and lipophilicity tend to bioaccumulate in food chains. Many of them are known xenobiotics of high toxicological significance with an aryl hydrocarbon (Ah) receptor mediated mechanism of toxicity. The most toxic representative is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD or TCDD). The toxicities of other individual halogenated POPs are expressed relative to 2,3,7,8-TCDD. This toxic equivalency factor (TEF) concept is used to determine the toxic equivalent concentration (TEQ) in a sample, providing the information on overall toxicological hazard caused by "dioxin-like" (DL) contaminants present in the sample. In this context, DL-contaminants refer to substances that have similarities to 2,3,7,8-TCDD in terms of structure, physicochemical properties, and in the toxic responses they elicit. It has been suggested that for including a compound in the TEF concept, it should: (i) share certain structural relationships to the PCDD/Fs; (ii) bind to the Ah receptor; (iii) elicit Ah receptor-mediated biochemical and toxic responses; (iv) be persistent and accumulate in the food chain [5,6].

Only 17 out of the 210 PCDD/Fs and 12 out of the 209 PCBs have been assigned TEFs and meet the stated criteria. Nevertheless, a wide range of other potential DL-contaminants and other

toxicants could be present in the samples at high levels and alter their contamination status. Many laboratories have developed effective analytical protocols for the isomer-specific analysis of toxicologically significant 2,3,7,8-chlorine substituted PCDD/Fs and DL-PCBs in environmental samples, and many other DL-compounds may be included in the scope of analysis in the future. The lack of analytical and toxicological data precludes the assignment of TEFs to many DL-compound candidates. The toxicological data [7-10] on PBDD/Fs, PXDD/F, and PXBs reveal a similar mode of Ah receptor mediated toxicity seen in PCDD/Fs, with similar or, in some cases, higher relative potencies [11]. Therefore, the elaboration of reliable complex methodologies that are capable of simultaneously covering different classes of priority POPs, including organobromines, such as BFRs, PBDD/Fs, PXDD/Fs, PXBs, and their chlorinated analogs, is of great significance in terms of providing a complete information on the contamination status of analyzed samples.

The aim of the work. Elaboration of an efficient and reliable analytical scheme for the simultaneous analysis of seven target groups of POPs (PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs, and BFRs (including PBDEs and hexabromocyclododecanes (HBCD)) in fish by a single sample extraction procedure using high resolution mass spectrometric (HRMS) detection.

The approach used. To achieve the aim of the work, several tasks were proposed:

i) Development of an efficient sample extraction and clean-up procedure, and selection of optimal instrumental conditions for the analysis of compounds of interest;

ii) Validation of the elaborated method for fish matrix;

iii) Application of the developed method for analysis of selected POP groups in fish from the Baltic region;

iv) Characterization of contamination of fish from the Baltic region with selected POPs, including the evaluation of the contribution of the less studied PBDD/Fs, PXDD/Fs, and PXBs to the overall contamination status.

Scientific novelty.

i) Improvement of analytical methodologies for the determination of brominated and mixed brominated/chlorinated POPs using HRMS;

ii) Implementation of a new type of HRMS (Orbitrap-HRMS) in the analysis of POPs;

iii) Comparative evaluation of Orbitrap-HRMS versus conventional MS detection approaches in the analysis of BFRs;

iv) Characterization of the contamination status of fish from the Baltic region with largely unexplored brominated and mixed brominated/chlorinated contaminants.

Practical application of the work. The elaborated analytical scheme could be applied to performing continuous European monitoring programs for the selected POPs in fish and fish products**.**

Scientific publications.

1. **Zacs, D.;** Bartkevics, V.; Frank, H. Levels and congener profiles of PCDD/Fs and dioxin-like PCBs in Baltic wild salmon (*Salmo salar*). *Toxicol. Environ. Chem.*[1] **2012**, *94*, 1502-1510.
2. **Zacs, D.;** Bartkevics, V.; Viksna, A. Content of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls in fish from Latvian lakes. *Chemosphere*[2] **2013**, *91*, 179-186.
3. **Zacs, D.;** Rjabova, J.; Bartkevics, V. Occurrence of Brominated Persistent Organic Pollutants (PBDD/DFs, PXDD/DFs, and PBDEs) in Baltic Wild Salmon (*Salmo salar*) and Correlation with PCDD/DFs and PCBs. *Environ. Sci. Technol.*[3] **2013**, *47*, 9478-9486.
4. **Zacs, D.;** Bartkevics, V. Levels of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls in food and feed samples collected in Latvia in 2009 – 2011. *Food Addit. Contam. B*[4] **2014**, ***7***, 186-201.
5. **Zacs, D.;** Rjabova, J.; Bartkevics, V. New perspectives on diastereoselective determination of hexabromocyclododecane traces in fish by ultra high performanceliquid chromatography-high resolution orbitrap mass spectrometry. *J. Chromatogr. A*[5] **2014**, *1330*, 30-39.
6. **Zacs, D.;** Rjabova, J.; Pugajeva, I.; Nakurte, I.; Viksna, A.; Bartkevics, V. Ultra high performance liquid chromatography – time-of-flight high resolution mass spectrometry in the analysis of hexabromocyclododecane diastereomers: Method development and comparative evaluation versus ultra high performance liquid chromatography coupled to Orbitrap high resolution mass spectrometry and triple quadrupole tandem mass spectrometry. *J. Chromatogr. A*[5] **2014**, *1366*. 73-83.
7. **Zacs, D.,** Rjabova, J., Viksna, A., Bartkevics, V. Method development for the simultaneous determination of polybrominated, polychlorinated, mixed polybrominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans, polychlorinated biphenyls and polybrominated diphenyl ethers in fish. *Chemosphere*[2] **2015**, *118*, 72-80.

[1] *Peer reviewed journal, imprint of Taylor & Francis (IF=0.723 (2013)), ISSN: 0277-2248*
[2] *Peer reviewed journal, imprint of Elsevier (IF=3.499 (2013)), ISSN: 0045-6535*
[3] *Peer reviewed journal, imprint of American Chemical Society (IF=5.481 (2013)), ISSN: 0013-936X*
[4] *Peer reviewed journal, imprint of Taylor & Francis (IF=0.914 (2013)), ISSN: 1939-3210*
[5] *Peer reviewed journal, imprint of Elsevier (IF=4.258 (2013)), ISSN: 0021-9673*

8. **Zacs, D.;** Rjabova, J.; Fernandes, A.; Evans, D.; Bartkevics, V. Characterization of brominated (PBDD/DFs and PBDEs), chlorinated (PCDD/DFs and PCBs) and mixed brominated/chlorinated (PXDD/DFs and PXBs) persistent organic pollutants in European eels (*Anguilla anguilla*) from Latvian lakes. *Environ. Pollut.*[6] (submitted 12.04.15)

List of conferences.

1. Dioxin 2014, Madrid, Spain, 2014. **Zacs, D.;** Rjabova, J.; Viksna, A.; Bartkevics, V. Analytical capabilities of ultra performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry in analysis of hexabromocyclododecane diastereomers. *Organohalogen Compd,* **2014**, *76*, 33-36. (in book of abstracts/oral presentation/availability at " Organohalogen database " (www.dioxin20xx.org)).
2. 72nd University of Latvia conference, Riga, Latvia, 2014. **Zacs, D.;** Rjabova, J.; Nakurte, I.; Viksna, A.; Bartkevičs, V. Nolidošanas laika un Orbitrap tipa masspektrometrijas izmantošana heksabromociklododekāna noteikšanai zivīs (oral presentation).
3. 6th International Symposium on Recent Advances in Food Analysis (RAFA 2013), Prague, Chech Republic, 2013. **Zacs, D.;** Rjabova, J.; Viksna, A.; Bartkevics, V. Elaboration of the GC-HRMS method for simultaneous determination of polybrominated, polychlorinated, mixed polybrominated/chlorinated dibenzo-p-dioxins and dibenzofurans, polychlorinated biphenyls and polybrominated diphenyl ethers in fish samples (in book of abstracts/poster presentation).
4. 4th International Conference on Laboratory Diagnostics in Veterinary Medicine, Food and Environmental Safety, Riga, Latvija, 2013. **Zacs, D.;** Rjabova, J.; Bartkevics, V. Levels and congener profiles of polybrominated diphenyl ethers in Baltic wild salmon (*Salmo salar*) (in book of abstracts/oral presentation).
5. Rīgas Tehniskās universitātes 54. Studentu zinātniskā un tehniskā konference, Riga, Latvija, 2013. A. Kokina, J. Rjabova, D. Začs. Polibromēto dibenzodioksīnu un dibenzofurānu noteikšana pārtikas produktos (in book of abstracts).
6. Nutrition and Health 2012, Riga, Latvija, 2012. **Zacs, D.;** Bartkevics, V. Levels of polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls in food and feed samples collected in Latvia in 2009-2011 (oral presentation).

[6] *Peer reviewed journal, imprint of Elsevier (IF=3.902 (2013)), ISSN: 0269-7491*

1. LITERATURE REVIEW

1.1. Compound classes included in the scope of the study

1.1.1. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs)

The impact of PCDD/Fs on the environment has been a source of great concern since the beginning of the 1970s. PCDD/Fs commonly referred to collectively as "dioxins", are two classes of "quasi-planar" tricyclic aromatic ethers representing 210 different compounds (congeners) in total, including 75 PCDDs and 135 PCDFs. The general structures of these classes of compounds are given in Figure 1.1.

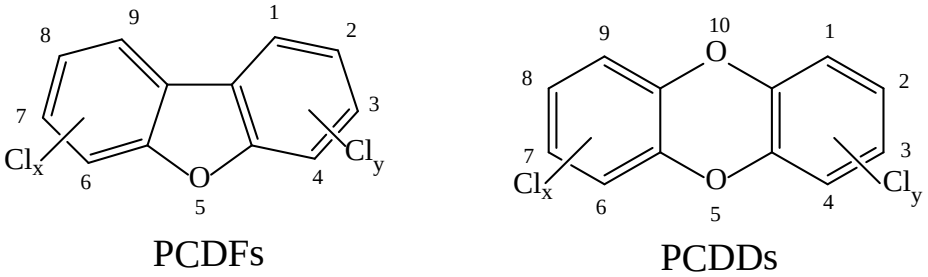


Fig. 1.1. **The general molecular structure of PCDD/Fs**

PCDD/Fs have never been commercially produced, however they are present as trace contaminants in various industrial chemicals and technical products (chlorophenols, PCB formulations, "*Agent Orange*", etc.), or are formed unintentionally in various combustion processes such as waste incineration, in the production of metals, in the bleaching of pulp with chlorine gas [2,12,13]. The improvements and optimization of the treatment of industrial emissions resulted in reduced release of PCDD/Fs into the environment, and the relative significance of sources such as open burning of waste or secondary sources such as the release of pollutants from "hot spots" has increased [2,13,14]. A number of accidents, such as the explosion in Seveso, Italy and the Yusho accident in Japan when PCDD/Fs were released into the environment during the late 1960s and 1970s increased public awareness and concern about these pollutants.

PCDD/Fs have similar chemical and physical properties, and their toxicity is highly dependent on the chlorination degree and the position of the chlorine substituents. Congeners which are fully chlorinated at the positions 2, 3, 7, and 8 (Figure 1.1) are the most toxic ones, including 7 PCDDs and 10 PCDFs. The most toxicologically significant congener 2,3,7,8-TCDD is one of the most toxic chemicals ever described [15]. The extreme toxicity of 2,3,7,8-TCDD was first showed in acute toxicological experiments with guinea pigs where low lethal doses (LD_{50}) of 0.6 – 2.5 μg kg^{-1}

bodyweight (orally ingested) were determined for this congener [16]. The toxic action of the 2,3,7,8-substituted congeners is mediated through binding to the Ah-receptor, which is present in most vertebrate tissues [6] and results in multisite carcinogenicity and other adverse effects. The difference of affinity of individual PCDD/F congeners to the Ah-receptor resulted in their different toxicities. In order to facilitate risk assessments and regulatory control, the concept of TEFs was proposed [5,6]. According to this approach, the toxicity of different congeners relates to the toxicity of the most toxicologically significant 2,3,7,8-TCDD which has a TEF 1, while other representatives have values ≤1. A number of TEF-systems have been developed (e.g., the Nordic-TEF and the I-TEF), however the World Health Organization TEF (WHO-TEF) is the internationally used system today and the latest re-evaluation of the TEFs in 2005 provides TEF values ranged from 1 for 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD to 0.0003 for OCDD and OCDF [6]. In order to express the toxicity of the sample due to the presence of PCDD/Fs, the concentration of each individual congener is multiplied by its respective TEF value, and all products are added, to give a single 2,3,7,8-TCDD equivalent (toxic equivalent or TEQ). This approach can be described mathematically by the equation 1.1.

$$TEQ = \sum_{n=1}^{k} C_n \times TEF_n \quad (1.1)$$

where: *TEQ* – toxic equivalent;

C_n – concentration of the individual congener in the sample;

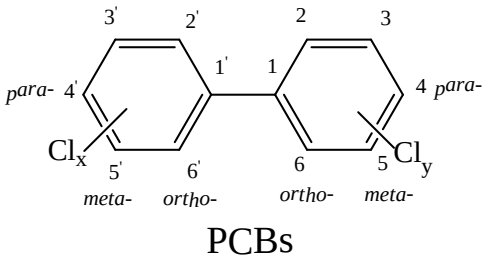
TEF_n – established TEF for the individual congener.

1.1.2. Polychlorinated biphenyls (PCBs)

PCBs are a class of organic compounds characterized by two benzene rings linked by a C-C bond, which were first discovered in the late 1800s and first commercially synthesized in 1929 [17]. PCBs comprise a group of 209 structurally distinct congeners with 10 PCB homologue groups (mono- to deca-CBs) with different numbers of isomers. The general molecular structure and nomenclature of the PCBs is shown in Figure 1.2. Today, a numbering system (PCB 1 to PCB 209) developed in 1980 [18] is used for the identification of individual PCB congeners.

Crude PCB formulations have been widely used in transformers, capacitors, heat transfer fluids, e.g., in the production of asphalt, sealants in the construction of buildings, as additive to paint and in carbonless copy paper. Properties like thermal stability, high resistance to oxidation and reduction, electrical conductance, low flammability, resistance to acids and alkali were advantageous in all of these applications. The total accumulated volume of crude PCBs produced in the world has been estimated to be more than 1.3 million tons [3], with the peak annual production

around 1970. PCBs are lipophilic and several of those are stable enough to be strongly bioaccumulative in wildlife and humans, and they are ubiquitously distributed almost in every matrix including air, water, soil, and sediments [19].



PCBs

Fig. 1.2. **The general molecular structure of PCBs**

The degree of lipophilicity (octanol/water partition coefficient (log K_{ow}) increases and solubility in water decreases with higher degree of chlorination (PCB 1, log K_{ow} = 4.46; PCB 209, log K_{ow} = 8.18) [20]. PCBs can be divided into sub-groups depending on the number of chlorine atoms at the *ortho*- positions (2, 2´, 6, 6´ positions) of the biphenyl ring system. These sub-groups (non-, mono-, di-, tri-, and tetra- *ortho* PCBs) include 20, 48, 72, 48 and 21 congeners, respectively. One of the most important conformational characteristic of PCB molecules is their ability to rotate around the phenyl-phenyl (1, 1') bond, attaining a planar configuration (co-planar PCBs), similar to the PCDD/Fs. Some of these non- and mono-*ortho* substituted PCBs bind to the Ah-receptor and are therefore referred to as the dioxin-like PCBs (DL-PCBs), all having chlorine atoms in the *meta*- and *para*- positions. Likewise, 17 PCDD/Fs and twelve DL-PCBs were included into the TEF concept with assigned TEF values [5,6]. Four of them are congeners that lack chlorine atoms at the *ortho*-position, the non-*ortho* PCBs (77, 81, 126, 169), and eight have one chlorine substituent at the *ortho*-position, the mono-*ortho* PCBs (105, 114, 118, 123, 156, 157, 167, 189). The non- and mono-*ortho* PCBs overall have lower TEFs than the PCDD/Fs (0.1 – 0.00003) [6], but since the concentration of PCBs in environmental samples often far exceeds the concentration of PCDD/Fs, their contribution to the total TEQ can be significant [21].

1.1.3. Polybrominated diphenyl ethers (PBDEs)

PBDEs, also known as polybrominated diphenyl oxides, are flame retardants used as additives in a variety of plastics, textiles, surface coatings, foams, and man-made fibers. Like PCBs, the theoretical number of possible congeners is 209, and the same numbering system as proposed for PCBs is used [18]. The general molecular structure and nomenclature of the PBDEs is shown in

Figure 1.3.

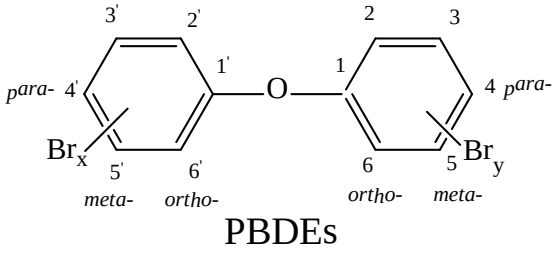
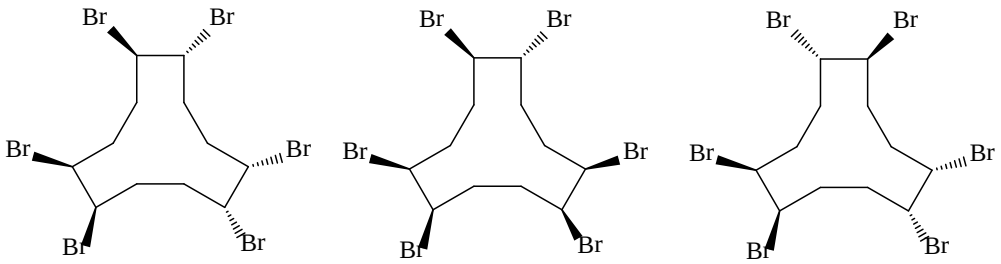


Fig. 1.3. **The general molecular structure of PBDEs**

There are three major technical products of PBDEs: pentabromodiphenyl ether (Penta-mix), octabromodiphenyl ether (Octa-mix), and decabromodiphenyl ether (Deca-mix), which are mixtures of congeners with differing degree of bromination, similar to the commercial crude products of PCBs. Because of the chemical properties of the oxygen directing the bromine to *para*- and *ortho*-positions, and the steric hindrance of the substituted bromines, only a limited number of congeners are present in commercial mixtures [22]. The major congeners in the Penta-mix are tetra- and penta-substituted BDEs, Octa-mix is composed mainly of hepta- and octa-BDEs, and in the Deca-mix formulation the main component is decabromodiphenyl ether or PBDE 209. Towards the end of the 20th century, great concerns over PBDEs grew due to observations of bioaccumulation and toxic properties, and of rapidly rising concentrations in environmental and human tissue samples world-wide [23,24]. PBDEs were one of the most utilized group of BFRs until the mid-2000s when strict bans were imposed on the production of PBDE formulations due to their persistency and bioaccumulation properties [25]. Nevertheless, significant quantities of PBDEs have been released into the environment, and as a result, these compounds have became ubiquitous and are found in various matrices (e.g., water, sediments, biota, and humans) [26]. Recent data show that PBDE levels tend to decrease in biota in Europe [27], while the concentrations of tetra- to hepta-BDEs in the Arctic region are increasing or possibly stagnating during the last 2 – 5 years, and BDE 209 concentrations are still increasing in the Arctic air [28]. A growing number of published reports show that PBDEs can elicit toxicity and biological effects via several different mechanisms [29]. The developmental neurotoxicity of these substances currently is little understood [30]. It is believed that PBDEs present a risk to the developing brain [31]. The other possible source of concern attributed to the use of PBDEs is that these technical mixtures may contain highly toxic impurities, such as polybrominated dibenzo-*p*-dioxins and furans (PBDD/Fs), which have similar toxicities and long-range transport properties as their chlorinated analogues [7]. It was found that combustion processes in the presence of PBDEs can also lead to the production of PBDD/Fs [32].

1.1.4. Hexabromocyclododecane (HBCD)

Hexabromocyclododecane (HBCD, Figure 1.4.) is an aliphatic brominated flame retardant. HBCD is seen by manufacturers as a possible replacement to PBDEs. HBCD is an off-white, crystalline, free-flowing solid. It is soluble in organic solvents and has a melting range of 185 – 195°C. HBCD is thermally quite labile, breaking down through dehydrobromination reactions between 240 and 270°C, forming a myriad of products, typically via a tribromocyclododecatriene intermediate [26]. HBCD was introduced to the market in the 1960s. HBCD is an additive flame retardant that is incorporated in synthetic materials and is one of the most widely used compounds among the BFRs with annual usage in Europe of 6000 tons [33]. The commercially available crude HBCD is a mixture of 1,2,5,6,9,10-hexabromocyclododecane isomers, consisting mainly of α-, β-, and γ-HBCD diastereomers [26]. The physical and chemical properties of HBCDs are similar to those of POPs, and HBCD was classified in 2008 by the European Commission (EC) as persistent, bioaccumulative, and toxic compound [34]. Discarding of HBCD-containing materials results in the release of this chemical to the environment, and HBCD has thus become a ubiquitous contaminant found in water, soil, sediments, fish, birds, mammals, and people [26,35].



α-HBCD β-HBCD γ-HBCD

Fig. 1.4. **The structure of HBCD diastereomers**

HBCD is chemically closely related to the pesticide hexachlorocyclohexane and, as such, it is persistent and bioaccumulative [31]. The environmental transport and fate of HBCD is uncertain. It has been hypothesized that HBCD is unlikely to leach from most polymeric matrices and will only contaminate discrete areas surrounding point sources. Recent evidence has suggested that combustion of HBCD in the presence of aluminum can trigger polycondensation reactions during HBCD decomposition, leading to the formation of high molecular weight, highly toxic polyaromatic compounds such as PAHs and brominated polycyclic compounds [36]. It is worth noting that many modern automotive parts are manufactured of aluminum, with interiors being heavily flame retarded with BFRs, such as HBCD. It has been stated that HBCD may be a potential mutagen and liver toxicant [31]. HBCD caused statistically significant increases in intragenic

recombination of mammalian cells indicating that it may induce cancer via a nonmutagenic mechanism, similarly to other environmental contaminants, such as DDT and PCBs [37].

1.1.5. Polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs).

The general structures of PBDD/Fs are shown in Figure 1.5. Similarly to PCDD/Fs, they are co-planar compounds with two aromatic rings coupled through one or two oxygen bridges. There are eight possible positions for bromine substitution, which gives 75 different PBDD congeners and 135 different PBDF congeners, taking all bromination degrees into account.

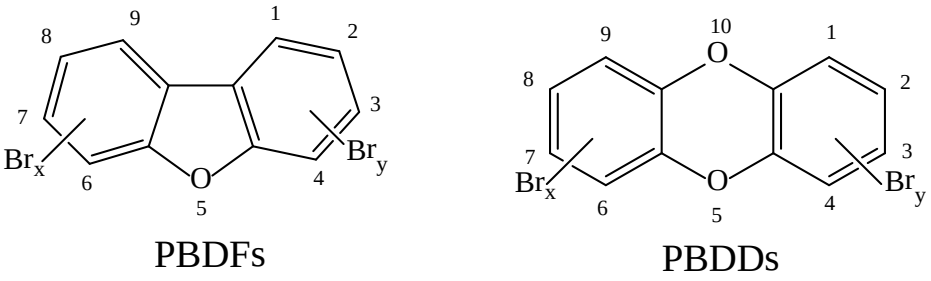


Fig. 1.5. **The general molecular structure of PBDD/Fs**

The main sources of PBDD/Fs are combustion processes and the substantial use of BFRs, where these contaminants have been found to be present as by-products [38] or formed through photolytic [39,40] and thermal degradation of BFRs [41-43]. Chemical precursors, such as brominated phenols [44] or PBDEs form PBDD/Fs during reactions at 250 – 500°C temperatures on catalytically active surfaces, or also spontaneously at the relevant temperatures (Figure 1.6.). PBDD/Fs levels were found to correlate positively with PBDE levels in the atmosphere, revealing the pollution sources associated with using of these crude formulations [45]. Moreover, the detection of elevated PBDD/Fs concentrations, in relation to PCDD/Fs levels in industrialized areas near the factories of electric machinery and equipment [46,47], supports the connection between the use of BFRs and the PBDD/F emissions to the environment.

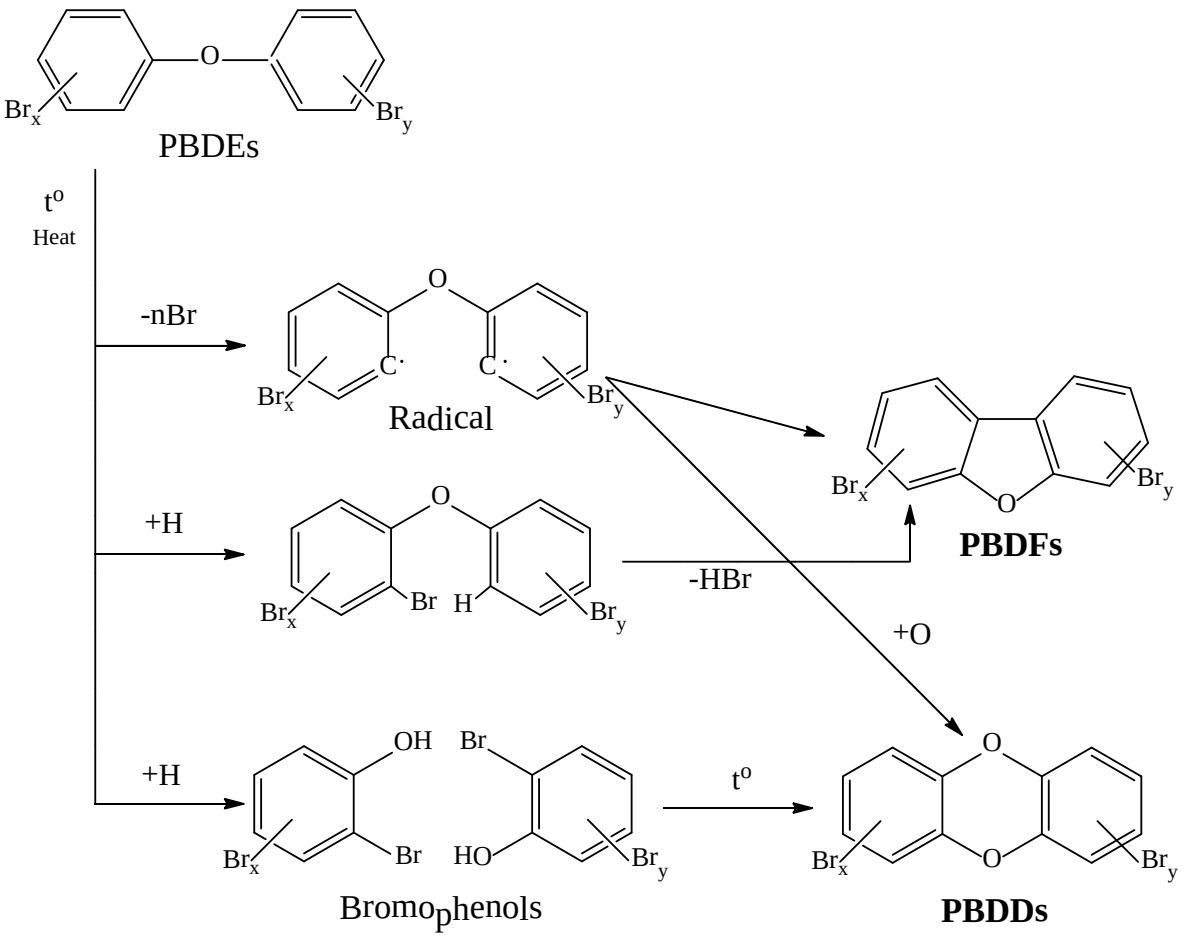


Fig. 1.6. **Possible mechanisms for the formation of PBDD/Fs from PBDEs [26]**

The concentrations of PBDD/Fs in environmental and human tissue samples have begun to reach detectable levels and have been determined in various matrices, including ambient air [45,46,48] at electronic waste dismantling areas and in plastics [49], fuel gases [50,51], fly ash [52,53], sediments [54,55], food samples [56], shellfish [57,58] and fish [59,60], adipose tissue [61], and human breast milk [48].

The number of studies on the formation of PBDD/Fs during incineration of bromine containing waste shows that the formation is significant, although not as high as PCDD/F formation [51,62]. The majority of these studies were small scale experiments or on-line measurements during the incineration process, and few authors have investigated the PBDD/F levels that were emitted from the incineration facilities. The lower brominated dioxins were found to be produced by algae and cyanobacteria due to eutrophication, and assimilated in blue mussels in the Baltic Sea [63]. Nevertheless, these were mono- to tri- substituted PBDDs and would not contribute significantly to the toxic burden of PBDD/Fs.

PBDD/Fs exhibit somewhat different properties than the PCDD/Fs, due to the larger size of the bromine atom, as well as the weaker C-Br bond, compared to the C-Cl bond. PBDD/Fs have higher molecular weights, higher melting points, lower water solubilities and vapour pressures than PCDD/Fs. PBDD/Fs are believed to bioaccumulate similarly to their chlorinated homologues, but appear to be less persistent in the environment due to the higher sensitivity towards UV degradation. This is suggested to result from the fact that bromide is a better leaving group than chloride. Nonetheless, the toxic properties of these brominated contaminants are not completely known, some studies have shown that toxicity of 2,3,7,8-TBDD is even higher in comparison to 2,3,7,8-TCDD, and PBDD/Fs seem to be more resistant against mammalian metabolism, compared to chlorinated analogs [8]. Moreover, PBDD/Fs show the same DL toxicity as their chlorinated analogues in both human and mammalian cell lines [9,64].

Despite the structural similarity to PCDD/Fs, the analysis of PBDD/Fs is more difficult due to the differences in physico-chemical properties. The unavailability of isotopically labeled standards and analytical interference from degradation products of PBDEs (i.e., brominated furans) also obstruct the analytical determination.

1.1.6. Mixed bromo/chloro polyhalogenated dibenzo-*p*-dioxins and dibenzofurans (PXDD/Fs) and mixed bromo/chloro polyhalogenated biphenyls (PXBs).

Relatively little is known about the environmental and toxicological significance of PXDD/Fs and PXBs. While chlorinated analogues have been studied for several decades, mixed bromo/chloro analogues are less known. However, they have also been identified as potentially toxic, environmental pollutants. Little research has been carried out on the toxicity of PXDD/Fs and PXBs, and limited studies have suggested an equivalent or even higher toxicity and biological activity of these compounds compared to chlorinated analogues [9,11]. By analogy to PCDD/Fs and PBDD/Fs, combustion and incineration in the presence of halogens are also the typical processes for the formation of PXDD/Fs [65]. A wide range of electrical equipment contains both chlorinated substances like polyvinyl chloride or polyvinylidene chloride and brominated flame retardants, which serve as precursors for PXDD/F formation. Because these organic pollutants are highly lipophilic and persistent, they tend to migrate and accumulate in the food chain.

In contrast to PBDD/Fs and PCDD/Fs, and PCBs for which 210 and 209 congeners are theoretically possible, PXDD/Fs and PXBs (the respective structures are shown on Figure 1.7.) are far larger groups with 4600 and 9179 possible congeners, including about one thousand of potentially significant 2,3,7,8-substituted PXDD/F congeners.

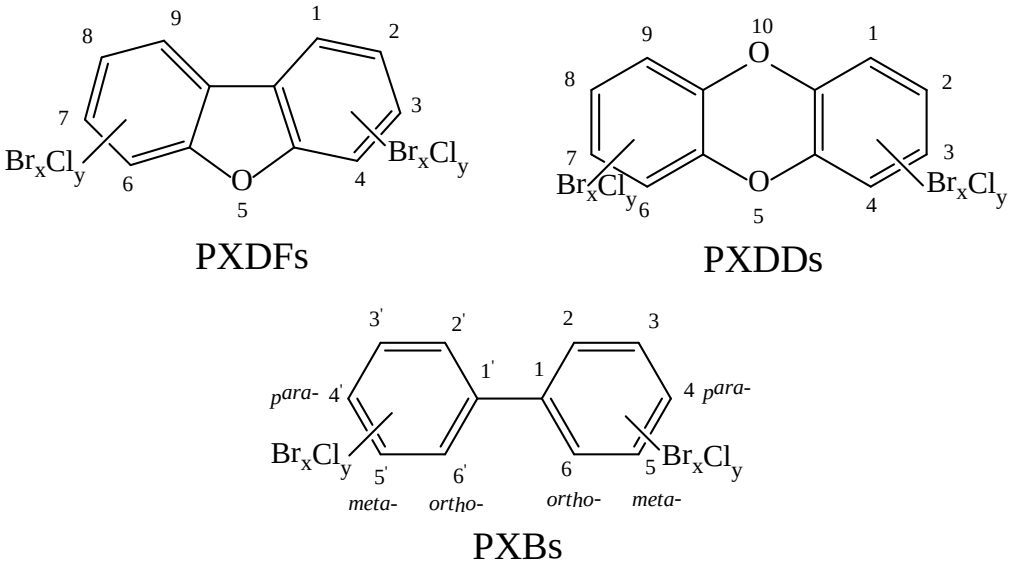


Fig. 1.7. **The general molecular structure of PXDD/Fs and PXBs**

The limited toxicological data on these compounds reveals a similar mode of Ah receptor mediated toxicity seen in PCDD/Fs, with similar or in some cases higher relative potencies [9]. This is confirmed by more recent studies [10,66], and in particular [11], that characterizes a number of PXDD/Fs and PXBs, and the proposal to introduce the TEF concept (as used for PCDD/Fs) to define the toxicity of PBDD/Fs [67].

The literature on these contaminants is scarce. A limited amount of the available data confirms the occurrence of these compounds in incineration process emissions [68-72], biota and food products [60,66,73,74]. The majority of this data deals with unspeciated homologue groups, relating to the different substitution pattern of chlorine and bromine on the parent dibenzo-*p*-dioxin, dibenzofuran or biphenyl molecule, and reflects the analytical difficulties in congener-specific determination due to the chromatographic and spectrometric interferences caused by the presence of other POPs, which in some cases could be isobaric compounds and are present in the samples at much higher levels (e.g., PCBs and PBDEs).

1.2. The analytical approaches for determination of POPs

The analytical methodologies for POP analyses are especially complex due to the diversity of the mixtures of possible congeners of interest (especially if mixed bromo/chloro-substituted POPs are included in the scope of the analysis) and the low detection limits required (from ppb to sub-ppq). In addition, time consuming sample preparation and clean-up steps are needed because of the presence of matrix components and a large number of interfering compounds. Overcoming these analytical problems has only been possible with the application of rigorous clean-up protocols and

the use of highly selective and sensitive approaches, such as capillary GC, HPLC or UHPLC coupled to low resolution MS (LRMS) or HRMS. A principal analytical protocol to determine POPs is shown in Figure 1.8. The clean-up steps allow a suitable removal of the bulk matrix and some interfering compounds; GC or LC methods provide an appropriate separation between the different congeners or groups; and, finally, MS affords a sensitive and selective detection. In order to provide reliable and accurate quantification, a stable isotope dilution technique based on the use of internal standardization with ^{13}C- or ^{2}H-isotopically labeled surrogates is commonly used.

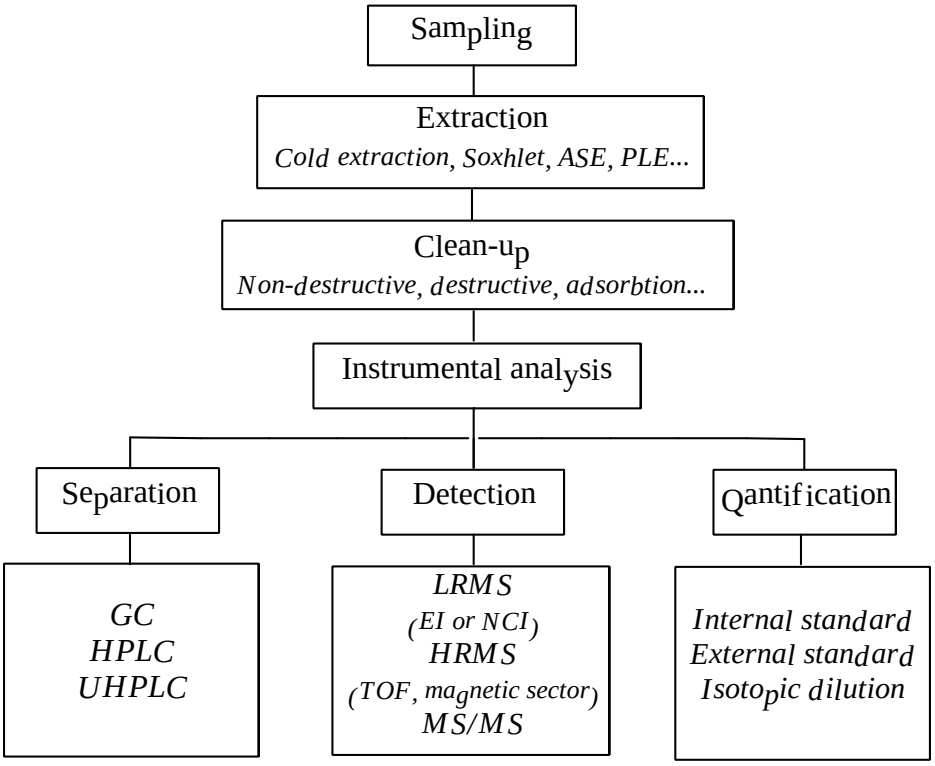


Fig. 1.8. **The principal analytical scheme used for the quantification of POPs**

1.2.1. Extraction

For trace analysis of DL-contaminants, Soxhlet extraction is accepted as one of the most efficient approaches. However, the main drawback of this technique is the time required and the considerable consumption of solvents used for extraction procedure, which necessitates the evaporation of a large amount of usually hazardous liquids (such as dichloromethane) before the subsequent clean-up. Recent developments in extraction techniques with reduced solvent volumes, shorter times, and high levels of automation provide further advancements in the analysis of POPs. Microwave-assisted extraction (MAE) [75,76] and pressurized or accelerated liquid extraction (PLE or ASE) [77,78] have been applied by several laboratories in the extraction of priority POPs, such as of PCDD/Fs and PCBs. These advances resulted in a considerable shortening of extraction time

(to 1 hour) and low solvent consumption (approx. 30 mL) by the aforementioned techniques, in comparison with a conventional Soxhlet extraction. Although several parameters influence the extraction efficiency (e.g., extraction solvents, extraction time, pressure and temperature), these parameters should be optimized for complete extraction of the pollutants from matrix [79,80].

1.2.2. Clean-up procedures

The extract clean-up procedures should remove the potentially large quantities (up to a gram or even more) of organic material selectively, while retaining as much of the desired analytes as possible. This is a quite challenging task, especially for the DL-compounds, where the concentration factors of 10^6 or 10^8 are typically needed to meet the very low detection limits required for these compounds (in most cases sub-ppt (picogram per gram) for soils/sediments/biota and sub-ppq (picogram per liter) for aqueous samples). The basic principles and procedures behind sample clean-up in POP analyses have not changed in many years and are usually based on solid-liquid destructive or non-destructive adsorption chromatography in open columns utilizing the combination of different sorbents, such as silica, Florisil, alumina, and various types of activated carbon [81,82]. Size exclusion chromatography (mostly gel permeation chromatography (GPC)) is also a frequently used method for the removal of the bulk of high molecular weight compounds (e.g., lipids) from the sample extracts. The most commonly applied sorbents are polystyrene – divinylbenzene based copolymeric materials (e.g., Bio-beads SX-3) [83-85]. The presence of isobaric compounds that could interfere with instrumental analysis is another problem in the analyses of DL-compounds, requiring intensive extract fractionation in order to achieve selective separation of the POP groups in several fractions [74,86,87]. Despite the fact that the typically used manual clean-up approaches are time- and labor-consuming, these are the most common routine procedures for the determination of POPs. Due to the advent of automated sample-handling techniques, automated clean-up systems are more preferable and recently have been developed and applied for routine purposes [88]. In most of the cases, sample extracts must be concentrated prior to clean-up, therefore a variety of sample concentration procedures, including nitrogen or gas blow down, rotary evaporation, Kuderna-Danish concentration, or automated evaporative concentration systems have been proposed [81,82].

1.2.3. Instrumental analysis

The PCDD/F, PCB, and PBDE analysis involves the detection of multiple congeners at the levels from ppb to sub-ppq, for which isotope dilution techniques using GC-HRMS are the current methods of choice (US EPA Method 1613, US EPA Method 1668, US EPA Method 1614) [89-91].

Conventionally, HRMS was used in the electron ionization (EI) mode, with the electron energy usually between 36 – 38 eV at a mass resolving power of 10 000. However, the analysis of mixed bromo/chloro-substituted compounds requires higher MS resolution due to the potential presence of isobaric compounds in the sample extracts [74,87]. Typically, MS systems are operated in selective ion recording mode (SIR) to achieve the best possible selectivity and sensitivity, while additionally applying the isotope dilution approach for reliable and accurate quantification.

Regarding the chlorinated POPs, alternative techniques are electron capture detector (ECD) (for mono-*ortho* PCBs, but with possible selectivity problems due to the co-elution risks) [92], electron capture negative ionization (ECNI) coupled with LRMS (for non-*ortho* CBs) [93, 94], ion-trap tandem mass spectrometry (IT-MS/MS) and two dimensional GC (GC×GC) coupled with time-of-flight MS (TOF-MS) [95]. The optimization of collision characteristics in IT-MS/MS provided an improvement of the instrumental limit of detection (i-LOD) for 2,3,7,8-TCDD up to 50 fg, which satisfies the sensitivity requirements for screening methods [96]. The GC×GC-μECD and GC-IT-MS/MS procedures were subjected to an extensive validation against the GC-HRMS technique [97], and it was found that for real samples the accuracy, precision, and limit of quantification (LOQ) were in the same range (fish oil, fish) or slightly lower (milk, pork), compared to the GC-HRMS results [95,97,98], confirming the potential of these alternative techniques. Nevertheless, it should be pointed out that despite the lower investments for GC×GC-μECD and GC-IT-MS/MS equipment, the samples may require longer preparation times due to additional clean-up steps, the instrumental part requires more frequent maintenance of the MS systems (GC-IT-MS/MS) or more data treatment time to evaluate the complex chromatograms (GC×GC-μECD) [95]. Moreover, the space-charging effect in using of IT-MS/MS is a considerable disadvantage that affects the reproducibility.

For BFRs such as PBDEs or HBCD, the most common detection techniques are EI-HRMS and ECNI-LRMS [99,100], but ECD or μECD could be used as well [101]. ECNI-MS provides a good sensitivity and selectivity for the detection of bromine containing compounds. Despite the poor specificity, the most commonly monitored ions are *m/z* 79 and 81, representing the two stable bromine isotopes [99]. Such a choice is explained by the low yields of molecular ions during the ionization process, resulting in insufficient sensitivity. For deca-brominated BDE (PBDE 209), the sufficiently strong *m/z* 485 and *m/z* 487 peaks can be monitored as well, and HBCD *m/z* 561 can be used as a qualifier ion. In EI-MS, the most commonly monitored ions are $[M\text{-}Br_2]^+$ and $[M]^+$. They provide a good selectivity, but a lower sensitivity, especially for the higher brominated PBDE congeners [100]. Moreover, selectivity problems could be caused by the presence of PCBs in the samples, which could interfere with the GC-MS analysis of PBDEs [102]. EI-MS techniques, such

as GC-LRMS, GC-HRMS, or GC-MS/MS enable the use of isotopically labeled standards, which allows applying internal standardization – the most powerful approach for a reliable and accurate quantification. HRMS provides a good sensitivity and selectivity, but at higher instrumentation investment and maintenance costs. Alternatively, TOF-MS may be used for the detection of PBDEs at a sensitivity comparable to other MS techniques [103,104], but the restricted linear range of the instrument limits the use of this type of MS [104].

One of the most critical issues in the analysis of BFRs, such as PBDEs and HBCD, is the thermal lability of these compounds. In contrast to GC, the application of LC eliminates the problem of thermal lability or interconversion of BFRs during the analysis, providing an effective chromatographic resolution of components. The selective detection by mass spectrometry positions the LC-MS combination as a method of choice for the analysis of HBCDs. Several studies reported the application of LC-MS based methods in diastereomer-specific analysis of HBCDs in various types of matrices, including sewage sludge [105], sediments [106], indoor and airborne dust [107,108], food products [109], and fish [110-113]. The most frequently used detection technique for diastereomer-specific analysis of HBCD is LC-MS/MS, which utilizes triple quadrupole or IT-MS analyzers operated in the selected reaction monitoring (SRM) mode [114]. Monitoring of the specific transition $[M-H]^-$ (*m/z* 639 and 641) → $[Br]^-$ (*m/z* 79 and 81) provides a selective analysis of trace levels of HBCDs. Typically, a sufficient ionization degree could be obtained by the means of using electrospray ionization (ESI) operated in negative mode.

Fewer studies have reported the analysis of PBDEs using LC-MS techniques, which may be attributed to the availability of well-established, sensitive, and efficient analytical methodologies for the determination of these compounds using GC-MS based techniques. However, LC-MS analysis has a major advantage for the analysis of highly brominated PBDEs, which may undergo thermal degradation and/or extensive fragmentation during a GC-MS analysis [115]. The ESI mode in the LC-MS analysis of PBDEs is limited due to poor ionization in this source [116]. Recently, an isotope-dilution method for the determination of 14 major tetra- to deca-BDEs using LC coupled to MS/MS system equipped with atmospheric pressure photoionization (LC-APPI-MS/MS) under the conditions of reversed-phase LC has been reported [117]. The method was applied the soft APPI technique, to obtain stable pseudomolecular ions $[M-Br+O]^-$ and $[M-2Br+O]^-$ in Q1, with further detection of $[Br]^-$ (*m/z* 79 and *m/z* 81) ions for selective detection in Q3. LC-APPI-MS/MS based methods were successfully applied for the analysis of PBDEs in dust [118], air [119], fish [110], and human breast milk [120]. The performance of atmospheric pressure chemical ionization (APCI) has been also tested in the analysis of PBDEs in wastewater samples under the conditions of LC-MS/MS [121], and it was found that the LC-APCI-MS/MS technique could provide 2 – 8 times

better sensitivity than APPI [122].

2. EXPERIMENTAL PART

2.1. General flow diagram of analytical methodology

A multi-group analytical procedure was developed and used for the analysis of fish samples from the Baltic region, including Baltic wild salmon (*Salmo Salar*) and eels (*Anguilla Anguilla*) collected from Latvian lakes, as well as for other freshwater and saltwater fish species. This analytical procedure allowed to perform the analysis of seven groups of POPs, including the little studied brominated and mixed bromo/chloro-substituted compounds with further possibility to expand the list of analyzed contaminants (e.g., including pesticides and BFRs other than PBDEs and HBCDs). The method development procedures are described in detail in the Papers 5 – 7, while application of the elaborated methods to real samples is presented in the Papers 1 – 3 and 8. To evaluate the possibility of extending the number of analyzed matrices, the elements of the method have been applied to the analysis of several samples other than fish matrices, including fish products, food samples, and animal feed samples. The study was carried out in the frame of the official monitoring programme with regard to PCDD/Fs and PCBs, for which MLs and ALs have been established by the European Commission and the results are presented in Paper 4.

An analytical flow diagram showing the principal clean-up and analysis steps for the various POP groups is showed in Figure 2.1. The analytical methodology was designed to enable the determination of various POPs in fish samples. The scheme is associated with the clean-up and analysis of planar DL-compounds (e.g. PCDD/Fs, PBDD/Fs, PXDD/Fs, and non-*ortho* PCBs and PXBs), and other DL and NDL contaminants, such as mono-*ortho* PCBs and PXBs, PBDEs, and HBCDs.

After the collection, the samples were homogenized, subjected to extraction and a sophisticated, multi-step clean-up procedure. The analytes of interest were elicited from the sample matrix using Soxhlet extraction. After the extraction, a 1/10 aliquot of the extract was subjected to clean-up using destructive acidic treatment and Florisil column chromatography (alternatively, non-destructive clean-up procedure could be applied by using of GPC for elimination of lipids from the sample extract) prior to the determination of HBCD diastereomers by means of LC-Orbitrap-HRMS. For the analysis of PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs, and PBDEs, a complex multi-column clean-up protocol was applied, including GPC, silica, and Florisil columns. The sample extract was split into three portions according to its potential toxicological significance, using carbon column chromatography in automated manner: the most toxicologically significant planar compounds (polyhalogenated DD/Fs), non-*ortho* substituted compounds which tend to attain

planar configuration, and the less toxicologically significant poly-*ortho* substituted contaminants. The final analysis of the target compounds was carried out using GC-HRMS, or either LC-Orbitrap-HRMS or LC-MS/MS systems.

2.2. Sample collection and storage

2.2.1. Baltic wild salmon (*Salmo Salar*) samples

A total of fifty three Baltic wild salmon specimens of various age, length, and weight were caught during the spawning period from the Daugava and Venta rivers from October 2010 and October 2012. Due to the large volume of POP data obtained during the practical application of the elaborated methodology (or its elements), only the results for the selected salmon samples are presented in this thesis. These were selected based on the frequency and levels of contamination, and in the case of the poorly studied POPs (e.g. PBDD/Fs and PXDD/Fs), also to emphasize the data not reported by other researchers. More detailed information on the analyzed samples that were measured as part of this work is presented elsewhere (Paper 1, 3 and 5).

Analyzed salmons were migrating for spawning to the Latvian rivers and therefore represented the open Baltic Sea region (River Venta) and Riga Gulf region (River Daugava). The selection of salmons for the study was carefully designed in order to achieve an even representation of two different regions and sexes and to have a maximum variation in weight and length of fishes. All the specimens had gonads at a stage of development typical for spawning. After individual laboratory codes were given for all the caught salmon, lengths and weights were measured, and the sex was noted. Age of the fish was determined according to squama characteristics. Samples were delivered to the laboratory, placed in polyethylene bags, and packed in ice. Thirteen male and twelve female specimens were collected. Average length and weight of female salmon was 79 cm (range from 59 to 86 cm) and 6.0 kg (range from 3.5 to 10.5 kg). Female fish had an age of 2 – 3 years, which is the typical spawning starting age for female wild Baltic salmon. Characteristics of male salmon were as follows: average length 68 cm (range from 56 to 93 cm) and average weight of 4.6 kg (range from 2.0 to 11.3 kg). Generally, the male specimens were 1 – 3 years old with the most common age of 1 year, which is typical for Baltic salmon; most males die during the spawning period. During the sample pretreatment the specimens were dissected, the edible fish fillets (including subcutaneous fat) were isolated and homogenized using a food blender (Kenwood FP101T, Kenwood Ltd., U.K.), and the homogenates were packed into polyethylene bags and stored at -18 °C until analysis. Baltic wild salmon samples were subjected for analysis of PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PBDEs and HBCDs.

2.2.2. Eel (Anquilla Anquilla) samples collected from Latvian lakes

Fifty eight eel (Anquilla anquilla) specimens of various length and weight were caught from Latvian freshwater lakes during the period from September 2013 to May 2014. To get an overview of the actual contamination levels of selected POPs in eels, sampling locations were selected to evenly cover all territory of Latvia. The selection of eels for this study was carefully designed in order to achieve an even representation of five of the most essential eel stocks over Latvia and to have a maximum variation in weight and length of fishes. At least five eel specimens were collected to represent each sampling site. Samples were packed in polyethylene bags and stored with ice during delivery to the laboratory. The samples were uniquely coded and lengths and weights were measured. Average length and weight of eels was 76 cm (range from 39 to 101 cm) and 1.0 kg (range from 0.1 to 1.9 kg). During the sample pretreatment the specimens were dissected, the edible fish fillets (including subcutaneous fat) were isolated and homogenized using a food blender (Kenwood FP101T, Kenwood Ltd, UK), and the homogenates were packed into polyethylene bags and stored at -18 °C until analysis. Eel samples were subjected for analysis of PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs, PBDEs and HBCDs and the results on the occurrence of these contaminants in analyzed samples are described in details in Papers 6 and 8.

2.2.3. Other fish species

To get an overview of the actual contamination levels of POPs in freshwater fish in Latvia, ten fish species were collected in Latvian freshwater lakes, at sampling locations selected to evenly cover all territory of Latvia. At least five specimens of each fish species of various weights were collected. Samples were packed in polyethylene bags and stored with ice during the delivery to the laboratory. Upon receiving at the laboratory, a unique code was given to each sample. To obtain a representative sample after the cutting and pooling of fish fillets, the material was homogenized in a food blender (Kenwood FP101T, Kenwood Ltd., UK), packed in polyethylene bags and stored at -18°C until the analysis. An appropriate sample amount was analyzed for the content of PCDD/Fs and PCBs. More detailed information is presented in Paper 2.

2.3. Chemicals and materials

All the solvents used were at least of pesticide purity grade. Hexane, toluene, dichloromethane, cyclohexane, and ethyl acetate were purchased from Lab-Scan (Glivice, Poland); silica gel (Kieselgel 60, 0.063 – 0.200 mm), Florisil, Celite-545, Carbopack B and Carbopack C were from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). Alumina (basic, 50 – 200 µm), sulfuric acid and sodium sulfate were obtained from Acros (New Jersey, USA). High-purity water

(18.2 MΩ) was prepared using Millipore Milli-Q purification system (Billerica, MA, USA). The native and isotopically-labeled standards for analyzed contaminants were purchased from Cambridge Isotope Laboratory, Inc. (CIL) (MA, USA), Wellington Laboratories, Inc. (Ontario, Canada) and AccuStandard, Inc. (New Haven, USA). Calibration solutions were prepared by serial dilution of stock solutions in toluene or nonane, evaporation of the organic solvent to dryness under gentle nitrogen stream and redissolving the residue in appropriate solvent or solvent mixture. The following seven groups of persistent organic pollutants were analyzed (for compounds given in bold, $^{13}C_{12}$-labeled surrogates were available and were used as internal or recovery standards):

1) Tetra-octa polychlorinated dioxins and furans (PCDD/Fs): **2,3,7,8-TetraCDF, 1,2,3,7,8-PentaCDF, 2,3,4,7,8-PentaCDF, 1,2,3,4,7,8-HexaCDF, 1,2,3,6,7,8-HexaCDF, 2,3,4,6,7,8-HexaCDF, 1,2,3,7,8,9-HexaCDF, 1,2,3,4,6,7,8-HeptaCDF, 1,2,3,4,7,8,9-HeptaCDF, OctaCDF, 2,3,7,8-TetraCDD, 1,2,3,7,8-PentaCDD, 1,2,3,4,7,8-HexaCDD, 1,2,3,6,7,8-Hexa-CDD, 1,2,3,7,8,9-HexaCDD, 1,2,3,4,6,7,8-HeptaCDD, OctaCDD**;
2) Tri-hepta polychlorinated biphenyls (PCBs): IUPAC numbers 18, **28**, 33, 47, 49, 51, **52**, 60, 66, 74, **77**, **81**, 99, **101**, **105**, 110, **114**, **118**, **123**, **126**, **138**, 153, **156**, **157**, **167**, **169**, 180, **189**;
3) Di-deca polybrominated diphenyl ethers (PBDEs): IUPAC numbers 7, **15**, 17, **28**, **47**, 49, 66, 71, 77, 85, **99**, 100, 119, 126, **138**, **153**, **154**, 155, 166, 181, **183**, 190, 203, **204**, 205, 206, **207**, **209**;
4) Tetra-octa polybrominated dioxins and furans (PBDD/Fs): **2,3,7,8-TetraBDF, 1,2,3,7,8-PentaBDF, 2,3,4,7,8-PentaBDF, 1,2,3,4,7,8-HexaBDF, 1,2,3,4,6,7,8-HeptaBDF, OctaBDF, 2,3,7,8-TetraBDD, 1,2,3,7,8-PentaBDD, 1,2,3,4,7,8-Hexa-BDD, 1,2,3,6,7,8-HexaBDD, 1,2,3,7,8,9-HexaBDD,1,2,3,4,6,7,8-HeptaBDD, OctaBDD**
5) Tetra-octa mixed bromo/chloro- polyhalogenated dioxins and furans (PXDD/Fs): 3-B-2,7,8-TriCDF, 2-B-3,7,8-TriCDD, **2,3-DiB-7,8-DiCDD**, 1-B-2,3,7,8-TetraCDF, **1-B-2,3,7,8-TetraCDD**, 2-B-1,3,7,8-TetraCDD, 2-B-3,6,7,8,9-PentaCDD, 1-B-2,3,6,7,8,9-HexaCDD, 1-B-2,3,4,6,7,8,9-HeptaCDD;
6) Six penta- to hexa-, mono- to tri-brominated/di- to penta-chlorinated PXBs with the arrangement of the halogen atoms in the molecules corresponding to the structures of DL-PCBs: **4'-B-3,3',4,5-TetraCB** (structure analog of non-*ortho* PCB 126), 3,4-DiB-3',4',5'-TriCB (analog of non-*ortho* PCB 126), 3',4',5-TriB-3,4-DiCB (analog of non-*ortho* PCB 126), **4'-B-2,3',4,5-TetraCB** (analog of mono-*ortho* PCB 118), 4'-B-2,3,3',4-TetraCB (analog of mono-*ortho* PCB 105), **4'-B-2,3,3',4,5-PentaCB** (analog of mono-*ortho* PCB 156);
7) Hexabromocyclododecane diastereomers: ***α*-HBCD**, ***β*-HBCD**, ***γ*-HBCD**

2.4. Sample preparation and clean-up

An overall diagram of clean-up procedure is shown in Figure 2.1. A sample aliquot was spiked with ^{13}C-labeled POP mixture solution in toluene and equilibrated for at least 1 h. The weight of the sample aliquot was dependent on the species of the fish and lipid content in the sample, and usually was equivalent to not more than 8 g of lipids. After equilibration, the sample was freeze dried for 48 h and the lipids were extracted using Soxhlet extraction with dichloromethane/*n*-hexane (1:1, v/v) mixture with extraction time at least 16 h. The extracts were filled into pre-weighed round-bottom flasks, and the solvent was removed using rotary evaporator at < 30°C. The lipid content was determined gravimetrically and the residue was diluted with cyclohexane/ethyl acetate (1:1, v/v) mixture to obtain the proportion of 1 g of lipids per 5 mL of the final extract volume. An aliquot A of 90% of the final extract volume was subjected to clean-up for the analysis by PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs, and PBDE, while the remaining 10% of the extract volume (aliquot B)) was reconstituted in dichloromethane/*n*-hexane mixture and treated prior to the analysis of HBCD diastereomers using destructive acidic clean-up procedure. Non-destructive clean-up procedure (GPC method) could be applied for the HBCD containing fraction as well, by means of taking 10% of the aliquot after GPC stage and treatment of this extract using Florisil column chromatography.

2.4.1. Procedures applied for aliquot A): PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs and PBDE

The high molecular substances were removed by gel permeation chromatography (GPC). The system was equipped with a glass column (50 x 2.5 cm) filled with 50 g of Bio-Beads SX-3 (Bio-Rad, Philadelphia, USA) stationary phase and eluted with cyclohexane/ethyl acetate (1:1, v/v) mobile phase at a flow rate of 5 mL min^{-1}. The automated GPC program was as follows: dump time 0 – 19 min, collection time 19 – 45 min; the collected eluate was concentrated by rotary evaporation at < 30 ^{o}C. The pre-purified sample extract was placed on top of a glass column (25 x 1.2 cm) filled with 2.5 g silica gel containing 50% of 18M sulfuric acid for degradation of remaining lipids. The analytes were eluted with 1.0 mL of toluene and subsequently with 25 mL of *n*-hexane, and the eluate volume was reduced by rotary evaporation to 0.5 mL. PBDD/Fs, PCDD/Fs and PXDD/Fs were chromatographically separated from PBDEs, PCBs and PXBs using a glass column (25 x 1.2 cm) filled with 6.0 g of Florisil deactivated with 3% water: first the fraction of the PBDEs, PCBs and PXBs was eluted with 80 mL of *n*-hexane, followed by 120 mL toluene for elution of the PBDD/F, PCDD/F and PXDD/F fraction. After solvent removal and concentration of each of the two fractions by rotary evaporation to 1.0 mL, additional clean-up and separation steps using active

carbon column were performed in an automated manner using Waters (Milford, USA) preparative chromatography system consisting of Controller 600, Autosampler 717 plus and Fraction collector III. For the PBDD/Fs, PCDD/Fs and PXDD/Fs a glass column (25 x 1.0 cm) was consequently filled with 0.5 g of Carbopack C/Celite-545 (10/90, w.w.) and 0.5 g of Carbopack B/Celite-545 (10/90, w.w.). After application of the sample extract to the top of the Carbopack C/Celite-545 layer, the column was washed with *n*-hexane/dichloromethane (65:35, v/v) mixture at a rate of 1.5 mL min^{-1} for 20 min and the PBDD/Fs, PCDD/Fs, and PXDD/Fs were eluted with toluene in the back flush mode at a flow rate of 2.5 mL min^{-1} for 40 min. PBDEs, DL-PCBs, NDL-PCBs and PXBs were separated in direct flow using a glass column (25 x 1.0 cm) filled with 2.0 g of a 1:1 (w/w) mixture of Carbopack B and Celite-545; PBDEs, mono-*ortho* PCBs and PXBs and NDL-PCBs were eluted with *n*-hexane at a flow of 2.0 mL min^{-1} within 25 min, the non-*ortho* PCBs and PXBs subsequently by elution with toluene at a flow rate of 2.5 mL min^{-1} within 30 min. Finally, to remove potentially remaining interferants such as PBDEs and PCBs from the fraction containing PBDD/Fs and PXDD/Fs, the fraction was purified using glass column filled with 3.0 g of basic alumina (activated at 450 °C for 16 h) as follows: the fraction containing PBDD/Fs, PCDD/Fs, and PXDD/Fs was evaporated on rotary evaporator and the solvent was exchanged to *n*-hexane without traces of toluene; after addition of the *n*-hexane extract to column, the column was rinsed with 20 mL of *n*-hexane/dichloromethane (90:10, v/v) mixture to waste and analytes of interest were collected with a 60 mL fraction of dichloromethane. After rotary evaporation to about 150 – 200 µL all three extracts were transferred to 2 mL chromatographic vials, treated with 37 N sulfuric acid (30 µL) and mixed. The mixture was allowed to stand for 20 min and centrifuged at 3000 rpm to separate the acid and organic layers. The acidic bottom layer was discarded and the organic layer was evaporated with addition of recovery standard solutions in *n*-nonane. As recovery standards, $^{13}C_{12}$-1,2,3,7,8-PentaBDF and $^{13}C_{12}$-1,2,3,7,8,9-HexaBDD were used for PBDD/Fs, while $^{13}C_{12}$-1,2,3,4-TetraCDD and $^{13}C_{12}$-1,2,3,7,8,9-HexaCDD were used for PCDD/Fs and PXDD/Fs. For PBDE, mono-*ortho* PCB and PXB, and non-dioxin-like PCB fraction, the $^{13}C_{12}$-PBDE 138, $^{13}C_{12}$-PCB 101, and $^{13}C_{12}$-PCB 138 surrogates were added as recovery standards, while for non-*ortho* PCB and PXB fraction the recovery standards were $^{13}C_{12}$-PCB 101 and $^{13}C_{12}$-PCB 138. The final volume of the PBDD/F, PCDD/F and PXDD/Fs fraction was 10 µL. The fraction containing PBDEs, mono-*ortho* PCBs and NDL-PCBs was evaporated to 50 µL. Non-*ortho* PCB fraction was also reduced to 50 µL. After the analyses of PBDEs and PCBs, the corresponding extracts were evaporated until 20 µL and analyzed on the content of PXBs.

2.4.2. Procedures applied for aliquot B): HBCDs

2.4.2.1. Destructive removal of high molecular compounds (acidic method)

The organic extract was evaporated to dryness, the residue was dissolved in 35 mL dichloromethane/*n*-hexane (1:1, v/v) mixture and treated with 10 mL of 18M sulphuric acid by intense shaking for 15 min. The mixture was allowed to stand for 15 min and centrifuged at 3000 rpm using a Falcon 6/300 benchtop centrifuge (MSE, London, UK). The bottom (aqueous) layer was discarded and the organic extract was treated with 10 g of silica gel impregnated with sulphuric acid (50% of 18M sulphuric acid). After intense shaking of the mixture, the organic phase was filtered and silica residue was washed with 20 mL of extraction solvent mixture. The filtrate and washings were combined and transferred to a round-bottom flask and concentrated to approximately 1 mL on a rotary evaporator, and the solvent was exchanged to 1 mL of *n*-hexane. The extract was further treated on a glass column (25 × 1.0 cm) filled with 3.0 g of Florisil deactivated with 3% of water: after the addition of the sample extract, the column was washed with 20 mL of *n*-hexane and the eluate was discarded. HBCD diastereomers were eluted from the column with 40 mL of *n*-hexane/dichloromethane (1:1, v/v) mixture, the fraction was concentrated by rotary evaporation, reconstituted in 200 µL methanol-water-acetonitrile mixture (60:20:20, v/v/v), transferred to an autosampler vial and analyzed on the content of HBCDs.

2.4.2.2. Non-destructive removal of high molecular compounds (GPC method)

A sample aliquot of 10% for determination of HBCDs was taken after the removing of the bulk of the high molecular components (e.g. lipids) by means of GPC. The solvent was evaporated and the sample was reconstituted in 1mL of *n*-hexane. Further clean-up procedure was performed using Florisil column according to section 2.4.2.1.

2.4.2.3. Preparation of the fish sample extracts for the comparative evaluation of the instrumental responses of UHPLC-Orbitrap-HRMS, UHPLC-TOF-HRMS and UHPLC-QqQ-MS/MS systems

For the establishing the influence of different sample clean-up steps on the instrument response to the analytes of interest, a butter fish homogenate was used as model matrix. The 5 g of the material was extracted with dichloromethane/*n*-hexane (1;1, v/v) using cold extraction and was treated according to the individual steps of the procedure, or their combinations (e.g. GPC method, acidic method and the addition to the scheme of Florisil column). The model matrix was previously analyzed for the content of HBCD diastereomers and found to contain only traces of *α*-HBCD (triplicate analysis of the material showed the average concentration of 11 pg g^{-1} sample fresh

weight (f.w.))whereas β- and γ-HBCDs were found to be below the LOQ. To evaluate the possible instrumental signal suppression by the matrix components, the sample extracts obtained after the corresponding clean-up stage were spiked with the appropriate volume of HBCD diastereomer solution in toluene and were evaporated until dryness under a gentle stream of nitrogen. The obtained samples were reconstituted in 200 µL of methanol-water-acetonitrile mixture (60:20:20, v/v/v), and transferred to an autosampler vial. The amount of the standard solution added before the instrumental analysis provided the final 10 pg μL^{-1} concentration for each HBCD stereoisomer. The samples for instrumental signal suppression experiments were prepared in triplicate (n=3) and each sample was injected in duplicate. It was found that the relative standard deviation (RSD) for the obtained instrumental responses for each set of samples corresponding to the evaluated sample preparation step or their combinations was below 20%.

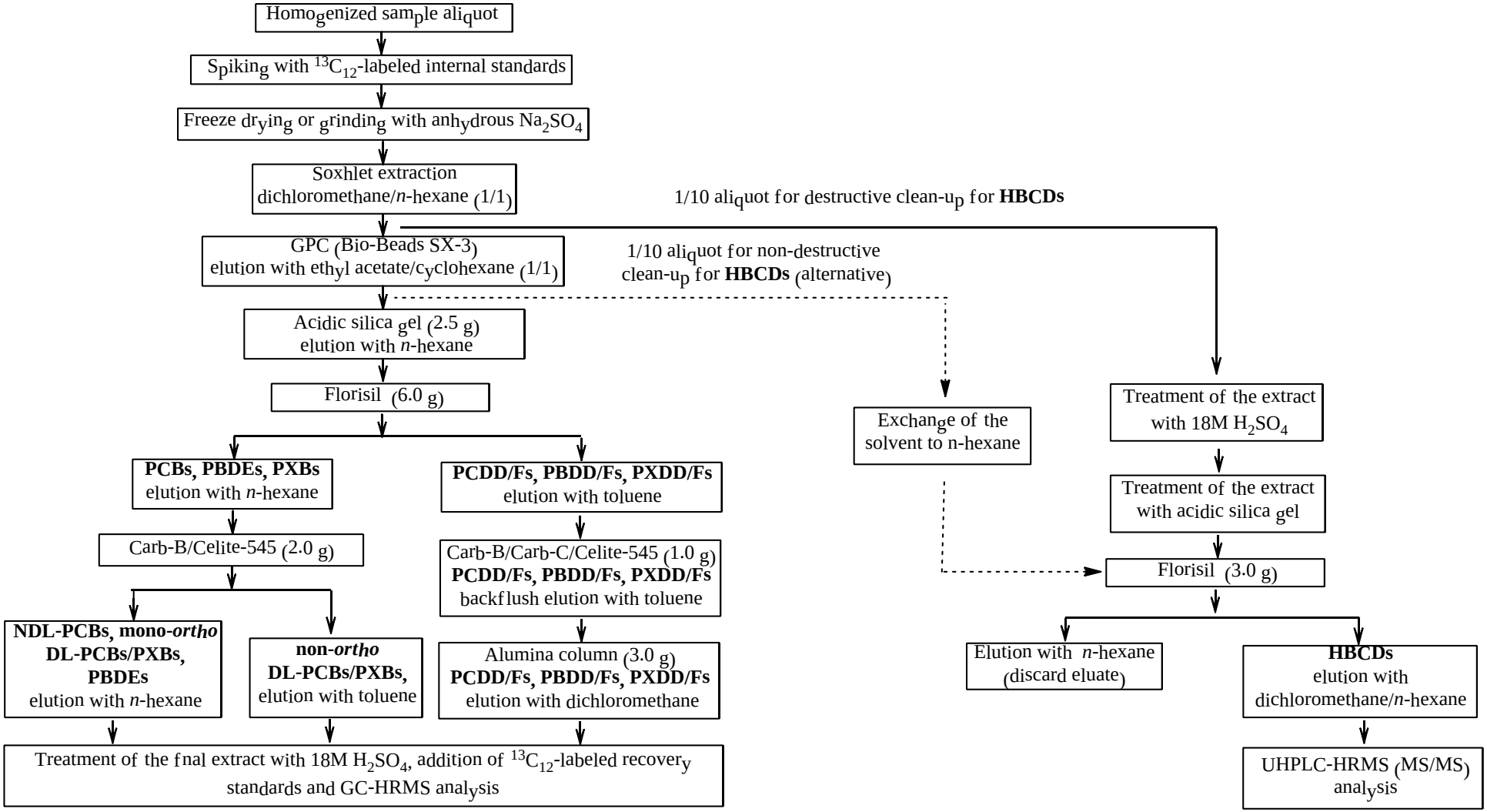


Fig. 2.1. **The analytical diagram, showing principal clean-up and analysis steps for PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs, PBDEs and HBCDs**

2.5. Instrumental analysis

2.5.1. Instrumental analysis and quantification of PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs and PBDEs by GC-HRMS

Selected analyte groups were analyzed by GC-HRMS. The measurements were performed on Micromass Autospec Premier high resolution mass spectrometer (Milford, USA) coupled with Agilent 6890 N gas chromatograph (Santa Clara, USA). Aliquots of 1 µL of the final sample extracts and calibration solutions were introduced into GC-HRMS system equipped with a silica capillary column and a split/splitless injector operated in splitless mode. The transfer line from the GC to the HRMS and the ion source temperatures were kept at 280 ^{o}C. Ion source was operated in the positive electron impact mode (EI^{+}) with electron energy of 36 eV and a trap current of 600 µA. The resolution of the mass spectrometer was better than 10 000 (at 10% peak valley) for all analyte groups with the exception of PXDD/Fs and PXBs, for which resolution was in the range of 13 500 – 15 000 (at 10% peak valley). Mass calibration for all analyzed compounds was obtained at acceleration voltage of 7.5 kV. Table 2.1 presents the GC conditions used for selected POP groups.

Table 2.1

GC conditions for the analysis of target compounds

PCDD/Fs	
Column	ZB-5MS, 60 m, 0.25 mm i.d., 0.25 µm film thickness (Phenomenex, Torrance, USA)
Temperature program	140 °C (held for 3 min), 15 °C min^{-1} to 200 °C, 3 °C min^{-1} to 235 °C (held for 15 min), 4 °C min^{-1} to 300 °C (held for 12 min)
Injection temperature	280 °C
Carrier gas	helium at flow rate of 1.0 mL min $^{-1}$
PCBs	
Column	ZB-5MS, 60 m, 0.25 mm i.d., 0.25 µm film thickness (Phenomenex, Torrance, USA)
Temperature program	75 °C (held for 2 min), 15 °C min^{-1} to 150 °C, 2.5 °C min^{-1} to 290 °C (held for 1 min)
Injection temperature	270 °C
Carrier gas	helium at flow rate of 1.0 mL min $^{-1}$
PBDD/Fs	
Column	DB-5MS, 18 m, 0.25 mm i.d., 0.10 µm film thickness (J&W Scientific, Folsom, USA)
Temperature program	100 °C (held for 4 min), 40 °C min^{-1} to 200 °C (held for 3.5 min), 10 °C min^{-1} to 320 °C (held for 2.5 min)
Injection temperature	260 °C
Carrier gas	helium at flow rate of 1.0 mL min $^{-1}$
PXDD/Fs	
Column	DB-5MS, 30 m, 0.25 mm i.d., 0.10 µm film thickness (J&W Scientific, Folsom, USA)
Temperature program	120 °C (held for 2 min), 20 °C min^{-1} to 240 °C, 5 °C min^{-1} to 320 °C (held for 4 min)
Injection temperature	250 °C
Carrier gas	helium at flow rate of 1.0 mL min $^{-1}$
PXBs	
Column	ZB-5MS, 60 m, 0.25 mm i.d., 0.25 µm film thickness (Phenomenex, Torrance, USA)

Table 2.1continuation

GC conditions for the analysis of target compounds

Temperature program	70 °C (held for 3 min), 100 °C min^{-1} to 140 °C, 15 °C min^{-1} to 210 °C ,4 °C min^{-1} to 310 °C (held for 10 min), 10 °C min^{-1} to 315 °C (held for 4 min)
Injection temperature	290 °C
Carrier gas	helium at flow rate of 1.0 mL min^{-1}
Di-hepta BDEs	
Column	DB-5MS, 30 m, 0.25 mm i.d., 0.10 µm film thickness (J&W Scientific, Folsom, USA)
Temperature program	100 °C (held for 3 min), 5 °C min^{-1} to 320 °C (held for 15 min)
Injection temperature	250 °C
Carrier gas	helium at flow rate of 1.0 mL min^{-1}
Octa-deca BDEs	
Column	DB-5MS, 15 m, 0.25 mm i.d., 0.10 µm film thickness (J&W Scientific, Folsom, USA)
Temperature program	100 °C (held for 3.5 min), 40 °C min^{-1} to 200 °C (held for 4 min), 10 °C min^{-1} to 320 °C (held for 3 min)
Injection temperature	280 °C
Carrier gas	helium at flow rate of 1.0 mL min^{-1}

All analyte groups were determined by selected ion recording (SIR) using the two most abundant ions of the respective molecular ion cluster of both the native and the $^{13}C_{12}$-labeled surrogates. The run was time segmented and SIR descriptors changed according to elution times for analytes of interest. Quantification was carried out using isotope dilution method applying the $^{13}C_{12}$-labeled surrogates as internal standards. Masslynx™ software was used for raw data interpretation and for targeting/quantification of selected contaminants.

2.5.2. Instrumental analysis and quantification of HBCD diastereomers

2.5.2.1. UHPLC separation of the target analytes

Within the framework of this study UHPLC separation of target compounds was carried out using Kinetex C_{18}, 100 mm × 2.1 mm, 1.7 µm reversed-phase analytical column at 25°C, applying a flow rate of 250 µL min^{-1} with a mobile phase gradient based on (A) methanol-water (75:25, v/v) and (B) acetonitrile. The effective gradient began at the initial composition (A/B) of 20:80 (v/v) that was maintained for 1.0 min and then ramped to 55:45 over 0.1 min, where it was held for 6.0 min before returning to the initial conditions over 1.0 min. The column was equilibrated for 2.0 min between the runs. The injection volume of 10 µL was used both for the standard solutions and sample extracts.

2.5.2.2. UHPLC-Orbitrap-HRMS system

The UHPLC-Orbitrap-HRMS system used in this study consisting of Thermo Accela UHPLC system (Zwingen, Switzerland) coupled to an Orbitrap Q Exactive mass spectrometer (Bremen,

Germany) equipped with heated electrospray ionization (HESI-II) interface. The negative ion mode was used for acquisition of the mass spectra. During the tuning procedure the signal of HBCD diastereoisomers was preliminarily optimized for the highest response of the $[M\text{-}H]^-$ ion. Direct introduction of the target compounds (native and $^{13}C_{12}$-labeled α-, β-, and γ-HBCDs, 1 ng μL^{-1} of each in methanol) into the HESI-II interface of MS system was performed at 10 µL min^{-1}using a Chemyx Fusion 100T (Stafford, USA) infusion pump. Orbitrap-MS detection in targeted selected ion monitoring (t-SIM) mode was used for a quantitative determination of selected compounds using the two $[M\text{-}H]^-$ most abundant ions of the respective molecular ion cluster for both the native and the $^{3}C_{12}$-labeled surrogates. The channels monitored for HBCD diastereoisomers were *m/z* 640.6374 (quantification) and *m/z* 638.6396 (confirmation) for the native components, and *m/z* 652.6782 (quantification) and *m/z* 650.6804 (confirmation) for the $^{13}C_{12}$-labeled surrogates. External calibration of the Orbitrap-MS system was performed before each batch of samples over the mass range of *m/z* 50 – 2000 according to the guidelines provided by the instrument supplier. The details of the optimized instrumental conditions are summarized in Table 2.2. Thermo Xcalibur™ and TraceFinder™ 3.0 software were used for raw data interpretation and for targeting/ quantification of selected contaminants.

2.5.2.3. UHPLC-TOF-HRMS system

The UHPLC-TOF-HRMS instrument consisted of an Agilent Technologies 1290 Infinity UHPLC system coupled to a 6230 TOF mass spectrometer (Santa Clara, CA, USA) equipped with a heated ESI interface. The negative ion mode was used for the acquisition of mass spectra. A TOF-HRMS detection in the scan mode over the *m/z* range 600 – 700 was used for a quantitative determination of selected compounds using the two most abundant $[M\text{-}H]^-$ ions of the respective molecular ion cluster for both the native and the $^{13}C_{12}$-labeled surrogates. The nominal channels monitored for HBCD diastereomers were the same as for Orbitrap-HRMS. External calibration of the TOF-HRMS instrument was performed before each batch of samples over the mass range of *m/z* 100 – 3200 and achieving mass resolving power greater than 10 000 FWHM according to the guidelines provided by the instrument supplier. For the raw data treatment and targeting/ quantification of selected contaminants, Agilent Technologies MassHunter Workstation software was used. The details of the optimized instrumental conditions are summarized in Table 2.2.

2.5.2.4. UHPLC-QqQ-MS/MS system

For MS/MS analyses an AB SciexQTrap 5500 mass spectrometer (AB SCIEX, Framingham,

MA, USA) equipped with heated ESI interface and a Waters Acquity UHPLC system (Waters, Milford, MA, USA) were used. The data acquisition was performed in the negative SRM mode to obtain sufficient number of quantification points for the confirmation of each HBCD diastereomer. Analyst® software was used to control all the components of the instrument and for the data acquisition and processing. The channels monitored for the three HBCD diastereomers were *m/z* 640.6 → 78.9 (quantification) and *m/z* 640.3 → 80.9 (confirmation), and the internal standard channels were *m/z* 652.6 → 78.9 and *m/z* 652.6 → 80.9. External calibration of the mass spectrometer was performed according to the manufacturer requirements. Detailed instrumental conditions of the system are summarized in Table 2.2.

Table 2.2

The optimised instrumental conditions for determination of HBCD diastereomers using different MS techniques

	Orbitrap-HRMS	TOF-HRMS	QqQ-MS/MS
ESI^- conditions			
Drying gas temperature	-	260°C	-
Drying gas flow	-	13 L min^{-1}	-
Nebulizer pressure	-	25 psig	-
Sheath gas temperature	-	280°C	-
Capillary voltage	-	3.0 kV	-
Nozzle voltage	-	3.0 kV	-
Fragmentor voltage	-	150 V	-
Skimmer voltage	-	75 V	-
Sheath gas flow/pressure	15 a.u.*	12 L min^{-1}	60 psi
Auxiliary gas flow/pressure	5 a.u.*	-	30 psi
Capillary temperature	250°C	-	-
Source heater temperature	250°C	-	400°C
Spray voltage	4.5 kV	-	4.5 kV
S-lens radio frequency	50	-	-
MS conditions			
Maximum injection time	100 ms	-	-
Automatic gain control (AGC target)	5×10^4	-	-
MS resolution	35 000 FWHM	15 000 FWHM	UNIT
Detection mode	t-SIM	FULL SCAN	SRM
Declustering potential	-	-	90 V
Collision energy	-	-	60 V

a.u.* – arbitrary units.

2.6. Quality assurance and quality control

Before use, all the glassware was solvent washed to remove possible background contamination. To prevent the degradation of potentially photolabile compounds such as HBCDs, PBDD/Fs and PBDEs, all stages of analytical procedure, including sample extraction, purification and handling of the final extracts were performed under ‘‘UV-protect’’ conditions (e.g. using amber

glassware or wrapping the glassware with aluminum foil). To compensate the losses of the analytes during the extraction and clean-up steps, each sample was spiked before the analytical procedure with $^{13}C_{12}$-labeled congeners and quantification was carried out on the basis of stable isotope dilution and internal standardization. Recovery standards added after clean-up procedure were used for internal standard recovery control and calculation. Five to six point calibration curves were used for quantification of congener concentrations in each sample run. At the end of the analytical run, a reference standard solution was analyzed to check the system performance and calibration validity. Linearity of the calibration curves was checked with relative response factors (RRFs).

The following internal quality control criteria for the positive identification of analytes of interest were applied:

i) The retention time of native compound should be within a window of +3 to 0 seconds compared to the corresponding $^{13}C_{12}$-labeled internal standard.

ii) The isotope ratios of the two molecular ions of the halogen ion distribution cluster analyzed in SIR-mode should be within ± 15% of the theoretical values.

iii) The signal-to-noise ratio should be equal to or greater than 3 ($S/N \geq 3$);

iv) Procedural blanks and quality control samples should be run in each sample sequence, consisting of not more than 10 samples;

v) Recovery of $^{13}C_{12}$-labeled internal standards was in the range of 60 – 120% (with exception of HBCDs, for which recovery of internal standards was not evaluated for each analyzed sample).

To control the ongoing precision and recovery of the selected POPs, quality control samples were routinely added in each sample sequence. Quality control samples consisted of “in house” prepared fish oil or freeze-dried salmon fillet naturally contaminated with PCDD/Fs, PCBs, PBDEs, and HBCDs which was tested by four different laboratories. To control the ongoing precision and recovery of the PBDD/Fs, PXDD/Fs and PXBs quality control samples were spiked with the analytes of interest. To reveal the performance in analysis of POPs, laboratory participates on a regular basis in international proficiency tests on determination of PCDD/Fs and PCBs in food and feed matrices.

3. RESULTS AND DISCUSSION

3.1. Clean-up and fractionation

The sample preparation procedure applied in the present study was based on the dehydratation of fish tissue using freeze drying (lyophilization) followed by the extraction of lipophilic target compounds into a dichloromethane/*n*-hexane mixture. Alternatively, the dehydration procedure could be performed with anhydrous sodium sulphate or other drying agent (e.g., magnesium sulphate) suitable for these purposes. Extraction of POPs from the samples was performed using Soxhlet extraction approach as one of the simplest and most effective methods, which does not require sophisticated and expensive equipment [81]. The sample extract was split into two portions after the extraction step: 90% was subjected to the clean-up and fractionation steps required for the analysis of PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs, and PBDEs, while the remaining 10% portion was used for the analysis of HBCDs.

3.1.1. Clean-up and fractionation for the fraction containing PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs and PBDEs

Because of the structural similarity of PBDD/Fs and PXDD/Fs to PCDD/Fs, and PXBs to PCBs, for which a robust method has been successfully used over the course of many years for food and feed matrices, a similar analytical approach with modifications to better suit PBDD/F and PXDD/F analysis was used. Taking into account the typically low levels (ppt or sub-ppt) at which PBDD/Fs and PXDD/Fs are represented in food products and environmental matrices, relatively high sample aliquots were taken for analysis with a concentration factor up to 10 000 to provide better sensitivity for PBDD/Fs PXDD/Fs and PXBs. The most commonly used procedures to remove the bulk of high molecular compounds from the samples of animal origin are destructive (acid/base treatment) and nondestructive methods (size exclusion chromatography (e.g. GPC), dialysis through the semi-permeable membranes) [123]. Although under the conditions of well established analytical procedures for POPs destructive methods give reliable results, there are some potential risks. On one hand, the acid treatment of large sample amounts with high fat content leads to a risk of losing of analytes of interest by adsorption on the treated carbonaceous matrix. On the other hand, sample digestion with alkali could cause the breakdown of highly halogenated compounds resulting in formation of respective congeners with lower halogenation pattern [124]. Moreover, the acid treatment alone is not effective for some types of matrices (e. g. materials of plant origin), due to the presence of waxes, which are relatively stable to hydrolysis. To prevent the possible risks related to the usage of destructive lipid elimination methods, GPC was used to

remove the bulk (up to 95%) of high molecular compounds from the sample. Furthermore, GPC presented several advantages: this type of chromatography is flexible and robust; a column could be used repetitively without regeneration and could be easily operated in automated manner. Additionally an acidic silica column was used for the degradation of the remaining part of lipids, as well as for retaining the polar matrix components.

As it was reported previously [41,74,125-127], the separation of PBDEs and PCBs from PBDD/Fs and PXDD/Fs has been of great concern to prevent the mass spectral interference from similar fragments during the ionization. Table 3.1 shows the example of possible interference of PCBs in mass spectral analysis of monobromo-trihloro and monobromo-tetrachloro DD, and dibromo-dichloro and dibromo-trichloro DFs.

Table 3.1

Possible example of mass spectral interference of PCBs in analysis of PXDD/Fs

Component	Isotopic fragment and mass (*m/z*)		Isotope ratio (± 15%)	Required MS resolution (R)
Br_1Cl_3DD	$[M]^+$ 363.8455	$[M+2]^+$ 365.8430	0.51 (± 0.08)	> 35 000
HexaCB	$[M+6]^+$ 363.8351	$[M+8]^+$ 365.8321	4.1 (± 0.62)	
Br_1Cl_4DD	$[M+2]^+$ 399.8040	$[M+4]^+$ 401.8013	1.2 (± 0.18)	> 37 000
HeptaCB	$[M+8]^+$ 399.7932	$[M+10]^+$ 401.7902	5.2 (± 0.78)	
Br_2Cl_2DF	$[M+2]^+$ 393.7878	$[M+4]^+$ 395.7955	1.1 (± 0.17)	> 110 000
HeptaCB	$[M+2]^+$ 393.8020	$[M+4]^+$ 395.7991	1.0 (± 0.15)	
Br_2Cl_3DF	$[M+2]^+$ 427.7588	$[M+4]^+$ 429.7563	0.93 (± 0.14)	> 113 000
Br2Cl3DF	$[M+2]^+$ 427.7630	$[M+4]^+$ 429.7601	0.89 (± 0.13)	

On one hand, it is clearly seen that the interference of PCBs could not be distinguished by using conventional MS with the high resolution of R = 10 000. On the other hand, increasing of the MS resolution in magnetic sector HRMS instruments dramatically decreases the sensitivity. The isotope ratios of the two molecular ions of the halogen ion distribution cluster, which are constant for the selected groups of compounds, serve as a clear indicators of the presence of the component of interest in the sample. Nevertheless, in the case of the presence of selected components in the sample at ultra-trace levels, isotope ratios could be affected due to the background of the instrument, leading to an incorrect interpretation of the SIR chromatograms. Moreover, overlapping of the retention ranges of different classes of compounds prevents unequivocal chromatographic identification of the analytes. As it could be seen from Papers 1 – 4, typically, fish

is the matrix most contaminated with PCBs and PBDEs among the food products and the latter compounds reach ppb or even higher concentrations on f.w. basis. Due to the significant difference of concentration profiles of target compound groups in the samples and the ability of some compounds to affect or interfere with instrumental analysis, these groups should be eliminated from the extracts as completely as possible during the clean-up procedure, thus extensive sample fractionation should be used. In our study an elaborated clean-up procedure with three-step fractionation for the fraction containing PBDD/Fs and PXDD/Fs was used in order to provide a more complete exclusion of possible interferants. The first fractionation stage involved a Florisil column chromatography, taking advantage of the almost complete separation of PCBs, PXBs and PBDEs from PBDD/Fs, PCDD/Fs and PXDD/Fs [127,128]. The elution profile for all these classes of analytes is shown in Figure 3.1.

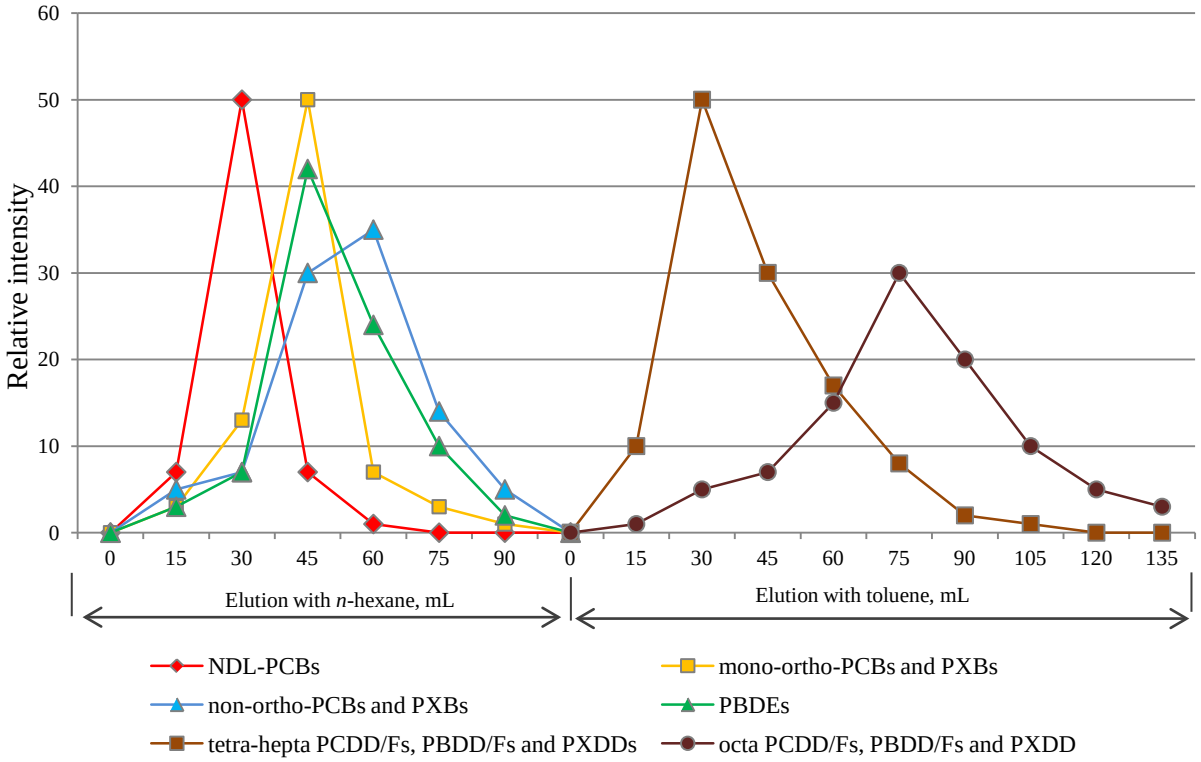


Fig. 3.1. **Elution profiles of PCBs, PXBs, PBDEs, PCDD/Fs, PBDD/Fs and PXBs in Florisil column**

Although the Florisil column was capable of separating up to 99.5% of PBDEs from the sample extract, taking into account the differences in typical levels of PBDEs and PBDD/Fs (ppb and ppt or sub-ppt, respectively), the remaining 0.5% of PBDEs could provide undesirable GC-MS interference. Due to the different retention characteristics of planar and non-planar aromatic molecules on activated carbon, based on different interactions between the *p*-electrons of the

aromatic molecules and *p*-electrons of the carbon graphite structure, carbon column chromatography was suggested for the separation of PCBs and PBDEs from PBDD/Fs and PXDD/Fs [50,57,74,86], and it was applied in this study in automated manner as a second fractionation step. Polyhalogenated dioxins and furans have planar aromatic structures and tend to interact stronger with the carbon surface in comparison to the mono- to tetra-*ortho* substituted PCBs and PBDEs, which have restricted rotation around the phenyl-phenyl bond and remain relatively non-planar. This results in weak or intermediate retention of PCBs and PBDEs on activated carbon and by using moderately polar solvent mixtures (e.g., *n*-hexane/dichloromethane) it is possible to isolate these potential interferants from the extract. Moreover, the using of dual-layer reversible carbon column consisting of activated carbon sorbents with different surfaces areas (Carbopack C with surface area of 10 m^2 g^{-1} and Carbopack B with surface area of 100 m^2 g^{-1}) provides a better and more reproducible recoveries for $^{13}C_{12}$-labeled hepta- and octa-substituted dioxins and furans, which is common issue for these compounds [129]. The benefit is associated with the fact that the sample extract is applied on the additional Carbopack C layer, which has weaker adsorption strength to low chlorinated congeners, compared to Carbopack B, while the highly chlorinated congeners are retained on this material with further possibility to elute them with smaller volumes of solvent. The comparison of the recoveries for $^{13}C_{12}$-PCDD/Fs obtained by using conventional and dual-layer carbon columns is shown in Figure 3.2.

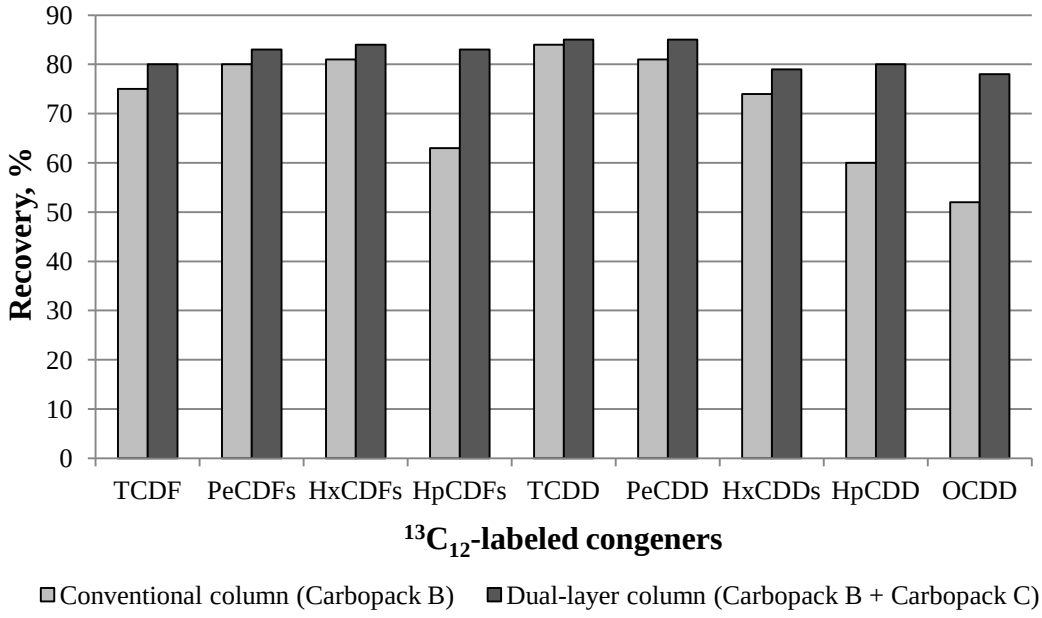


Fig. 3.2. **Improvement of the recoveries for $^{13}C_{12}$-PCDD/Fs obtained by using of dual-layer carbon column**

The final purification stage for fraction containing polyhalogenated DD/Fs utilizes a basic alumina column to exclude the possible remaining mass interferants, and this step is particularly useful in analysis of matrices with high PCB and PBDE contamination (e.g., Baltic wild salmon and other fish from the Baltic Sea).

Finally, due to the fact that non-*ortho* PCBs can attain a planar configuration, these compounds are more toxic in comparison with ortho PCBs and PBDEs [6]. Taking into account the toxicological significance of non-*ortho* PCBs and the occurrence of these compounds in the samples at lower concentrations compared to other PCBs, this group of contaminants was isolated as an individual fraction using carbon column, to improve the sensitivity and robustness of the method.

3.1.2. Clean-up and fractionation for the fraction containing HBCDs

For analysis of HBCDs after the extraction procedure an aliquot of the sample extract was subjected to the destructive clean-up procedure with additional purification stage on a Florisil column. This approach was chosen as the simplest and less time consuming in comparison with non-destructive methods such as GPC in development of a target-oriented method where the analytes (HBCDs) are stable under the aggressive conditions. Although the bulk of high-molecular compounds (e.g., lipids) were decomposed with concentrated sulphuric acid, polar matrix components could remain in the sample extract, thus the additional treatment with acidic silica gel was introduced in order to remove these interferants. Fish is a matrix known to be potentially highly contaminated with brominated organic compounds [130,131], and some of those (e.g., PBDEs) could interfere with the mass spectrometric analysis of HBCD by giving rise to similar mass fragments during the ionization of the sample extract. In order to remove these potentially isobaric mass interferants from the sample extract, additional clean-up on a Florisil column was used with the successful elution of analytes of interest from the column with a medium polarity solvent mixture (dichloromethane/*n*-hexane). Moreover, a three-stage sample clean-up procedure provided the final sample extracts of the desired purity, which was essential for the analysis of organobromine compounds, since the matrix components tended to adhere on the hot surfaces of the ion source and the thermally labile compounds such as HBCDs were destroyed on these spots resulting in a drop of method sensitivity. Since the clean-up procedure might become the most critical step, elution profiles of the analytes of interest were tested first and found to acceptably provide the absolute recoveries of the HBCD diastereoisomers in the range of 70 – 110%, without correction for the isotopically labeled internal standards.

Alternatively, clean-up for analysis of HBCDs could be performed under conditions of non-destructive clean-up protocol by means of using of GPC as the first step for the elimination of high-molecular compounds. Such approach could be more affordable with respect of extension of the list of the analytes by ensuring of more flexible conditions of the method and to minimizing the degradation of susceptible compounds. Moreover, sample extracts obtained in such way could be analyzed in full scan modes using either UHPLC-Orbitrap-HRMS or UPLC-TOF-HRMS with the possibility of retrospective post-run evaluation of the instrumental raw data and screening of unknown compounds (e.g. other BFRs).

3.2. Instrumental analysis

3.2.1. GC-HRMS in analysis of PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs and PBDEs

Although the increase in the halogenation degree of dibenzo-*p*-dioxins and furans is linked to a decrease in their potential toxicity, some of the PBDD/Fs with high degree of bromination (e.g., hepta- and octa-brominated congeners) might be of high toxicological concern. Unfortunately, in most studies data on highly brominated congeners is scarce. Although the sensitivity of reported GC-HRMS methods for analysis of PBDD/F congeners with low degree of substitution, such as di- to penta-brominated dioxins and furans, is comparable to the methods reported for chlorinated analogs, the analysis of highly brominated components is often complicated, resulting in increased LOQs for these toxicologically significant compounds. Due to the thermal lability of highly brominated congeners, the transit time through the GC column becomes critical for these compounds. To avoid thermal degradation problems during the GC separation of brominated POPs, such as PBDD/Fs and PBDEs, shorter columns with a thinner internal coating are more preferable [86,115,132]. In this study an 18 m GC column with phase coating of 0.10 µm that offered both adequate chromatographic resolution and sensitivity for PBDD/Fs was used. Taking into account the possibility of thermal degradation of PXDD/Fs, especially at high degree of halogenation, the latter were checked by application of two columns with different lengths and phase loadings (a 60 m column with phase loading of 0.25 µm and 30 m column with phase loading of 0.10 µm). As was expected, the 30 m column with film thickness of 0.10 µm provided much better sensitivity for hepta- and octa-halogenated PXDDs without significant loss of chromatographic resolution for low halogenated congeners. Since only penta- and hexa-substituted PXBs were included in the scope of the study, no sensitivity problems due to the thermal degradation were obtained for these compounds during the GC run, thus 60 m GC column with phase loading of 0.25 µm was used with regard to provide better selectivity. For chromatographic separation of PBDEs, where thermal

degradation was of great concern during the GC-MS analysis [115,132], columns with phase loading of 0.10 μm were used to improve the sensitivity.

In spite of the extensive multistep sample clean-up and fractionation, the possible presence of mass interferants (e.g., PBDEs and PCBs) in the final PBDD/F and PXDD/F extracts could not be excluded. During the analysis of PBDD/Fs, possible thermal degradation of potentially present PBDEs and debromination with release of two bromine atoms can be expected, resulting in the formation of intensive fragment peaks with the same number of bromines and molecular weight as the PBDF congeners [86]. To prevent the false-positive identification of PBDFs, simultaneous monitoring of the fragment ions of PBDEs with one and two extra bromines in comparison with PBDFs of interest was carried out and no signals with the retention times corresponding to the analytes of interest were found. Taking into account previous studies [74,133], the corresponding ions for PXDD/Fs and PXBs were chosen in the way to prevent the overlapping of the analyte ions with the main mass interferants (PBDEs and PCBs) as much as possible. In addition, higher mass resolution (13 500 – 15 000) was used during the analyses of PXDD/Fs and PXBs. Within the scope of this study $[M]^+$ fragments for GC-MS determination of di- to hepta-BDEs were chosen, although for octa- to deca-BDEs $[M-2Br]^+$ type ions were used because of the higher peak intensity in the mass spectrum compared to ions from $[M]^+$ ion cluster [134]. The selected mass descriptors for measurement of PBDD/Fs, PBDEs, PXDD/Fs and PXBs are overviewed in Annexes 1, 2 and 3. Specific *m/z* fragments for the determination of PCDD/Fs and PCBs were selected according to the standard methods US EPA 1613 and US EPA 1668A [89,90].

3.2.2. LC-Orbitrap-HRMS in analysis of HBCD diastereomers

3.2.2.1. LC separation

Until this study there was no available literature on the analysis of BFRs or other contaminants using Orbitrap-HRMS based methods, thus special attention was paid on the capabilities of this emerging type of mass spectrometry in analysis of POPs. Since an analytical approach based on LC-MS for determination of HBCD was proposed [135], several studies have documented the chromatographic separation of HBCD diastereoisomers under reversed-phase conditions [106,112,113,136,137]. In this study the three HBCD diasteromers found to be baseline separated under the UHPLC conditions described in Section 2.5.2., using methanol, acetonitrile, and water gradient on C18 reversed-phase analytical column.

3.2.2.2. Q Exactive Orbitrap-HRMS system

The Q Exactive Orbitrap-HRMS used in this study consists of five main modules: ion source,

quadrupole mass filter for precursor ion selection, intermediate storage device (C-Trap) for short pulse injection, collision cell for performing HCD (Higher Energy Collisional Dissociation) experiments and Orbitrap analyzer for Fourier transform mass analysis. After the chromatographic separation, sample components are introduced into the heated ionization source. The observed ions are transferred into the C-Trap through the quadrupole rod assembly which operates as ion transmission device with the possibility to filter the transmitted ions according to its *m/z* ratios. In the C-Trap, the ions are accumulated and their energy dampened using a bath gas (nitrogen). The ions are then injected using a lens system into the Orbitrap analyzer where mass spectra are acquired via image current detection. In the case of operation of the instrument in targeted-MS2 (t-MS2) mode, before the injection into the Orbitrap analyzer ions are passed through the C-Trap into the HCD cell where higher energy collision induced dissociation takes place. In combination with the quadrupole mass filter this allows MS/MS experiments. After the ions have been fragmented in the HCD cell, the HCD cell voltages are ramped up and the ions are transferred back into the C-Trap from where they are injected into the Orbitrap analyzer for detection. The principal scheme of the Q Exactive Orbitrap-HRMS system is shown on Figure 3.3.

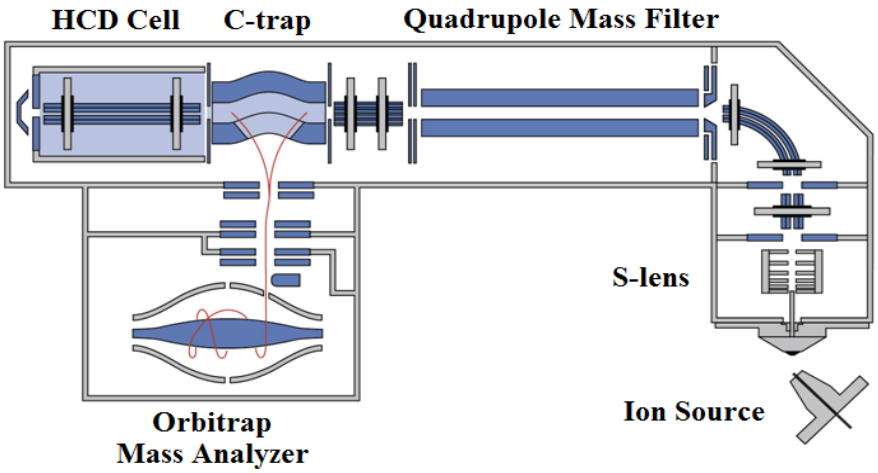


Fig. 3.3. **Principal scheme of the Q Exactive Orbitrap-HRMS system**

3.2.2.3. Detection mode selection

To gain the optimal MS sensitivity, infusion experiments were performed in a full scan mode over the *m/z* range of 50 – 700. The $[M\text{-}H]^-$ peaks with different bromine isotopic content were predominant in the MS spectra of HBCD after the pair of the ions with *m/z* 78.9173 and *m/z* 80.9152 corresponding to $[Br]^-$ ions with different isotopic pattern. No other specific peaks were found to be present, which is in accordance with previous studies [135,136]. Two different targeted detection modes were attempted during the method development process: t-MS2 and t-SIM (analog

of SIR mode) modes. Initially the t-MS2 mode was checked in order to achieve a potentially better selectivity of the method in comparison to t-SIM detection. This type of detection utilized the tandem MS option and comprised a full MS scan done by the Orbitrap analyzer with a defined isolation window set by the quadrupole mass filter followed by a data dependent scan with the fragmentation energy applied and selected mass resolution adjusted. The fragmentation of the precursor ions of *m/z* 638.6396 and *m/z* 640.6374 in the HCD collision cell resulted in the intensive formation of *m/z* 78.9173 and *m/z* 80.9152 pair corresponding to $[Br]^-$ and, these specific transitions were used for evaluation of the t-MS2 mode efficiency in analysis of HBCD. Contrary to t-MS2 mode, application of the t-SIM mode did not rely on the tandem mass spectrometry option. This type of scanning comprised a full MS scan with an adjusted mass resolution within a defined isolation window, and further registration of target ions with selected *m/z* values. The results of analysis of the standard solutions using above mentioned detection modes showed a strong prevalence of the t-SIM mode over the t-MS2 mode in terms of sensitivity. The t-SIM method provided at least ten times lower LOQs for selected HBCDs in comparison to the t-MS2 method. The chromatograms of spiked butter fish sample (0.40 pg g^{-1} f.w. of each HBCD diastereoisomer) analyzed in the t-MS2 and t-SIM modes are shown in Figure 3.4. The decreased sensitivity of the t-MS2 mode can be logically explained by taking into account the losses/incomplete fragmentation of precursor ions during the collision process in HCD cell, or different detector response factors for ions with different *m/z*. During the analysis of spiked samples no selectivity problems or interferences were observed under the t-SIM conditions. Taking into account the aforementioned results, the t-SIM mode with the registration of two specific fragments corresponding to the $[M-H]^-$ peaks of the respective molecular ion clusters both for the native and $^{13}C_{12}$-labeled HBCDs, and the adjusted mass resolution of 35 000 FWHM was considered to be the most suitable option for the application of high-resolution Q Exactive Orbitrap-HRMS system in analysis of these contaminants.

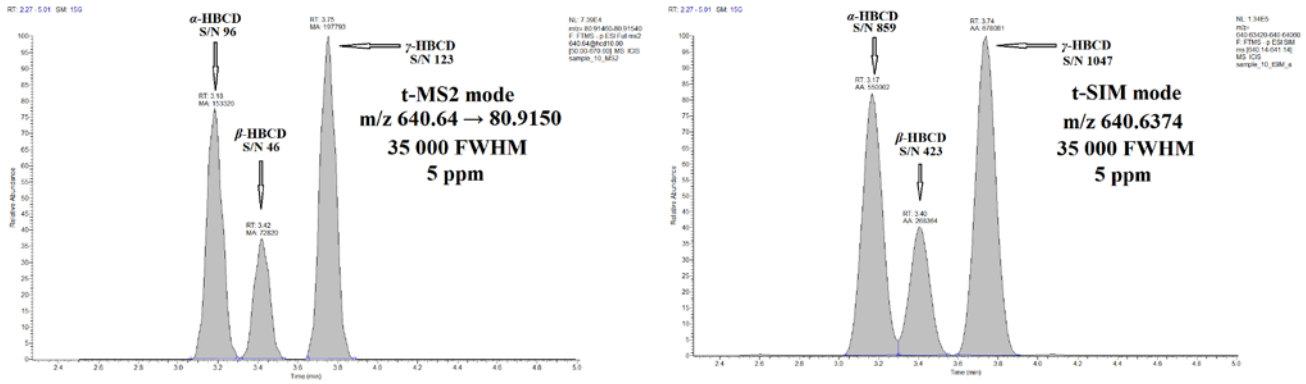


Fig. 3.4. **The chromatograms of spiked butter fish sample (0.40 pg g^{-1} f.w. of each HBCD diastereoisomer) analyzed in the t-MS2 and t-SIM modes**

3.2.2.4. Ion population in the Orbitrap mass analyzer

To provide the most accurate data in terms of spectral resolution, the ion population transferred into the Orbitrap mass analyser should be controlled. This can be achieved by the curved C-trap operated in different modes according to the trapped ion count. The insufficient ion count leads to the signal and spectral instability; on the contrary, if the ion count is too high, the overcharge per unit area results in the "space charging" effect, thus a shift in mass accuracy could be observed. The optimal ion count of 5×10^4 controlled by the automatic gain control (AGC target) option was determined on the basis of S/N ratio, peak area, peak shape, and reproducibility of the results in the analysis of fortified fish oil samples at background concentrations.

3.2.2.5. Orbitrap-HRMS resolution

The use of high resolution mass spectrometry involves a compromise between the selectivity and sensitivity. Increasing of mass resolution provides higher mass accuracy resulting in better selectivity of the developed MS conditions. However, because of the reduced scanning speed provided by the Q Exactive MS system at maximum possible mass resolution mode, selecting the higher possible resolution of 140 000 FWHM in combination with the processing of the data with appropriate scan filter option and smoothing of the raw chromatograms affects the sensitivity and reproducibility of the results obtained at background contamination levels compared to those obtained at lower mass resolution modes. On the contrary, the application of lower resolution modes creates higher background noise and could lead to reduced S/N ratios and false positive/negative results because of the degraded mass accuracy. On the basis of the results obtained in analysis of the spiked samples and naturally contaminated salmon samples employing different mass resolution modes (17 500, 35 000, 70 000 and 140 000 FWHM), a resolution of

35 000 FWHM was found to be optimal for the analysis of HBCD diastereoisomers as a compromise between selectivity, sensitivity and reproducibility of the analytical data. Figure 3.5 illustrates the chromatograms of spiked fish oil sample obtained at different mass resolution modes. The raw data were processed by application of the Fourier Transform Mass Spectrometry (FTMS) filter option and negative ion profile signal acquired in the single stage t-SIM mode within the mass window of *m/z* 640.14 – 641.14. The raw chromatograms were treated employing Gaussian smoothing with the degree of 13 points.

3.2.2.6. Mass extraction window

For confirmation of the analytes of interest in the analyzed samples the exact masses of analyte fragments were extracted within a specific mass window after the UHPLC-MS analysis in t-SIM mode. On the one hand, the narrowing of the mass extraction window during the interpretation of the raw data resulted in increased intensity of selected peaks in comparison to the background, thus improving the S/N ratio for selected fragments and providing better results in terms of selectivity. On the other hand, the significantly narrowed mass extraction window potentially could decrease the sensitivity and provide false negative results. In this study the difference of 5 ppm between the mass window and the theoretical mass of selected fragments was used with no significant matrix interference at the levels around the limit of quantification (LOQ). Figure 3.6 shows the effect of different mass extraction windows during the processing of the raw chromatogram of salmon sample contaminated with β- and γ-HBCDs at background levels.

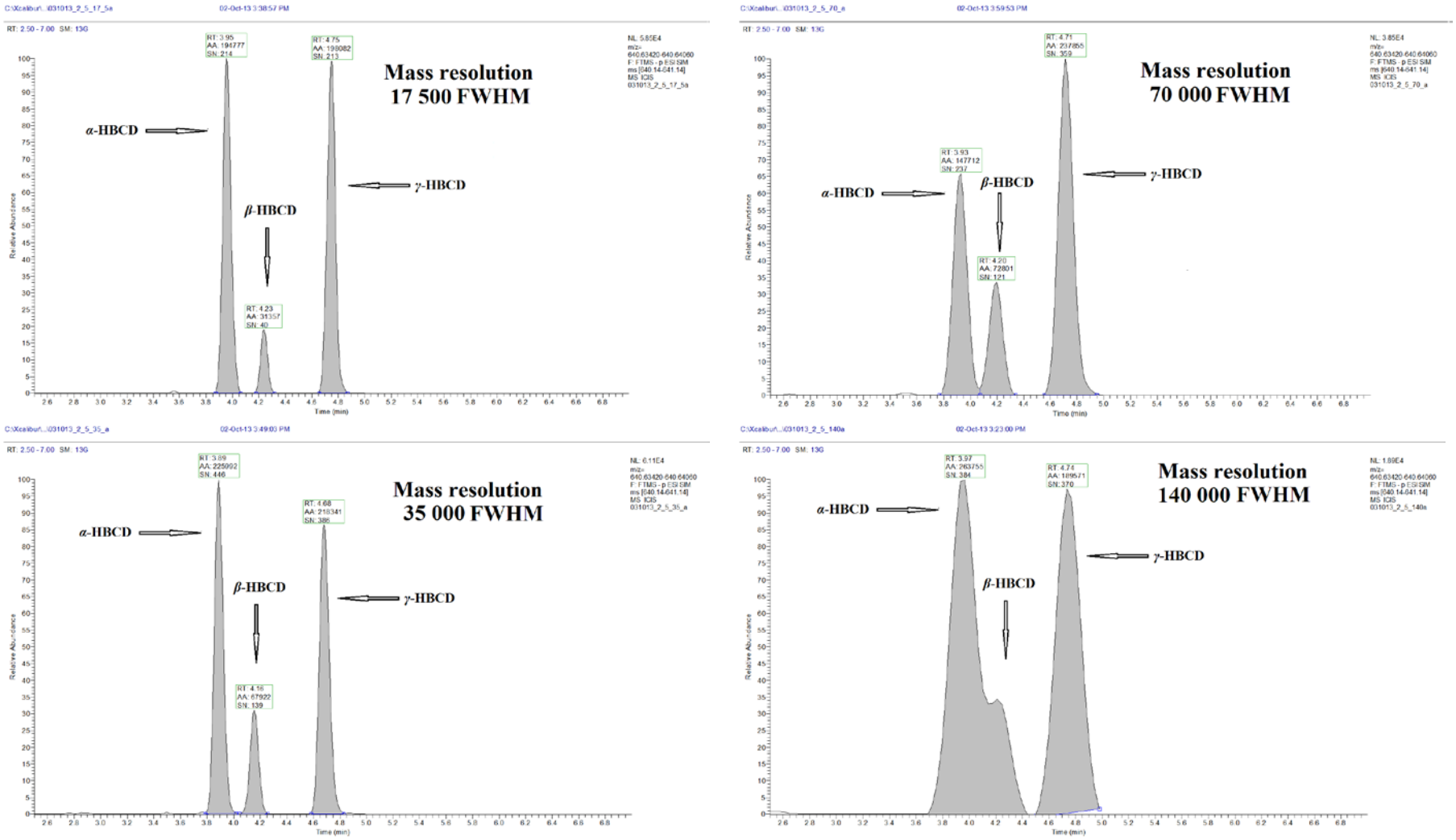


Fig. 3.5. **The effect of using different mass resolution modes in analysis of spiked fish oil at the level corresponding to injection of 10 pg of each HBCD diastereoisomer on the column (t-SIM chromatograms for *m/z* 640.6374 (quantification ion))**

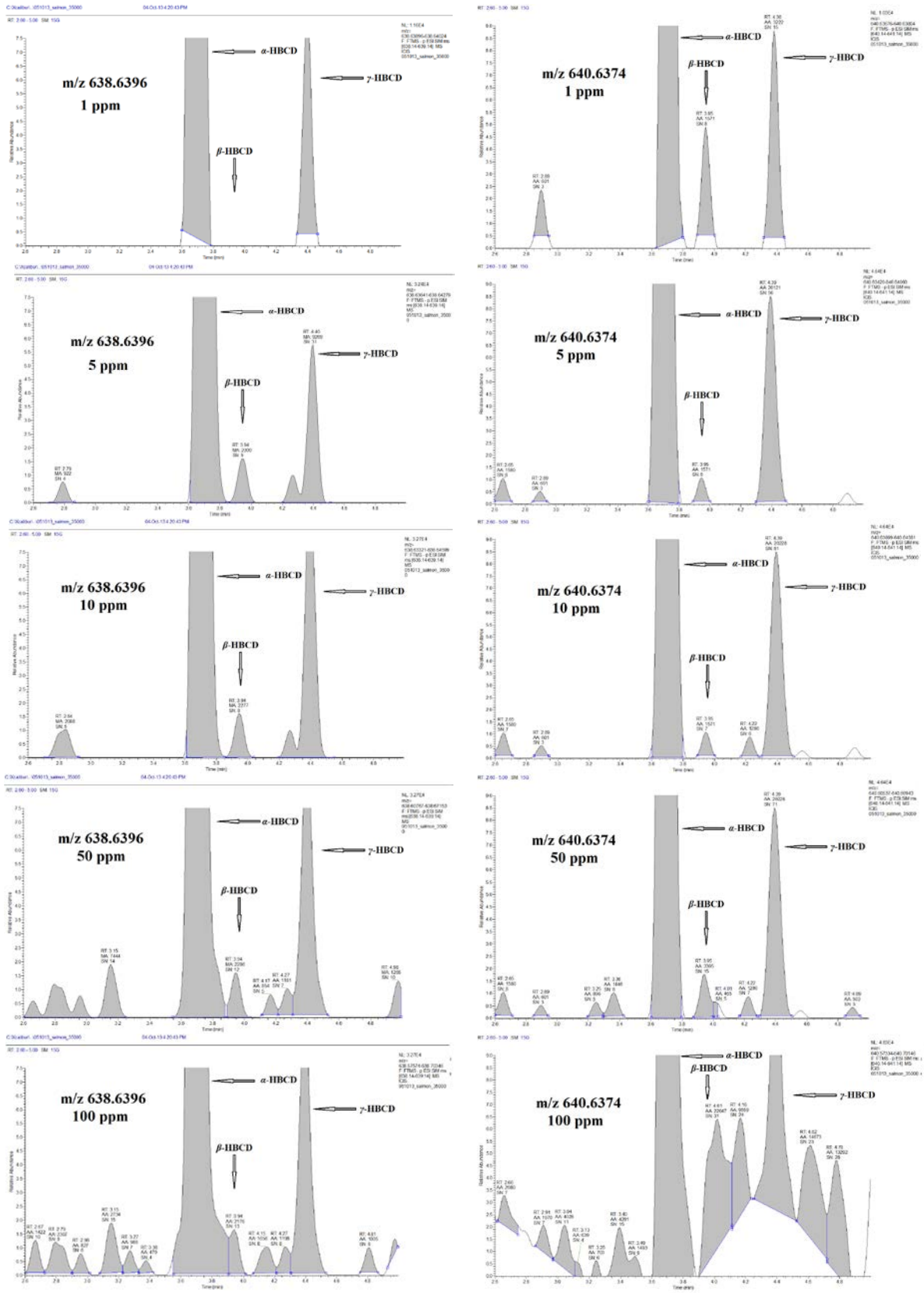


Fig. 3.6. **The effect of different mass extraction windows in the processing of the raw chromatogram of naturally contaminated salmon sample (t-SIM chromatograms for *m/z* 640.6374 (quantification ion) and *m/z* 638.6396 (confirmation ion))**

3.2.3. Orbitrap-HRMS full scan mode capabilities

After targeted analytical method for determination of HBCD diastereoisomers was successfully established, the perspective of HBCD detection in a full scan mode was evaluated. The principal aim of these experiments was to establish the method capability for the detection of target analytes at the levels of interest using non-targeted full scan detection mode, with the opportunity to store and post-process the raw data. The retrospective post-targeted evaluation of experimental data offered the possibility to screen for non-targeted analytes and offered the possibility to simultaneously perform a screening of virtually unlimited number of compounds. The analyses of standard solutions, as well as spiked samples of fish oil and fish tissue were performed using both the t-SIM mode and a full scan mode over the *m/z* range of 50 – 750. The results were compared in terms of S/N ratio for chromatographic peaks of target compounds, by replicated analyses of the same samples using different detection modes. The obtained analytical data showed the possibility of reliable detection and confirmation of selected HBCDs in a full scan mode without degradation of the sensitivity. Thus, it could be concluded that considerable extension of the analyte list and post-targeted detection of new compounds is possible. Moreover, the possible modification of clean-up procedure with the exchange of destructive matrix component removal methods (e.g. acidic digestion) to non-destructive treatment of the sample (e.g. gel permeation chromatography) would provide the possibility to analyse compounds that are labile in the conditions of destructive sample clean-up (e.g. organophosphorus flame retardants) and persistent contaminants in one run, thus a more complete information on the sample contamination status could be obtained.

3.2.4. Comparative evaluation of Orbitrap-HRMS versus TOF-HRMS and MS/MS in analysis of HBCD diastereomers

In order to provide a more complete information regarding the analytical capabilities of the UHPLC-Orbitrap-HRMS additionally to optimization of the instrumental conditions, importance of sample clean-up stages in the detection of HBCD was evaluated. Moreover, taking into account the absence of comparative evaluation of the most frequently used LC-MS based HBCD detection techniques, a detailed information on the analytical capabilities of UHPLC-TOF-HRMS and UHPLC-QqQ-MS/MS systems in the analysis of this contaminant in fish samples, including the influence of sample clean-up steps on the response of the employed MS systems is presented. To compare the examined LC-MS techniques for the analysis of HBCD, the previously mentioned clean-up and UHPLC conditions (Sections 2.4.2.1, 2.4.2.2 and 2.5.2.1) were kept constant and three types of MS systems – TOF-HRMS, Orbitrap-HRMS, and QqQ-MS/MS were applied for the

detection of this BFR in fish. The elaborated analytical methods were robustly validated and used for the analysis of eel samples collected in Latvia, and these results were compared.

One of the most important factors that can affect the performance of the LC-MS system is the signal suppression caused by matrix components. Several papers in the field of LC-MS are devoted on this effect, and it could be seen from the literature that MS systems coupled to ESI are more vulnerable to signal suppression in comparison to atmospheric-pressure chemical ionization (APCI) or atmospheric-pressure photo-ionization (APPI) techniques [138,139]. The signal suppression effect depends mostly on the efficiency of ionization interface of the LC-MS system and is not connected to the type of MS analyzer. The possible reasons of the signal suppression are: 1) the charge competition between the analyte and signal suppressing substances, resulting in a decreased conductivity of the liquid phase and reduced ionization of the analyte; 2) the reduction of the droplet evaporation efficiency due to the increasing surface tension and viscosity of the liquid phase in the presence of large amounts of signal suppressing substances; 3) the reduction of the ionization efficiency due to the reactions in gas phase between the analyte and signal suppressing molecules [138]. Due to the complexity of the food and environmental matrices, the primary concern of the signal suppression phenomenon in LC-MS is the severe decrease of the sensitivity of the method. An investigation of the importance of proposed clean-up steps was carried out by evaluation of the signal suppression for three LC-MS techniques. The influence of the signal suppression effect was estimated by adding known amounts of analytes of interest to the final sample extracts, which were processed using different clean-up steps (or their combinations). The influence of the sample weight taken for analysis was evaluated as well. The S/N ratios, which were calculated for the chromatographic peaks of HBCD diastereomers obtained for the investigated extracts, were compared to those obtained for standard solution of equivalent analyte concentrations in the mobile phase. The data for the analyte solution in mobile phase provide a relative 100% response (S/N) value, whereas the data for the same amount of compound added to processed samples show the effect of sample matrix on MS response and on the obtained S/N. The Figure 3.7 shows decreasing S/N values obtained by the investigated LC-MS techniques for each HBCD diastereomer, by application of different clean-up steps or their combinations and using different sample amounts for analysis.

Firstly, the efficiency of destructive (acidic treatment) and non-destructive (GPC) approaches for removing matrix components was evaluated. Both methods and their combinations are well known in the sample preparation for POP analysis (particularly for HBCD [115]). Acidic treatment was chosen as one of the most efficient and simplest ways to remove high molecular matrix components (e.g., lipids) from the sample extract. However, it should be noted that such destructive

approach could be used only when the stability of the compounds of interest under the harsh conditions of acidic treatment is assured. On the contrary, GPC provides a non-destructive procedure for high molecular compound removal, which is based on the separation of the sample components according to molecular size, and this type of chromatography offers great advantages in the sample preparation for potentially labile compounds, particularly for non-targeted analysis [81]. Nevertheless, the efficiency of the separation of high molecular matrix components is usually not more than 95%, therefore additional clean-up steps are needed in some cases. The results of our study indicate that significant signal suppression effects could be observed in the analysis of HBCD using the examined LC-MS methods. The UHPLC-TOF-HRMS system seems to be most influenced by the signal suppression in comparison with UHPLC-QqQ-MS/MS and UHPLC-Orbitrap-HRMS. Application of the one-stage clean-up protocol including only acidic treatment of the sample extract or GPC caused a more than 90% sensitivity drop for the UHPLC-TOF-HRMS system, while the analytical response of the UHPLC-QqQ-MS/MS and UHPLC-Orbitrap-HRMS systems was suppressed by about 50%. No significant differences between these analytical techniques in terms of signal suppression were obtained neither with destructive nor non-destructive clean-up.

A significant improvement of S/N values was achieved by implementing an additional adsorption chromatography clean-up stage on a Florisil column. For UHPLC-TOF-HRMS system the S/N ratio for chromatographic peaks due to analytes of interest could reach up to 50% of the instrumental response obtained for standard solution. For the UHPLC-QqQ-MS/MS and UHPLC-Orbitrap-HRMS systems, the application of a two-stage clean-up procedure including removal of high molecular substances and Florisil column chromatography could provide S/N ratios up to 80 – 90% of the ratios obtained for standard solution. Florisil column chromatography provides better sensitivity of analysis because the signal suppressors originating from the matrix could be either fractionated or permanently adsorbed during this clean-up stage. Moreover, taking into account the fact that fish samples usually contain significant amounts of organobromines (e.g. PBDEs) which could potentially interfere with the mass spectrometric analysis of HBCD by providing similar mass fragments during the ionization of the sample extract, the using of Florisil column could ensure an additional benefit due to the ability to isolate these potential mass interferants in separate fractions.

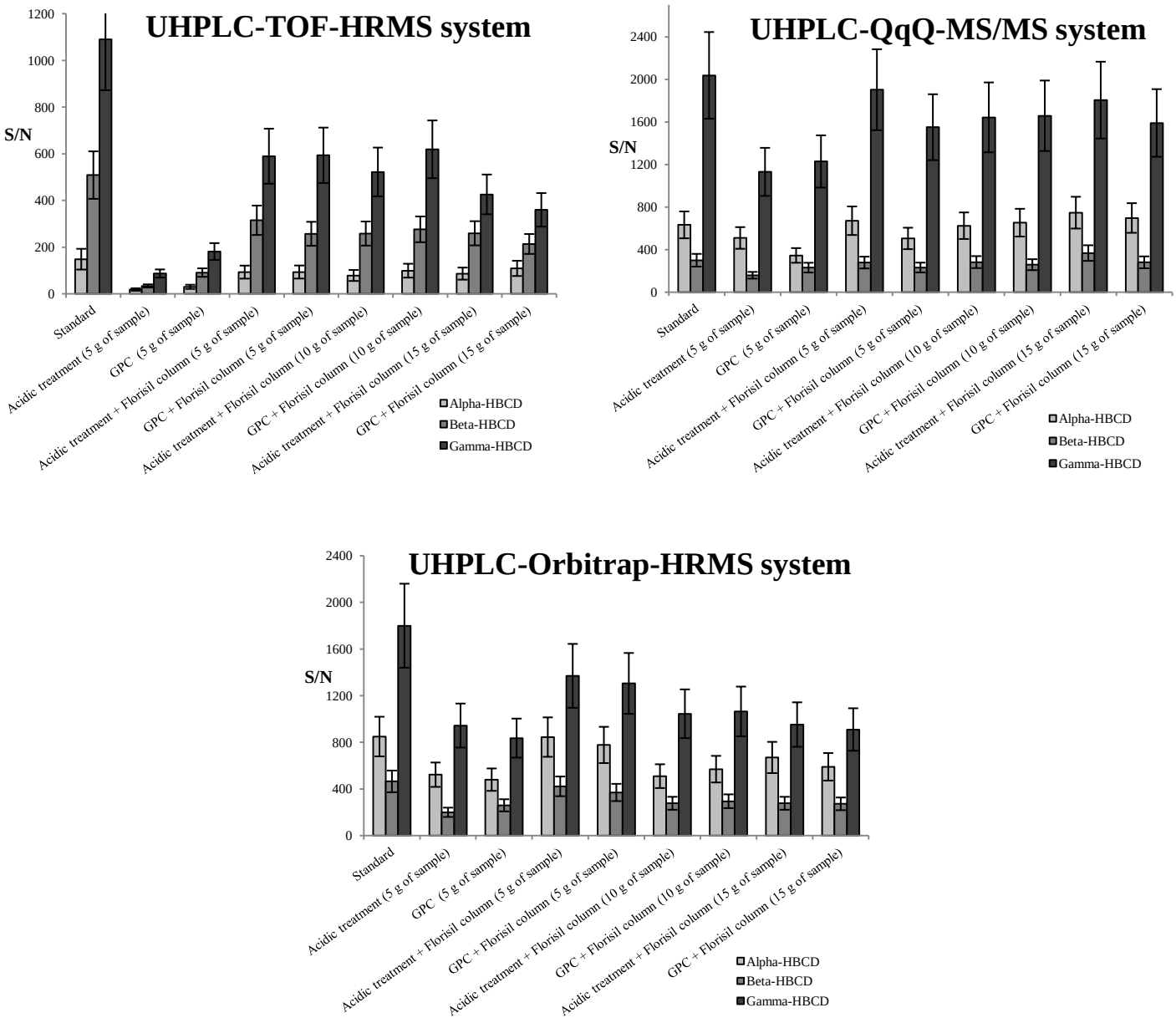


Fig. 3.7. **Changes of the S/N values obtained for the investigated LC-MS techniques for each HBCD diastereomer by application of different clean-up steps or their combinations and different sample amounts taken for analysis**

In addition to evaluating the importance of the proposed clean-up steps or their combinations, the influence of the sample amount taken for analysis was investigated for aliquots of 5, 10, and 15 grams of butter fish homogenate. The observed degree of signal suppression indicates that this effect plays a significant role, and it was more expressed for UHPLC-TOF-HRMS and UHPLC-Orbitrap-HRMS systems, while the UHPLC-QqQ-MS/MS system seems to be more robust in terms of this phenomenon (Figure 3.7).

3.3. Validation of the developed method

3.3.1. Validation of GC-HRMS method for analysis of PCDD/Fs, PBDD/Fs, PXDD/Fs, PCBs, PXBs and PBDEs

Since there were no specific requirements or guidelines for the validation of analytical procedures for determination of PBDD/Fs, PXDD/Fs, PXBs and PBDEs a validation protocol based on the criteria of Commission Regulation (EU) No 252/2012 which related to the criteria for PCDD/Fs and PCBs was used [129]. The validation study was performed in terms of recovery, precision and limit of quantification. The experiments were carried out at two spiking levels with six parallel samples and each fortification level was repeated on two different days. The precision, repeatability and recovery were calculated for fish oil spiked with native compounds. The detailed validation results are summarized in Table 3.2. For reproducibility estimation of the developed method within laboratory, in-house prepared reference material (freeze-dried salmon tissue) was used which was naturally contaminated with PCDD/Fs, PCBs and PBDEs and was fortified with native PBDD/Fs, PXDD/Fs and PXBs. Repeated analyses of this material were performed in different routine sample sequences. The results showed good consistency at selected fortification levels. Reproducibility for selected compounds appeared to be similar to that found for PCDD/Fs and PCBs in routine analyses. There were no proficiency tests or certified reference materials for PBDD/Fs, PXDD/Fs and PXBs, but in order to confirm the performance in persistent organic pollutant analysis, our laboratory successfully participated in international proficiency tests for determination of PCDD/Fs, PCBs, PBDEs and HBCDs in food and feed matrices organized by European Union Reference Laboratory for Dioxins and PCBs in Feed and Food, Freiburg, Germany.

In spite of multi-stage sample preparation and fractionation, typical quantitative recoveries of used $^{13}C_{12}$-labeled internal standards were in the range 60 – 110% with exception of $^{13}C_{12}$-labeled octa-brominated and octa-chlorinated dibenzo-*p*-dioxins and furans and octa- through deca-brominated diphenyl ethers, for which typical recoveries were in the range of 30 – 50%. The reagent blanks were free of PBDD/Fs and PXDD/Fs. Some insignificant concentrations of PCDD/F, PBDE and PCB congeners were usually found in the reagent blank extracts and the calculated concentrations in the samples were subjected to correction. The limits of quantification (LOQ) of selected POPs for the developed analytical procedure were calculated on the basis of investigated unspiked matrix. As it was anticipated, the sensitivity of the method decreased with higher degree of halogen substitution in the molecules of investigated compounds. This phenomenon was particularly emphasized for PBDD/Fs and, to a lesser extent, for PXDD/Fs, as expected from the thermal lability of highly halogenated organobromines [86,140]. The sensitivity of the elaborated

method for PBDD/Fs and PXDD/Fs was in the range of 0.03 – 1.6 pg g^{-1} fat, which was sufficient for determination of these contaminants in fish, while taking into account the toxicological properties and typical distribution of these compounds in aquatic biota.

Table 3.2.

Validation results for PBDD/F, PCDD/F, PXDD/F, PXB, PCB and PBDE groups

Congener group	Linearity of measurement, pg	LOQ [a], pg g^{-1} product	1st spiking level				2nd spiking level			
			Spiking level [a], pg g^{-1} fat	Recovery (n=2) [b], %	Intra-day presision, (n=2) [c], %	Inter-day presision (n=2) [d], %	Spiking level [a], pg g^{-1} fat	Recovery (n=2) [b], %	Intra-day presision, (n=2) [c], %	Inter-day presision (n=2) [d], %
PBDD/Fs	0.05 – 30	0.04 – 1.6	0.53 – 4.5	86 – 117	5 – 23	4 – 24	1.1 – 8.9	81 – 117	3 – 18	3 – 17
PCDD/Fs	0.05 – 200	0.02 – 0.11	0.25 – 2.5	90 – 120	3 – 13	3 – 14	0.50 – 5.0	94 – 119	2 – 10	2 – 10
PXDD/Fs	0.05 – 3.8	0.03 – 0.10	0.33 – 1.3	92 – 106	7 – 12	8 – 14	0.67 – 2.7	89 – 114	3 – 15	4 – 14
PXBs	0.05 – 20	0.03 – 0.11	0.33 – 1.3	89 – 108	4 – 14	4 – 15	0.67 – 2.7	87 – 117	2 – 8	3 – 9
DL-PCBs	0.10 – 200	0.17 – 0.34	100	94 – 115	2 – 8	4 – 9	200	93 – 114	2 – 4	3 – 5
NDL-PCBs	1.0 – 700	0.25 – 0.65	620 – 940	81 – 120	4 – 9	6 – 9	1200 – 1900	84 – 120	6 – 9	6 – 10
PBDEs	0.50 – 500	0.11 – 2.2	190 – 940	75 – 123	3 – 10	4 – 11	380 – 1900	79 – 120	5 – 10	6 – 10

[a] – parameters differ for individual congeners depending on halogenation degree;
[b] – recovery range (%) for selected contaminant group for corresponding fortification level calculated from the data obtained on two different days;
[c] – intraday precision range (%) for selected contaminant group for corresponding fortification level calculated from the data obtained on two different days;
[d] – interday precision range (%) for selected contaminant group for corresponding fortification level calculated from the data obtained on two different days.

3.3.2. Validation of UHPLC-Orbitrap-HRMS method for analysis of HBCDs and comparison of performance characteristics versus UHPLC-TOF-HRMS and UHPLC-QqQ-MS/MS

Since a target-oriented approach was used for the determination of HBCD diastereomers, destructive acidic treatment clean-up protocol with additional purification stage on a Florisil column was chosen for validation exercises as the simplest and less time consuming in comparison with a GPC based clean-up procedure. Essential analytical characteristics such as linearity, accuracy (recovery), repeatability (intra-day precision), intermediate precision (inter-day precision), instrumental limit of quantification (i-LOQ) and method limit of quantification (m-LOQ) were examined in order to evaluate the analytical performance of the compared analytical procedures. The methods were provisionally validated using butter fish homogenate, and the performance of the methods was evaluated by run-to-run (n=5) and day-to-day (n=3) analyses of spiked matrix at three concentration levels (200, 1000, and 2000 pg g^{-1} f.w. of each HBCD diastereomer). The repeatability and intermediate precision were expressed as RSD from the results obtained during the recovery experiments. Table 3.3 outlines the above mentioned analytical performance parameters for the three applied MS systems.

During the linearity experiments, both the matrix matched and solvent matched calibration experiments were performed at five calibration levels from 1.0 to 100 pg μL^{-1}, and each calibration solution was analyzed in triplicate. The working range was selected by taking into account the typical distribution profiles (e.g., strong predominance of α-HBCD in comparison to β-and γ-HBCDs in biota samples and predominance of the γ-HBCD for environmental objects), and the levels of HBCD diastereomers in the most frequently analyzed objects, as well as sample intake and the amount of analyte injected on-column limited by the final volume and injection volume of the sample extract. For the Orbitrap-HRMS and QqQ-MS/MS instruments, the equations of the calibration curves were fitted to a linear function and the relationship obtained by internal standard method was found to be rectilinear with correlation coefficients of 0.995 or greater, and residual values less than 15% for both matrix matched and solvent matched calibration experiments. There were no differences in the plots of calibration curves obtained by matrix matched and solvent matched linearity experiments. Contrary to the Orbitrap-HRMS and QqQ-MS/MS techniques, for TOF-HRMS the non-linear calibration curve was observed within the examined working range from 1.0 to 100 pg μL^{-1} for all analyzed compounds, thus a quadratic type of the curve was selected as an appropriate fit. There are several mentions in the literature on non-linear behavior of the TOF-HRMS techniques, which could be attributed to analyte "saturation" effect during the charge competition phenomena inside the analyzer [141-143]. The Figure 3.8 shows the typical matrix

matched quadratic type standard curve for *α*-HBCD. Although the non-linear relationship for the TOF-HRMS technique may at first glance be considered as a disadvantage for quantitative determination, correlation coefficients of 0.999 or greater were obtained for the quadratic type calibration curves for all three HBCD diastereomers within the working range, and these were successfully used for quantification purposes during the validation experiments and analyses of real samples. Similarly to Orbitrap-HRMS and QqQ-MS/MS techniques, in the case of TOF-HRMS system no difference was observed between the matrix matched and solvent matched calibration plots.

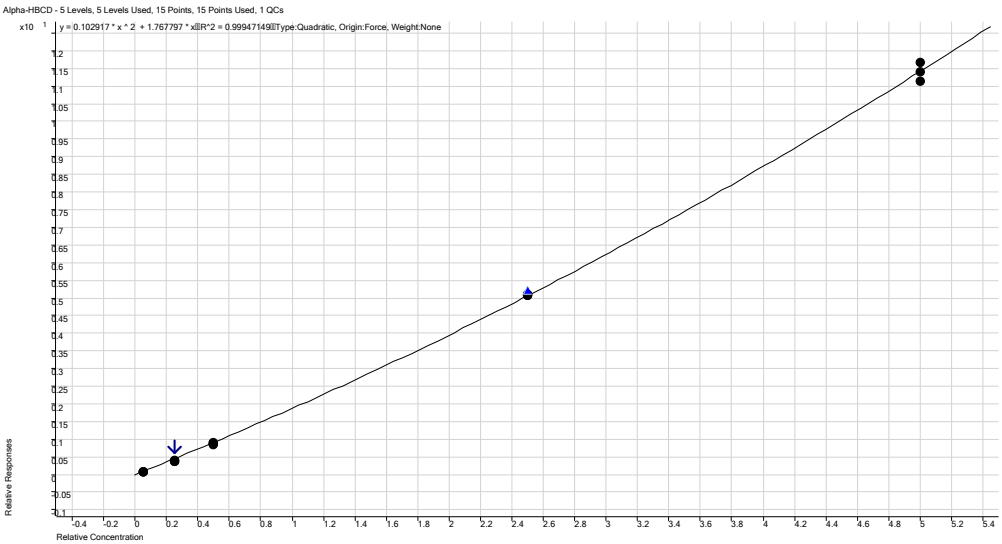


Fig. 3.8. **An example of the matrix matched quadratic type standard curve for *α*-HBCD within the working range from 1.0 to 100 pg µL^{-1}, generated by the TOF-HRMS system**

There was a good agreement between the studied LC-MS techniques in terms of recovery, repeatability, and intermediate precision. According to the obtained results, the evaluated instruments demonstrated similar performance in diastereoselective analysis of HBCD. All three applied analytical systems provided adequate recovery values at 200, 1000, and 2000 pg g^{-1} f.w. for each HBCD diastereomer, which were as follows: *α*-HBCD 102 – 114%, *β*-HBCD 99 – 116%, and *γ*-HBCD 99 – 112%. The UHPLC-QqQ-MS/MS system provided a slightly better repeatability and intermediate precision in comparison to the other two studied techniques. The RSD values of run-to-run and day-to-day validation experiments were 1 – 7% and 2 – 8%, respectively.

The i-LOQ values for the studied HBCD diastereomers were all within a similar range and suitable for confirmatory purposes. The S/N ratio used for the calculation of i-LOQ values was 10:1. The calculated values for i-LOQ for the studied LC-MS techniques were expressed as analyte amount injected on-column and were the following: 1.1 – 4.5 pg for *α*-HBCD, 1.4 – 3.0 for *β*-

HBCD, and 0.7 – 1.4 pg for γ-HBCD, respectively. The m-LOQ values were assessed by calculations taking into account the sample preparation procedure (sample weight taken for analysis and the final volume of the sample extract), and the signal suppression effect obtained by using acidic treatment procedure with additional purification on Florisil column. The m-LOQs were expressed as pg g^{-1} of sample f.w., and were in the range from 4.0 to 29 pg g^{-1} for α-HBCD, from 7.0 to 13 pg g^{-1} for β-HBCD, and from 4.0 to 7.0 pg g^{-1} for γ-HBCD.

Table 3.3.

Analytical characteristics of the compared LC-MS techniques for analysis of HBCD diastereomers

Compound	Linearity of measurement, pg μL^{-1}	UHPLC-QqQ-MS/MS			UHPLC-Orbitrap-HRMS			UHPLC-TOF-HRMS		
		Detection traces	i-LOQ, pg	m-LOQ, pg g^{-1} f.w.	Detection traces	i-LOQ, pg	m-LOQ, pg g^{-1} f.w.	Detection traces	i-LOQ, pg	m-LOQ, pg g^{-1} f.w.
α-HBCD	1.0 - 100	640.6→78.9/80.9	1.3	5.0	638.6396/640.6374	1.1	4.0	638.6396/640.6374	4.5	29
β-HBCD	1.0 - 100	640.6→78.9/80.9	2.1	9.0	638.6396/640.6374	3.0	13	638.6396/640.6374	1.4	9.0
γ-HBCD	1.0 - 100	640.6→78.9/80.9	0.9	4.0	638.6396/640.6374	1.4	7.0	638.6396/640.6374	0.9	7.0
1st validation level										
Compound	Spiking level, pg g^{-1} f.w.	UHPLC-QqQ-MS/MS			UHPLC-Orbitrap-HRMS			UHPLC-TOF-HRMS		
		Recovery (n=3)[a], %	Intra-day precision, (n=3)[b], %	Inter-day precision (n=3)[c], %	Recovery (n=3)[a], %	Intra-day precision, (n=3)[b], %	Inter-day precision (n=3)[c], %	Recovery (n=3)[a], %	Intra-day precision, (n=3)[b], %	Inter-day precision (n=3)[c], %
α-HBCD	200	109	3	4	114	4	4	104	5	5
β-HBCD	200	103	3	4	111	7	7	102	5	5
γ-HBCD	200	107	3	4	112	5	5	99	2	4
2nd validation level										
Compound	Spiking level, pg g^{-1} f.w.	UHPLC-QqQ-MS/MS			UHPLC-Orbitrap-HRMS			UHPLC-TOF-HRMS		
		Recovery (n=3)[a], %	Intra-day precision, (n=3)[b], %	Inter-day precision (n=3)[c], %	Recovery (n=3)[a], %	Intra-day precision, (n=3)[b], %	Inter-day precision (n=3)[c], %	Recovery (n=3)[a], %	Intra-day precision, (n=3)[b], %	Inter-day precision (n=3)[c], %
α-HBCD	1000	107	2	3	106	4	8	110	3	3
β-HBCD	1000	104	6	7	105	3	5	112	3	4
γ-HBCD	1000	103	2	2	105	2	3	106	3	3
3rd validation level										
Compound	Spiking level, pg g^{-1} f.w.	UHPLC-QqQ-MS/MS			UHPLC-Orbitrap-HRMS			UHPLC-TOF-HRMS		
		Recovery (n=3)[a], %	Intra-day precision, (n=3)[b], %	Inter-day precision (n=3)[c], %	Recovery (n=3)[a], %	Intra-day precision, (n=3)[b], %	Inter-day precision (n=3)[c], %	Recovery (n=3)[a], %	Intra-day precision, (n=3)[b], %	Inter-day precision (n=3)[c], %
α-HBCD	2000	104	1	3	102	3	5	105	6	6
β-HBCD	2000	99	4	4	103	2	3	116	7	8
γ-HBCD	2000	101	2	2	103	3	4	101	6	6

[a] – average recovery (%) for a selected diastereomer at the corresponding fortification level, calculated from the data obtained on three different days;
[b] – average intra-day precision (%) for a selected diastereomer at the corresponding fortification level, calculated from the data obtained on three different days;
[c] – average inter-day precision (%) for a selected diastereomer at the corresponding fortification level, calculated from the data obtained on three different days.

In order to compare the applicability of the elaborated LC-MS procedures for analysis of real fish samples, and to obtain the information on the actual levels of HBCD diastereomers in eels from Latvian lakes (Section 2.2.2), the determination of three HBCD diastereomers in eight samples was performed. The samples were extracted and purified according to the destructive acidic treatment clean-up protocol with the additional purification stage on Florisil column described in Section 2.4.2.1. The obtained extracts were analyzed using the above mentioned LC-MS systems as rapidly as possible after the sample preparation to prevent the possible changes of the final extracts. As it could be seen from the results, good agreement was observed between the analytical data obtained by applying the studied LC-MS systems for the analysis of fish samples contaminated with HBCD at sub-ppb levels (Figure 3.9). The maximum RSD between total-HBCD concentrations obtained for the analyzed samples was 9%. The RSD values between the concentrations obtained for α- and β-HBCDs were in the range of 3 to 9% and 3 to 27%, respectively. Higher deviations were obtained at concentrations near the m-LOQ. Thus, for γ-HBCD, which was generally found in the samples at concentrations near the m-LOQ, the RSD values varied from 5% up to 60%. The total-HBCD and individual diastereomer concentrations obtained with each studied LC-MS technique were statistically compared using a Friedman non-parametric statistical test and the probability value (*p*-value) was evaluated. The *p*-value was used to decide whether or not the null hypothesis (H_0) according to which the results come from the same population, was true. In the case if the *p*-value for some of components was smaller than the pre-established significance level ($\alpha = 0.05$), the H_0 was rejected, indicating that the alternative hypothesis could be true. No statistically significant differences were found for α-, β-, γ-, and total-HBCD with the *p*-values 0.197, 0.054, 0.065, and 0.233, respectively, and therefore these results indicated that the studied techniques produced adequate and similar results on HBCD content in fish samples. However, it should be pointed out that the Friedman test was performed only for samples in which all analytes were detected above the m-LOQ, in order to obtain more realistic results. In the case of calculating *p*-values for upperbound analyte concentrations (if the analyte was not detected in the sample or detected at concentrations below the m-LOQ, the m-LOQ was used for calculation), γ-HBCD showed *p*-value of 0.046, thus the H_0 for this component should be rejected. Nevertheless, the factor of statistical significance could not be estimated in such a way with high degree of confidence, because of the inability to provide the real analyte concentration in the sample, but giving only the most pessimistic approximation on the occurrence of the contaminant in the analyzed object. Moreover, the concentrations of β- and γ-HBCDs determined in the analyzed samples were in the range near the m-LOQ (especially for the γ-diasteroisomer), and this could be an additional source of increased dispersion of the results due to the higher measurement uncertainty at these low levels.

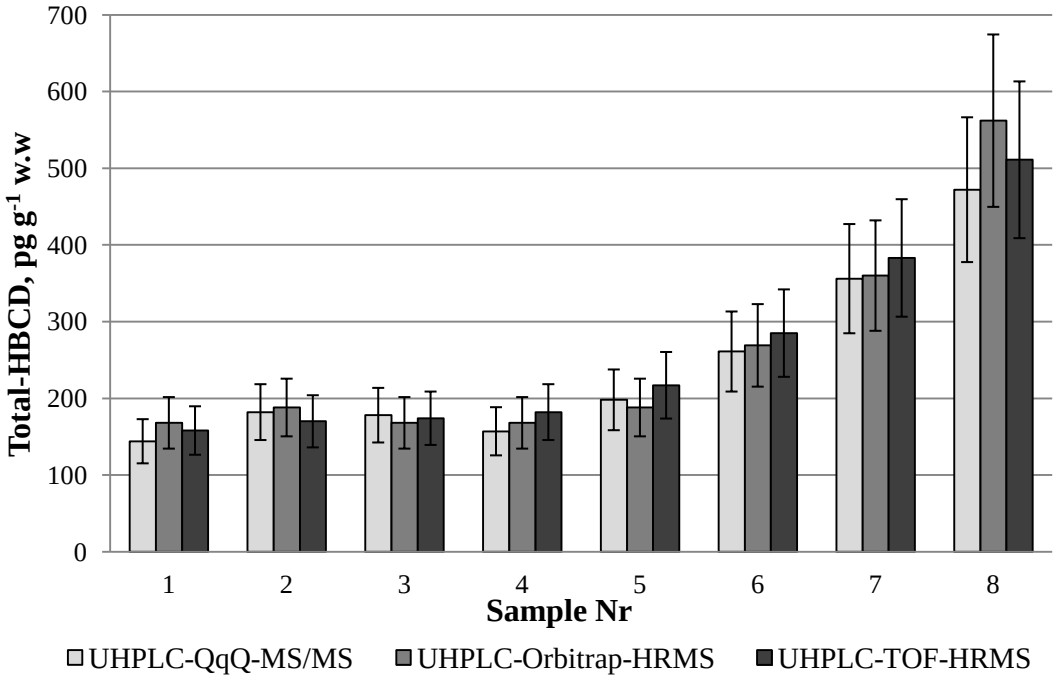


Fig. 3.9. **Comparison of the total-HBCD concentrations in eel samples obtained by applying different LC-MS techniques (error bars indicate measurement uncertianty of 20%)**

3.4. Application of the method to fish samples and evaluation of contamination status of fish from Baltic region

The developed methodology was used in a study of fish samples (Section 2.2) for the quantitative determination of selected persistent organic pollutants. Taking into account the bioaccumulative properties and formation mechanisms of poorly studied PBDD/Fs, PXDD/Fs and PXBs, the presence of these compounds in fish tissues might be expected. Due to the lack of analytical standards, from the wide range of possible bromo-, and particularly mixed bromo/chloro-substituted congeners, only selected PBDD/Fs, PXDD/Fs and PXBs were analyzed. For the 17 PCDD/Fs and 12 DL-PCBs, which had $TEFs_{2005}$ set by the WHO, the rounded result of each congener was multiplied by the corresponding TEF [6], and TEQs were calculated by summarizing the obtained values for selected POP groups. For comparative purposes for eel samples TEQ values for PCDD/Fs and DL-PCBs were additionally calculated using $TEFs_{1998}$ [5]. Because the TEFs have not been established for PBDD/Fs and PXDD/Fs, for tentative estimation of the possible toxicity risks of salmon tissue caused by the presence of these compounds, the TEQs for these contaminant groups were estimated using the corresponding $TEFs_{2005}$ of chlorinated analogs. Results were expressed as upperbound (UB) and lowerbound (LB) values and are expressed on a f.w. basis. Annexes 4 and 6 represent detailed PCDD/F and PCB congener concentrations in the

Baltic wild salmon. Length, weight, lipid content and place of sampling where the corresponding specimen was collected are also included. For salmon samples age and sex additionally reported.

3.4.1. Levels and congener profiles of POPs in Baltic wild salmon samples

PCDD/Fs and PCBs. The data showed an extremely high level of Baltic wild salmon contamination with PCDD/Fs and DL-PCBs, and this information is in good agreement with previous studies on Baltic wild salmon [144,145]. The congener profile typical of Baltic Sea fish was observed for all samples, and the major contributors to the total-WHO_{2005}-TEQ were tetra-, and penta-chlorinated DD/Fs and PCBs 118, 126 and 156. For most of the analyzed salmon samples, MLs established by European Commission [146] for PCDD/Fs and DL-PCBs were exceeded. The analyzed samples showed PCDD/F concentrations in the range of 2.1 – 6.1 pg WHO_{2005}-PCDD/F-TEQ g^{-1} f.w., with an average of 3.8 pg WHO_{2005}-PCDD/F-TEQ g^{-1} f.w., and DL-PCBs were found in the concentration range of 2.4 – 8.5 pg WHO_{2005}-PCB-TEQ g^{-1} f.w. Only for six samples out of the twenty five analyzed the ML of 6.5 pg total-WHO_{2005}-TEQ g^{-1} f.w. was not exceeded. The results are in agreement with the fact that salmon is the largest fish in the Baltic Sea, at the top of food chain, and tend to accumulate high levels of chlorinated POPs.

The NDL-PCBs represented in the samples had a wide range of concentrations, depending on the congener. The total sum of NDL-PCB concentrations was within the range of 26 – 65 ng g^{-1} f.w., with an average of 46 ng g^{-1} f.w. The congener pattern of NDL-PCBs in the samples consisted mainly of penta-, hexa-, and hepta-chlorinated biphenyls and was in good agreement with previous studies of the Baltic fish [130,147]. Out of the analyzed PCBs, the group of so called indicator PCBs (namely PCBs: 28, 52, 101, 138, 153 and 180) represented about 80% of total PCB concentration, with the main contributors PCB 153 and PCB 138. Other significant congeners were PCB 99 and PCB 110, which constituted about 13% of the total measured PCB concentration.

In 2011, the maximum level of 75 ng g^{-1} f.w. for $\sum_{ind.PCB}$ in fish was included in Commission Regulation (EU) No 1259/2011 [146]. Analyzed salmon samples show the levels for this parameter in the range of 20 – 51 ng g^{-1} f.w. with an average of 37 ng g^{-1} f.w. According to the obtained results, none of the analyzed salmon samples exceeded the ML. These levels are in line with a recent study [145], in which 2-year salmon samples collected in the open Baltic Sea showed the $\sum_{ind.PCB}$ in the range of 39 – 55 ng g^{-1} f.w., while the $\sum_{ind.PCB}$ concentration in salmon from the Gulf of Finland and Bothnian Sea was up to 2 times higher.

PBDD/Fs and PXDD/Fs. Deatiled information on the occurence of PBDD/Fs and PXDD/Fs in analyzed salmon samples is represented in Annex 5. The analyzed salmon samples contained a wide range of detectable PBDD/F congeners with the domination of PBDFs, which was in

accordance with the formation mechanisms of these compounds and reflected the typical composition of these contaminants in industrial emissions [47], food [57,58,74] and environmental matrices [148,149]. Three congeners (1,2,3,7,8-PentaBDF, 1,2,3,7,8-PentaBDD and OctaBDD) were not detected above the LOQ in any of samples. The most abundant congeners 2,3,7,8-TetraBDF, 1,2,3,4,6,7,8-HeptaBDF and OctaBDF were detected in the range of 0.16 – 0.92 pg g^{-1} f.w., on average 0.41 pg g^{-1} f.w.; 0.27 – 1.4 pg g^{-1} f.w., average 0.76 pg g^{-1} f.w. and 0.32 – 2.1 pg g^{-1} f.w., average 0.88 pg g^{-1} f.w., respectively. In most cases, the concentrations of PBDDs were below the LOQ, with the exception of 1,2,3,4,6,7,8-HeptaBDD, which was found in all samples. Potentially the most significant from toxicological point of view was 2,3,7,8-TetraBDD, which was detected above the LOQ in four of twenty five samples in the concentration range from 0.002 to 0.01 pg g^{-1} f.w., with an average of 0.01 pg g^{-1} f.w. Only one HexaBDF congener (1,2,3,4,7,8-HexaBDF) was analyzed in this study, and it was present in all samples in the range from 0.03 and 0.13 pg g^{-1} f.w., with an average value of 0.08 pg g^{-1} f.w. Chromatographically unseparated 1,2,3,4,7,8/1,2,3,6,7,8-HexaBDD congeners and 1,2,3,7,8,9-HexaBDD were each detected in four samples with average concentrations of 0.01 pg g^{-1}.

Due to the fact that very few references are available regarding PBDD/F content in biota, the systematization and evaluation of these data is problematic. Available references emphasized mainly the low brominated components (e.g. di-, through hexa-brominated congeners). Generally PBDD/Fs are far less represented in environmental samples and food products compared to their chlorinated analogs, and at the moment there are no established MLs for these contaminants. For tentative estimation of the possible toxicity risks of salmon tissue caused by presence of PBDD/Fs, TEQs were calculated as described above. Taking into account the $TEFs_{2005}$ [6], the analyzed salmon samples show PBDD/F-TEQ (UB) concentrations in the range of 0.04 – 0.14 pg g^{-1} f.w., with an average of 0.07 pg g^{-1} f.w. On the basis of the obtained results, it could be concluded that the presence of PBDD/Fs in the analyzed salmon samples is less pronounced than the chlorinated analogs, and therefore the PBDD/F-TEQ values are considerably lower compared to those found for PCDD/F-TEQs. The approximate calculations on the upper bound basis indicate that for the analyzed salmon PBDD/F-TEQ could contribute to the total PCDD/F-PBDD/F-TEQ in the range from 1% to 4%, with an average of 2%. Although the average contribution of PBDD/Fs to the aggregated POP contamination background of Baltic wild salmon is of secondary importance, calculation of individual congener concentrations and PBDD/F-TEQ values on lipid weight basis in some cases shows dramatically high levels of these contaminants. Some analyzed salmon samples reached contamination up to 4.8 pg PBDD/F-TEQ g^{-1} l.w., what might be a source of concern due to the tendency of such POPs to bioaccumulate and migrate through food chains.

The profile of thirteen 2,3,7,8-substituted PBDD/F congeners analyzed in tissue of Baltic wild salmon and the contribution of each congener to PBDD/F-TEQ is shown in Figure 3.10. The OctaBDF was the predominant congener, followed by 1,2,3,4,6,7,8-HeptaBDF and 2,3,7,8-TetraBDF (Figure 3.10 A). These congeners constitute more than 90% of the total sum of detected 2,3,7,8-substituted PBDD/F concentrations. Although the impact of the 2,3,7,8-TetraBDF (TEF 0.1) on the total sum of concentrations was only about 20%, due to its high toxicological significance compared to other contributors (TEF 0.001 – 0.0001) present in the samples at elevated concentrations, influence of this congener on the PBDD/F-TEQ reached up to 55% (Figure 3.10 B). The total contribution of 1,2,3,4,6,7,8-HeptaBDF and OctaBDF to the PBDD/DF-TEQ was about 20%. Several studies show the intensive formation of PBDFs, especially of highly brominated dibenzofurans, during the incineration and recycling of electronic waste [149,150]. High levels of 1,2,3,4,6,7,8-HeptaBDF and OctaBDF were reported as impurities in commercial PBDE mixtures as well [151]. According to the obtained results, the presence of bioaccumulated PBDFs in large aquatic top predators such as salmon indicates anthropogenic sources, although the metabolic persistence of such contaminants is under question.

Out of the nine analyzed PXDD/F congeners, only one compound (3-B-2,7,8-TriCDF) was present in quantifiable concentrations. It was found in all samples in the concentration range of 0.02 – 0.08 pg g^{-1} f.w., with an average of 0.04 pg g^{-1} f.w. Some of the analyzed samples showed the presence of potentially much more toxicologically significant 2-B-3,7,8-TriCDD and 2,3-DiB-7,8-DiCDD, but in all cases the concentrations were not quantifiable, although were above the LOD. Mixed bromo/chloro-substituted dibenzo-*p*-dioxins and furans are usually minor impurities formed during the unintentional formation of polyhalogenated dibenzo-*p*-dioxins and furans, and the obtained data are in line with the formation pattern and data on distribution of PXDD/Fs in the environment [152,153].

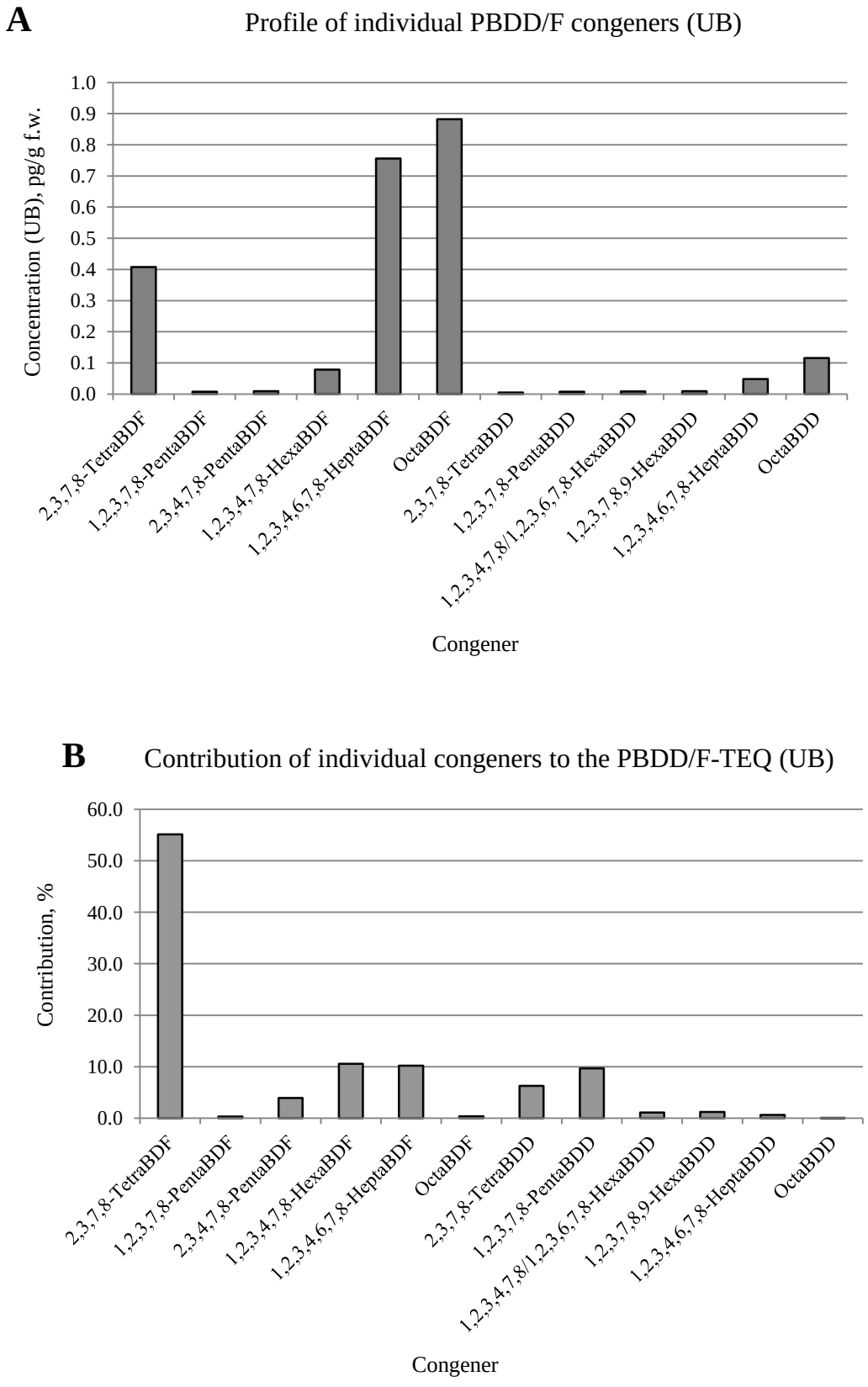


Fig. 3.10. **Congener profiles (A) of PBDD/Fs in Baltic wild salmon and contributions (B) of individual components to the PBDD/F-TEQ (UB)**

PBDEs. The total concentrations of twenty seven PBDE congeners in the analyzed salmon samples were in the range of 1.3 – 5.6 ng g^{-1} f.w., with an average of 3.3 ng g^{-1} f.w. (Annex 7). Tetra- through hexa-, and deca-brominated homologues were the predominant congeners contributing about 85% of the total PBDE load with the following order of contribution to the total sum of PBDEs: PBDE 47 > PBDE 49 > PBDE 100 > PBDE 99 > PBDE 154 > PBDE 209. The main contributor PBDE 47 was presented in the range of 0.47 – 2.1 ng g^{-1} f.w, with an average of 1.2 ng g^{-1} f.w. The levels of PBDEs in this study were comparable to those found for wild salmon from other references [131,154]. Generally, due to the strong contamination of the Baltic region with POPs, Baltic wild salmon shows higher levels of bioaccumulated PBDEs than salmon from other regions [155,156]. The obtained congener profile and distribution is typical for aquatic biota from the Baltic region found in other references [130,131] and is shown in Figure 3.11. Correlation of the total sum of PBDEs with concentrations of tetra-, and penta-brominated congeners (correlation of PBDE 47 with total PBDE sum up to $R^2 = 0.98$) reflects the source of contamination from widely used commercial "penta-BDE" mixture (Figure 3.11), in which PBDE 47, 49, 99 and 100 congeners are predominant [157]. Despite the widespread use of "octa-BDE" mixture containing mainly octa- and nona-brominated congeners, as well as the "deca-BDE" mixture containing mainly PBDE 209, contribution of those congeners to the sum of PBDEs was only about 6%, and there was no correlation between concentrations of octa-, nona-, and deca-brominated congeners with the total PBDE concentration. The explanation for such PBDE distribution in salmon was found in the different aquatic bioaccumulation and biotransformation potential of congeners with different extent of bromination. Highly brominated PBDEs are characterized by low uptake potential and are more susceptible to metabolic transformation, compared to congeners with low degree of bromine substitution [157,158]. There are reports on the short half-life times of highly brominated congeners in humans and biotransformation to lower brominated congeners in fish [159,160]. Species-selective bioaccumulation and biotransformation, probably via debromination of different PBDE congeners, was revealed for some types of fish and shellfish [58,161,162], and should be taken into account during the estimation of PBDE bioaccumulation and biotransportation in living organisms.

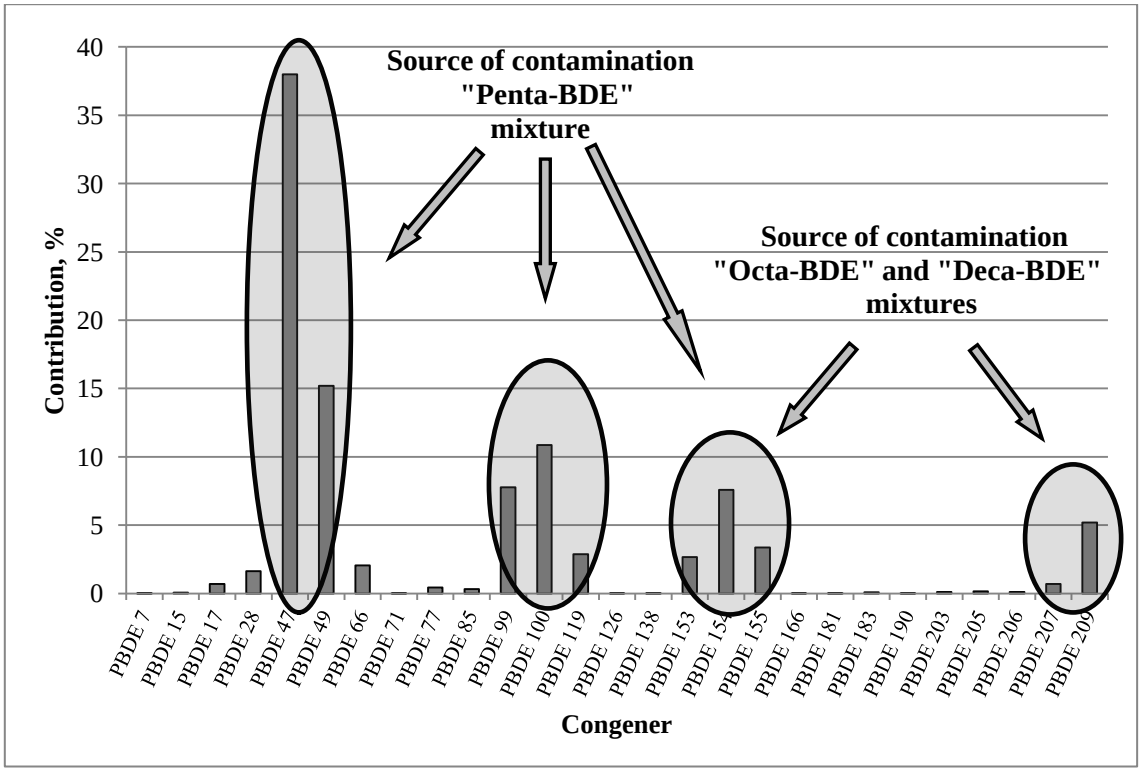


Fig. 3.11. **Congener profile of PBDEs in Baltic wild salmon and potential sources of contamination**

HBCDs. Twenty-five salmon samples were analyzed on the content of HBCD. The total-HBCD concentrations within the samples ranged from 390 to 3800 pg g^{-1} f.w, with an average of 1600 pg g^{-1} f.w. The biological parameters of the analyzed fishes, concentrations of individual HBCD diastereoisomers and the total HBCD for all analyzed samples are summarized in Annex 4. The concentrations determined in this study were of similar magnitude to those detected in fish from Baltic Sea in other studies [163-166]. The diastereomer pattern typical for aquatic biota was observed in all samples with strongly pronounced domination of α-HBCD over β- and γ-HBCDs (up to 90% of the total-HBCD) [163]. In contrast to environmental matrices like sediments, for which domination of γ-HBCD was usually observed due to the straight accumulation from the crude HBCD mixtures released from discarded polymers and related materials, selective metabolism of different HBCD enantiomers and/or biotransformation processes occur in fish and other aquatic biota [167,168]. The fact of close correlation between α- and γ-HBCD diastereoisomers indicates that processes of biotransformation or selective metabolism take place. Excluding the results of one outlier sample (sample 54505-11, Annex 4) the data showed correlation of R^2=0.93 between the latter parameters (Figure 3.12). In the case of β-HBCD, a weaker correlation with α-and γ-HBCDs was observed. Excluding the results for β-HBCD that were below the m-LOQ, correlation coefficients of R^2=0.76 and R^2=0.81 were observed for α- and γ-HBCDs, respectively (data not

shown). Unfortunately the data on the correlation between the *α*-, *β*- and *γ*-HBCDs in selected fish species are not provided in the available literature, although some studies indicate the absence of correlation between these diastereoisomers in the eggs of predatory sea birds [169,170]. Such behavior could be provided by the differences in long range transport properties of selected contaminants in the sequence: contamination source – feeding area – target species.

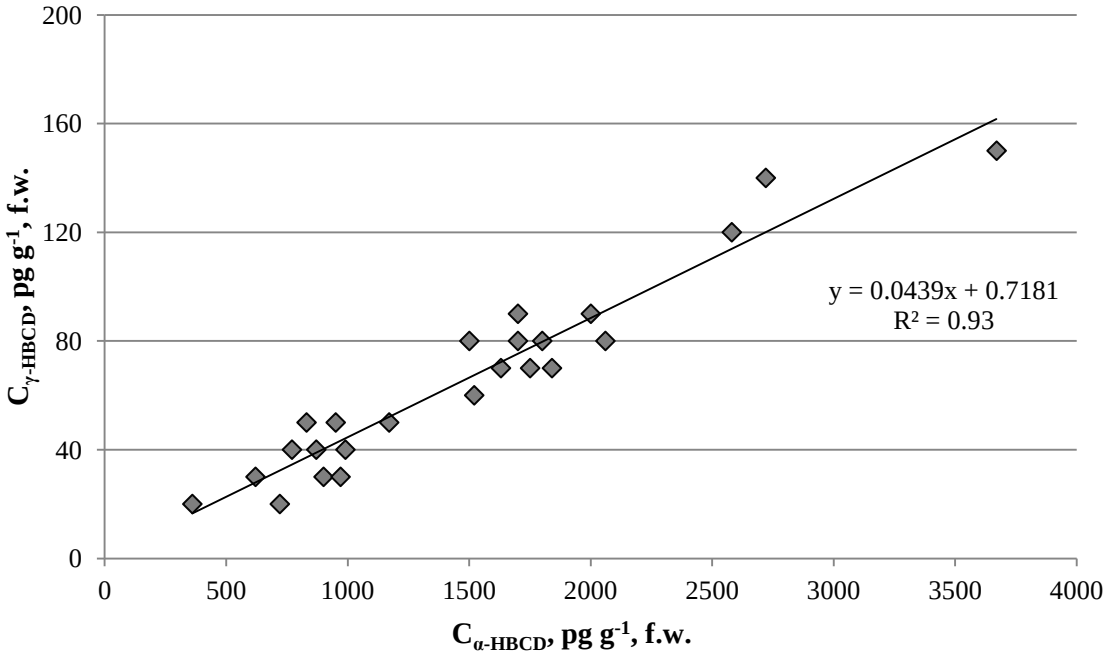


Fig. 3.12. **Correlations between *α*- and *γ*-HBCDs in the tissue of Baltic wild salmon**

3.4.2. Levels and congener profiles of POPs in eel samples from Latvian lakes

PCDD/Fs and PCBs. This study was the first research specially focused to characterize the levels of POPs in eels originated from Latvian eels and to evaluate the contamination status for selected species, obtained results should be comparative assessed in the context of the overall European situation. However, the majority of references [171-174] on PCDD/F and DL-PCB contamination in European eels do not provide detailed congener composition and report only TEQ values which in most cases are based on TEF_{1998} (5). Thus in order to facilitate the comparison both sets of WHO-TEQ (TEF_{1998} and TEF_{2005} based) values were calculated and presented in Annexes 8 and 10.

The available literature shows large variations in PCDD/F and DL-PCB contamination levels of eels depending on the sampling area. Levels of PCDD/Fs in the current study vary between 0.29 and 2.9 pg WHO_{2005}-PCDD/F-TEQ g^{-1} f.w., with an average of 1.2 pg WHO_{2005}-PCDD/F-TEQ g^{-1} f.w., and do not exceed the ML of 3.5 pg WHO_{2005}-PCDD/F-TEQ g^{-1} f.w established by the

European Commission [146] for eel tissue. When $TEFs_{1998}$ were used, PCDD/F-TEQs values ranging from 0.35 to 3.5 pg WHO_{1998}-PCDD/F-TEQ f.w. (average 1.4 pg WHO_{1998}-PCDD/F-TEQ f.w.) were obtained, with an increase of the PCDD/Fs-TEQ concentration value by about 18% on average, which is in line with previous reports [172]. In general, levels of the PCDD/F contamination obtained in our study were comparable or somewhat lower to those reported in the majority of studies for eels collected from other European sites. For example, levels in the range from 0.20 to 9.8 pg WHO_{1998}-PCDD/F-TEQ g^{-1} f.w. were found in eels from Flanders [175]; eels from Irish waters showed contamination in the range of 0.20 – 4.4 pg WHO_{1998}-PCDD/F-TEQ g^{-1} f.w. [176] and 0.40 – 5.9 pg WHO_{1998}-PCDD/F-TEQ g^{-1} f.w. were measured in eels from the Western Baltic Sea [147]. Higher concentrations of 0.48 – 22 pg and 5.0 – 23 pg WHO_{1998}-PCDD/F-TEQ g^{-1} f.w. were obtained for eels originating from the river Elbe and its tributaries, and from Greenland fjords, respectively [174,177]. Lower contamination with PCDD/Fs in the range 0.29 – 1.9 pg WHO_{1998}-PCDD/F-TEQ g^{-1} f.w. was observed for eels collected from Polish lagoons [173].

The DL-PCB concentrations ranged from 0.56 to 13 pg WHO_{2005}-PCB-TEQ f.w. with an average value of 3.5 pg WHO_{2005}-PCB-TEQ g^{-1} f.w. For some samples analyzed within the current study, application of $TEFs_{1998}$ resulted in a large increase (up to 250%) of the recalculated PCB-TEQs. An average increase of WHO-PCB-TEQ concentrations of 48% was observed, with the corresponding concentrations ranging from 0.71 to 27 pg WHO_{1998}-PCB-TEQ g^{-1} f.w. (average value of 5.2 pg WHO_{1998}-PCB-TEQ g^{-1} f.w.). The current observed levels of the DL-PCB contamination in Latvian eels represent the intermediate position in comparison to other European sites. Noticeably lower DL-PCB contamination was obtained for eels in Spain (0.09 pg WHO_{1998}-PCB-TEQ g^{-1} f.w. [178]; Greenland fjords (1.4 – 3.9 pg WHO_{1998}-PCB-TEQ g^{-1} f.w. [177]; and Ireland (0.17 – 1.2 pg WHO_{1998}-PCB-TEQ g^{-1} f.w. [176]. Somewhat similar DL-PCB levels were observed in eels originating from Poland (1.6 – 7.1 pg WHO_{1998}-PCB-TEQ g^{-1} f.w. [173]; Western Baltic Sea (0.93 – 15 pg WHO_{1998}-PCB-TEQ g^{-1} f.w. [147]; and France (0.24 – 14 pg WHO_{1998}-PCB-TEQ g^{-1} f.w. [172]. Considerably higher WHO_{1998}-PCB-TEQ concentrations were measured in eels originating from Germany (8.5 – 47 pg g^{-1}) [174]; and Belgium (up to 140 pg g^{-1}) [171].

Total-WHO_{2005}-TEQ values for analyzed samples varied between 0.85 and 16 pg g^{-1} f.w., with an average value of 4.7 pg g^{-1} f.w. Five of the analyzed eels (9% of all samples) exceeded the current European Commission ML of 10 pg total-WHO_{2005}-TEQ g^{-1} f.w. [146] for muscle of eels. The average total-WHO_{2005}-TEQ value for the samples exceeding the ML was 13 pg g^{-1} f.w., which exceeds the ML by 25%.

Overall, DL-PCB TEQ was much higher in comparison with PCDD/Fs. The contribution of individual PCDD/Fs and DL-PCBs to the WHO_{2005}-PCDD/F-TEQ, WHO_{2005}-PCB-TEQ and total-WHO_{2005}-TEQ values for the investigated lakes are shown in Figure 3.13.

The total sum of NDL-PCB concentrations in the eel samples ranged from 6.4 to 320 ng g^{-1} f.w., with an average of 51 ng g^{-1} f.w. (Annex 10). The congener pattern of NDL-PCBs was in good agreement with previous studies for Baltic fish [147,130] and for European eels in other studies [179-181]. The distribution showed a congener profile typical of widely used PCB mixtures (e.g. Aroclor 1254 and 1260) dominated by penta-, hexa- and hepta-chlorinated congeners. The indicator PCBs ($\sum_{ind.PCB}$ (namely PCBs: 28, 52, 101, 138, 153 and 180)) represented about 70% of total NDL-PCB concentration with values in the range of 4.8 – 190 ng g^{-1} f.w., with an average of 36 ng g^{-1} f.w. The ML for $\sum_{ind.PCB}$ in eel tissue of 300 ng g^{-1} f.w. specified in Commission Regulation (EU) No 1259/2011 [146] was not exceeded in any of the samples. The obtained results are consistent with previous studies where large variations of $\sum_{ind.PCB}$ in European eel samples were observed: 1.7 – 290 and 3.4 – 530 ng g^{-1} f.w. (PCB 118 was included with the indicator PCBs) in Polish studies [173]; 69 – 250 ng g^{-1} f.w. in France [172]; 1.9 – 18 ng g^{-1} f.w. in Ireland [176]; 16 – 1500 ng g^{-1} f.w. in Netherlands [182].

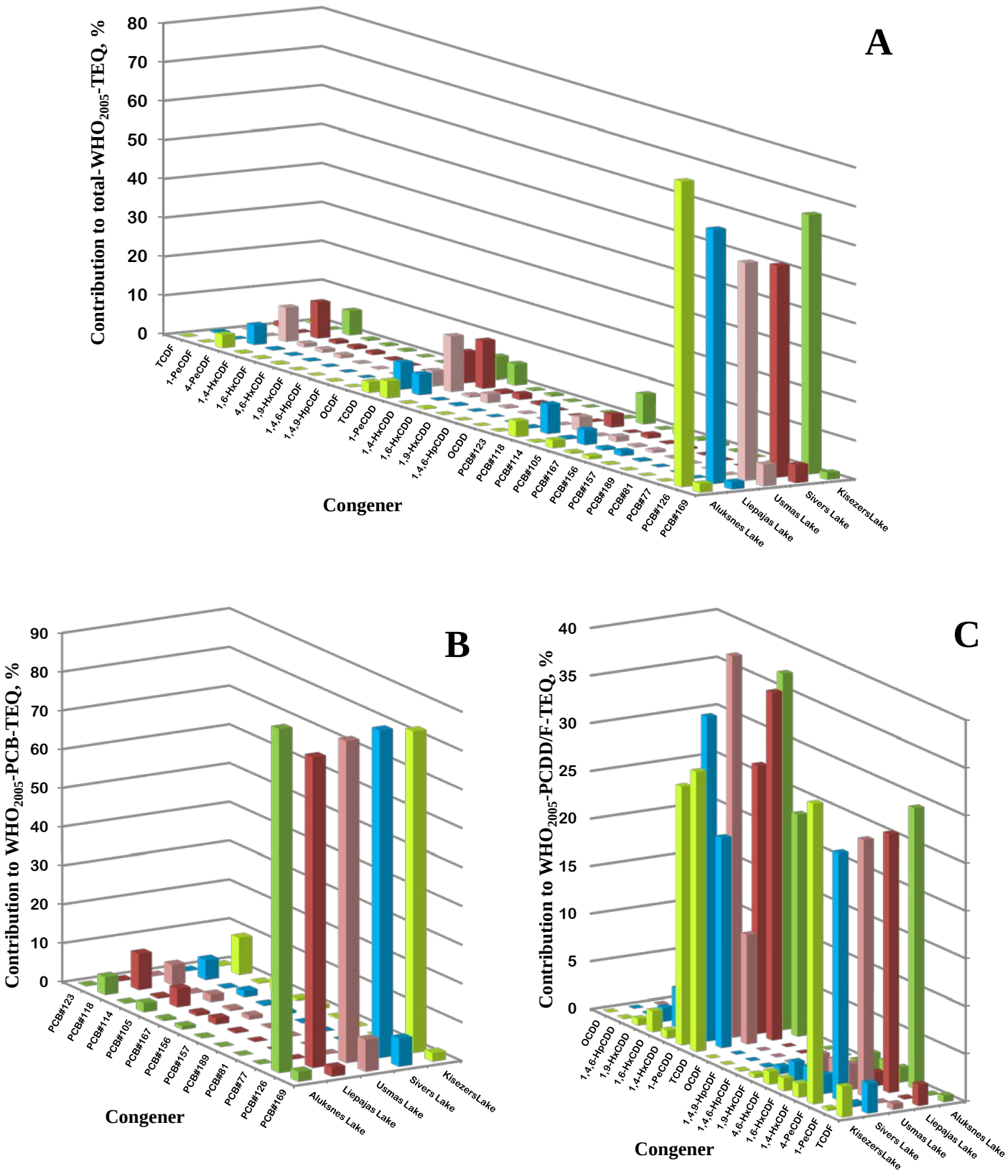


Fig. 3.13. **Contribution of individual PCDD/Fs and DL-PCBs to the total-WHO$_{2005}$-TEQ (A), WHO$_{2005}$-PCB-TEQ (B) and WHO$_{2005}$-PCDD/F-TEQ (C) values among the investigated lakes**

PBDD/Fs, PXDD/Fs and PXBs. One of the focuses in this study was to investigate the possible occurrence of the less studied POPs such as PBDD/Fs, PXDD/Fs and PXBs, in eel tissue. To the best of our knowledge this is the first attempt to characterize the contamination status of Latvian freshwater fish with these ecotoxicants. Taking into account the high fat content and typical diet of eels (e.g. scavenger fish) they are suspected to contain elevated levels of POPs in comparison to other fish species, thus detectable amounts of PBDD/Fs, PXDD/Fs and PXBs could be expected in tissue of analyzed specimens. The detailed information on the content of PBDD/Fs, PXBs and PXDD/Fs in analyzed eel samples is summarized in Annex 9.

The analyzed samples showed a wide range of detectable PBDD/F congeners with the domination of PBDFs, which was in accordance with the formation mechanisms of these compounds and reflects the typical composition of these contaminants in industrial emissions (47), food [57,58,183] and environmental matrices [148,149]. All of the PBDD/Fs included in the scope of the study were detected, however, in most cases the concentrations of PBDDs were below the LOQ. 2,3,7,8-TetraBDD and 1,2,3,7,8-PentaBDD were detected in three and one, respectively of fifty eight samples, showing maximum concentrations of 0.09 and 0.05 pg g^{-1} f.w. The most abundant congeners bwere 2,3,7,8-TetraBDF (detected in 52% of samples) and 1,2,3,4,6,7,8-HeptaBDF (detected in 100% of samples) and were observed in concentration ranges of 0.02 – 0.25 pg g^{-1} f.w. and 0.03 – 1.1 pg g^{-1} f.w., respectively. Highly brominated dibenzofurans were found to be the most abundant PBDD/F congeners in stack flue gases [150], and this anthropogenic emission may account for the universal occurrence of these congeners in our eel samples. Only one HexaBDF congener (1,2,3,4,7,8-HexaBDF) was analyzed in this study, and it was present in 19% of analyzed eels in the range from 0.04 to 0.20 pg g^{-1} f.w., with an average value of 0.11 pg g^{-1} f.w., while penta-brominated dibenzofurans, namely 1,2,3,7,8-PentaBDF and 2,3,4,7,8-PentaBDF, were present in 14% and 16% of samples, with maximum concentrations of 0.06 and 0.04 pg g^{-1} f.w., respectively.

Out of nine analyzed PXDD/Fs only three congeners (3-B-2,7,8-TriCDF, 1-B-2,3,7,8-TetraCDF and 2-B-3,6,7,8,9-PentaCDD) showed quantifiable concentrations, 3-B-2,7,8-TriCDF being the predominant congener presented in seven of fifty eight samples at maximum concentration of 0.04 pg g^{-1} f.w., whereas 1-B-2,3,7,8-TetraClDF and 2-B-3,6,7,8,9-PentaClDD were presented in one and five samples, respectively. Generally, detected PXDD/F congeners showed concentrations slightly above the LOQ and in accordance with the formation patterns from combustion/incineration sources and data on distribution of PXDD/Fs in the environment [152,153,184].

Among the above mentioned micro contaminants, three PXBs with the arrangement of halogen atoms corresponding to the non-*ortho* substitution pattern (4'-B-3,3',4,5-TetraCB (mono-bromo PXB 126), 3,4-DiB-3',4',5'-TriCB (di-bromo PXB 126) and 3',4',5-TriB-3,4-DiCB (tri-bromo PXB 126)) and three PXBs with mono-*ortho* configuration (4'-B-2,3,3',4-TetraCB (mono-bromo PXBs 105), 4'-B-2,3',4,5-TetraCB (mono-bromo PXB 118) and 4'-B-2,3,3',4,5-PentaCB (mono-bromo PXB 156)) were analyzed in twenty six of fifty eight samples (in 45% of all eel samples). The selection of the analytes of interest was based on the availability of reliable analytical standards and typical distribution and toxicity of corresponding PCB congeners (e.g. typical predominance of absolute concentrations of PCBs 118, 105 and 156 in fish samples and the highest toxicity of PCB 126 within the DL-PCB group). The most prominent congeners among the analyzed PXBs were mono-*ortho* PXB 118 and PXB 105 found in every analyzed sample in the ranges of 0.07 – 3.18 pg g^{-1} f.w. and 0.03 – 0.77 pg g^{-1} f.w., respectively followed by the mono-*ortho* substituted PXB 156 which was present in 58% of samples and showed concentrations from 0.04 to 0.30 pg g^{-1} f.w. A similar congener pattern following the order PXB 118 > PXB 105 > PXB 156 was observed in freshwater fish in a European study of foods in the UK showing the concentrations for the latter congeners of 3.4, 1.3 and 0.28 pg g^{-1} f.w., respectively [74]. Out of the three investigated non-*ortho* PXBs, only mono-brominated PXB 126 congener occurred in eels examined, while di-brominated and tri-brominated PXBs 126 were absent in all of the investigated samples. Mono-bromo PXB 126 was presented in 92% of samples in the concentration range of 0.01 to 0.05 pg g^{-1} f.w. which is consistent with results for freshwater fish from the UK where the latter component was observed at the level of 0.07 pg g^{-1} f.w. [74]. Despite the fact that usually congeners containing a single substituted bromine atom (mono-bromo congeners) are presented in food and biota samples [184], a Japanese study showed unexpectedly high concentrations of tri-brominated 3',4',5-TriB-3,4-DiCB (tri-bromo PXB 126) (up to 10 pg g^{-1} f.w.) in fish samples with unclear source of contamination [73].

Despite the extensive clean-up and fractionation procedures used for selective isolation of compounds of interest, it was not possible to identify some high intensity peaks that occurred on the SIR chromatograms of relevant *m/z* traces corresponding to the analyzed brominated or mixed bromo/chloro-substituted contaminants for which quality control criteria for positive identification (isotope ratio window ±15%) were fulfilled. This observation could reflect on the presence of potentially significant concentrations of unknown PBDD/Fs, PXBs and PXDD/Fs with different substitution patterns than those congeners included in this study. Figure 3.14 shows an example of an intensive peak of the unknown compound on the representative SIR chromatogram of eel sample for the *m/z* traces corresponding to TBDD congeners.

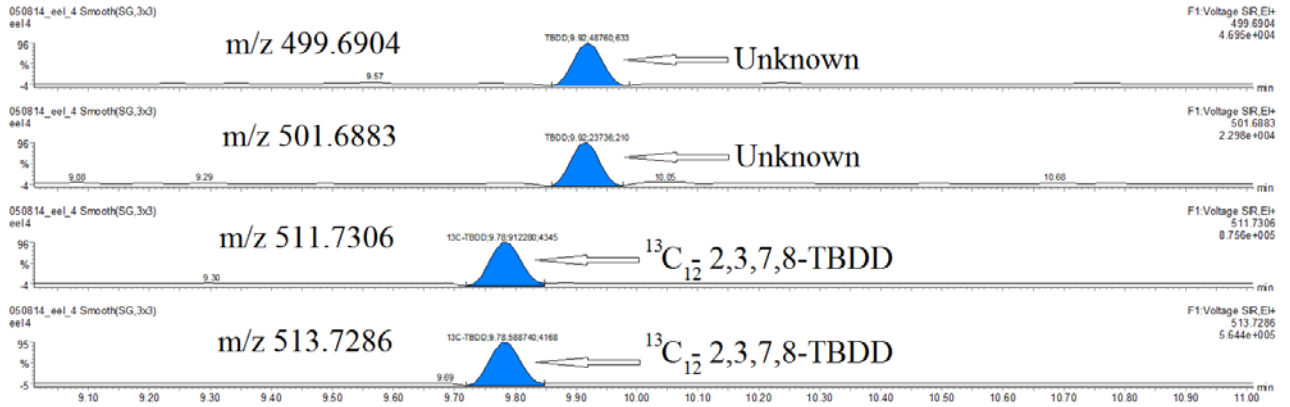


Fig. 3.14. **Representative SIR chromatogram of eel sample for the *m/z* traces corresponding to TBDD congeners (it should be noted however, in this example, that the peak may correspond to a non-2,3,7,8-substituted PBDD, of undefined toxicity)**

Currently there are very few data available on the PBDD/Fs, PXBs and PXDD/Fs content in aquatic biota; nevertheless these isolated references show that brominated and mixed brominated/chlorinated congeners occur to a lower extent in samples, in comparison to their chlorinated analogs. In the absence of established TEFs for PBDD/Fs, PXBs and PXDD/Fs, values for chlorinated analogues (WHO_{2005}-TEFs) have been used as suggested for PBDD/Fs [67], and although some REP values have been reported for PXDD/F and PXB compounds, they do not cover the higher mixed halogenated compounds measured in this work, so chlorinated analogue TEFs have also been used for these compounds. Thus the calculated TEQs obtained, provide only for a rough estimate of the potential toxicity presented by these contaminants. Using this approach, analyzed samples showed PBDD/F-TEQ (UB) concentrations in the range of 0.02 – 0.14 pg g^{-1} f.w., with an average of 0.05 pg g^{-1} f.w. Tentative TEQ values obtained for selected PXBs and PXDD/Fs were on average of 0.01 and 0.08 pg g^{-1} f.w., respectively. A comparison of the TEQ values shows that the brominated and mixed halogenated TEQ values are considerably lower compared to those obtained for PCDD/Fs and DL-PCBs. This estimation on the UB basis, indicates that these minor contaminants could contribute from 3% to 5%, to the total-WHO_{2005}-TEQ value with an average of 3%. With respect to TEQ levels provided by the presence of PCDD/Fs and DL-PCBs and taking into account the established ML levels for these groups of compounds, generally the contribution of PBDD/Fs is small, but nevertheless these data are of great importance in terms of providing of more complete information on the contamination status of the examined objects. The complete contribution of PXDD/F and PXB TEQ is currently unquantifiable, as unlike the PCDD/Fs and PCBs, it is not possible to quantify all toxicologically significant congeners at the present. Thus, as observed elsewhere [66] the current data form only a limited part of the total

potential toxicity from PXDD/Fs and PXBs. Nonetheless, the detection of these contaminants in inland lakes is an important complement to the observations made on occurrence of PXDDs in marine creatures [185] from the Baltic Sea.

PBDEs. Taking into account the recent EU recommendation on the monitoring of BFRs in food products [186], data on the occurrence of PBDEs in Latvian eels are of great relevance. The total concentrations of $\sum_{PBDE}$ (Annex 11) ranged from 0.28 to 27 ng g^{-1} f.w., with an average of 4.2 ng g^{-1} f.w. The obtained congener pattern with domination of tetra-, and penta-brominated BDEs is typical for aquatic biota from the Baltic region found in other studies [130,131] and for eels from other European countries [187]. PBDEs 47, 49 and 100 were the dominant congeners and contributed 79% on average to $\sum_{PBDE}$. Correlation of the $\sum_{PBDE}$ with concentrations of tetra-, and penta-brominated congeners (correlation of PBDE 47 with total PBDE sum was R^2 = 0.97 on average) most likely reflects the source of contamination as the widely used and recently banned commercial “penta-BDE” formulation (in which the latter congeners are predominant [26]. The more highly brominated PBDEs (e.g. octa-, nona-, and deca-brominated BDEs) were present in significantly lower concentrations. Despite the widespread usage of “octa-BDE” formulation (consisting mainly of octa- and nona-brominated congeners) and “deca-BDE” formulation (the main component is PBDE 209), the average contribution of these PBDE congeners to the $\sum_{PBDE}$ was less than 2% and was in accordance with the fact that highly brominated congeners are characterized by the low uptake potential by aquatic biota and are more susceptible to metabolic transformation, compared to congeners with low degree of bromine substitution [157,158,160]. A similar distribution for highly brominated congeners was observed in other studies for different fish species and particularly for eels [162,188]. Generally, comparable levels of PBDEs were obtained in our study in context of overall European occurrence for this type of recently used BFRs [176,187]. Nevertheless, some studies showed extremely high contamination levels in industrialized areas, for example in the Belgian study, levels of PBDEs collected in 2006, in highly contaminated areas near textile factories, were up to 790 ng g^{-1} f.w. [189].

HBCDs. Taking into account the diet of eels ("scavenger" fish) and the typically high fat content, elevated contamination levels of POPs are often encountered in such species [164,174,180]. Bioaccumulation of HBCD was confirmed in all analyzed eel samples. The total-HBCD concentrations within the samples ranged from 210 to 600 pg g^{-1} f.w, with an average of 300 pg g^{-1} f.w. The biological parameters of the analyzed fishes, concentrations of individual HBCD diastereomers, and the total-HBCD for all analyzed samples are outlined in Annex 8. As for salmons, the diastereomer pattern typical for aquatic biota was observed in all samples with the strongly pronounced domination of α-HBCD over β- and γ-HBCDs (up to 95% of the total-HBCD)

[163]. Generally, the concentrations determined in this study were significantly lower in comparison to those detected in eel samples analyzed in the majority of earlier studies [164,180,190]. Some studies indicate strong difference between the HBCD concentrations obtained for eel samples collected from nearby areas. For example, the HBCD concentration in samples collected in the highly polluted south-west region in Netherlands was up to magnitude higher in comparison with the samples collected in relatively remote areas or upstream of the production regions for which total levels of HBCD between 100 and 1000 pg g^{-1} were observed [112]. The relatively low HBCD contamination levels detected in eel samples collected in Latvia seem to be logical, since there are no BFR production factories or plastics processing facilities, which could cause an intensive emission of HBCD into the environment.

CONCLUSIONS

1. The developed analytical method presented in this study capable of the simultaneous determination of seven groups of priority POPs including PBDD/Fs, PCDD/Fs, PXDD/Fs, PBDEs, PCBs, PXBs, and HBCDs by applying a multistage clean-up and fractionation procedure and detection with GC-HRMS and UHPLC-Orbitrap-HRMS ensures a reliable detection of these contaminants at toxicologically significant levels.
2. We propose an extensive fractionation procedure involving a series of column chromatography steps with Florisil, dual layered activated carbon, and basic alumina, which provides more complete removal of non-planar interferants such as PBDEs and PCBs from the fractions containing PBDD/Fs and PXDD/Fs, thus significantly increasing the selectivity of the method for these compounds and resulting in a better recovery rates for planar compounds.
3. For the first time, a new rapid and reliable UHPLC-Orbitrap-HRMS based methodology was developed for the determination of HBCD diastereoisomers in fish matrices. This method provides a fast chromatographic separation, good selectivity, pg g^{-1} quantification levels, a wide range of linearity and acceptable precision, and it could be used as an effective tool to obtain detailed information on the HBCD diastereomer content in fish samples.
4. A comparative evaluation of the analytical performance of UHPLC-Orbitrap-HRMS technique versus UHPLC-TOF-HRMS and conventional UHPLC-QqQ-MS/MS systems revealed a good agreement between the studied approaches in terms of recovery, repeatability, and intermediate precision. No statistically significant differences were found for α-, β-, γ-, and total-HBCD concentrations obtained in the analysis of real samples with the examined LC-MS systems indicating that the newly proposed Orbitrap-HRMS technique produces adequate and similar results on HBCD content in fish samples, compared to conventional approaches.
5. Most of the analyzed Baltic wild salmon samples (75% of all samples) are highly contaminated with POPs above the MLs according to Commission Regulation No. (EC) 1259/2011 for PCDD/DFs and DL-PCBs, while freshwater fish, particularly eels, which due to its diet are proposed as a bioindicator for the environmental pollution, are far less contaminated with POPs, exceeding the MLs for PCDD/Fs and DL-PCBs in 9% of the analyzed samples.
6. The distribution of PBDE congeners in fish samples with dominance of PBDE 47, 49, 99, and 100 clearly reflects the bioaccumulation of these compounds from the recent usage of commercial PBDE mixtures.
7. For the first time, the universal presence of a number of PBDD/F, PXDD/F, and PXB congeners was confirmed in almost all fish samples obtained from the Baltic Sea and Latvian lakes. Although the DL-toxicity arising from the latter POP groups is low (1 – 5%) compared to the

chlorinated analogs, these data are of great importance in terms of providing of more complete information on the contamination status of the examined objects.

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ANNEXES

Annex 1

Mass descriptors used for measurement of PBDEs using GC-HRMS

PBDEs	Unlabeled congener *m/z*	Ion	Isotope ratio	$^{13}C_{12}$ – labeled congener *m/z*	Ion	Isotope ratio
Di-BDE	325.8942	M^+	0.51	337.9344	M^+	0.51
	327.8921	$[M+2]^+$		339.9324	$[M+2]^+$	
Tri-BDE	405.8027	$[M+2]^+$	1.03	417.8429	$[M+2]^+$	1.03
	407.8002	$[M+4]^+$		419.8409	$[M+4]^+$	
Tetra-BDE	483.7132	$[M+2]^+$	0.70	497.7514	$[M+4]^+$	1.54
	485.7111	$[M+4]^+$		499.7493	$[M+6]^+$	
Penta-BDE	563.6216	$[M+4]^+$	1.03	575.6619	$[M+4]^+$	1.03
	565.6196	$[M+6]^+$		577.6598	$[M+6]^+$	
Hexa-BDE	641.5322	$[M+4]^+$	0.77	655.5704	$[M+6]^+$	1.37
	643.5302	$[M+6]^+$		657.5683	$[M+8]^+$	
Hepta-BDE	721.4406	$[M+6]^+$	1.03	733.4809	$[M+6]^+$	1.03
	723.4386	$[M+8]^+$		735.4788	$[M+8]^+$	
Octa-BDE	639.5165	$[M-2Br+4]^+$	0.77	651.5567	$[M-2Br+4]^+$	0.77
	641.5144	$[M-2Br+6]^+$		653.5547	$[M-2Br+6]^+$	
Nona-BDE	719.4250	$[M-2Br+6]^+$	1.03	731.4652	$[M-2Br+6]^+$	1.03
	721.4229	$[M-2Br+8]^+$		733.4632	$[M-2Br+8]^+$	
Deca-BDE	797.3355	$[M-2Br+6]^+$	0.82	809.3757	$[M-2Br+6]^+$	0.82
	799.3334	$[M-2Br+8]^+$		811.3737	$[M-2Br+8]^+$	

Annex 2

Mass descriptors used for measurement of PBDD/Fs using GC-HRMS

PBDD/Fs	Unlabeled congener *m/z*	Ion	Isotope ratio	$^{13}C_{12}$ – labeled congener *m/z*	Ion	Isotope ratio
Tetra-BDF	483.6955	$[M+4]^+$	1.54	495.7357	$[M+4]^+$	1.54
	485.6934	$[M+6]^+$		497.7337	$[M+6]^+$	
Penta-BDF	561.6060	$[M+4]^+$	1.03	573.6462	$[M+4]^+$	1.03
	563.6039	$[M+6]^+$		575.6442	$[M+6]^+$	
Hexa-BDF	639.5165	$[M+4]^+$	0.77	651.5567	$[M+4]^+$	0.77
	641.5144	$[M+6]^+$		653.5547	$[M+6]^+$	
Hepta-BDF	719.4250	$[M+6]^+$	1.03	731.4652	$[M+6]^+$	1.03
	721.4229	$[M+8]^+$		733.4632	$[M+8]^+$	
Octa-BDF	797.3355	$[M+6]^+$	0.82	809.3757	$[M+6]^+$	0.82
	799.3334	$[M+8]^+$		811.3737	$[M+8]^+$	
Tetra-BDD	499.6904	$[M+4]^+$	1.54	511.7306	$[M+4]^+$	1.54
	501.6883	$[M+6]^+$		513.7286	$[M+6]^+$	
Penta-BDD	577.6009	$[M+4]^+$	1.03	589.6411	$[M+4]^+$	1.03
	579.5988	$[M+6]^+$		591.6391	$[M+6]^+$	
Hexa-BDD	657.5094	$[M+6]^+$	1.37	669.5496	$[M+6]^+$	1.37
	659.5073	$[M+8]^+$		671.5476	$[M+8]^+$	
Hepta-BDD	735.4199	$[M+6]^+$	1.03	747.4601	$[M+6]^+$	1.03
	737.4178	$[M+8]^+$		749.4581	$[M+8]^+$	
Octa-BDD	813.3304	$[M+6]^+$	0.82	825.3706	$[M+6]^+$	0.82
	815.3283	$[M+8]^+$		827.3686	$[M+8]^+$	

Annex 3

Mass descriptors used for measurement of PXDD/Fs and PXBs using GC-HRMS

PXDD/Fs PXBs	Unlabeled congener *m/z*	Ion	Isotope ratio	$^{13}C_{12}$ – labeled congener *m/z*	Ion	Isotope ratio
Br_1Cl_3DF	349.8491	$[M+2]^+$	1.54	-	-	-
	351.8461	$[M+4]^+$		-	-	
Br_1Cl_3DD	365.8440	$[M+2]^+$	1.54	-	-	-
	367.8410	$[M+4]^+$		-	-	
Br_2Cl_2DD	409.7935	$[M+2]^+$	1.12	421.8337	$[M+2]^+$	1.12
	411.7905	$[M+4]^+$		423.8308	$[M+4]^+$	
Br_1Cl_4DF	383.8092	$[M+2]^+$	1.20	-	-	-
	385.8071	$[M+4]^+$		-	-	
Br_1Cl_4DD	399.8041	$[M+2]^+$	1.20	411.8444	$[M+2]^+$	1.20
	401.8021	$[M+4]^+$		413.8423	$[M+4]^+$	
Br_1Cl_5DD	433.7651	$[M+2]^+$	0.99	-	-	-
	435.7631	$[M+4]^+$		-	-	
Br_1Cl_6DD	467.7262	$[M+2]^+$	0.85	-	-	-
	469.7241	$[M+4]^+$		-	-	
Br_1Cl_7DD	501.6872	$[M+2]^+$	0.74	-	-	-
	503.6851	$[M+4]^+$		-	-	
Br_1Cl_4B	369.8299	$[M+2]^+$	1.34	381.8701	$[M+2]^+$	1.34
	371.8279	$[M+4]^+$		383.8681	$[M+4]^+$	
Br_2Cl_3B	413.7793	$[M+2]^+$	1.03	-	-	-
	415.7783	$[M+4]^+$		-	-	
Br_3Cl_2B	457.7297	$[M+2]^+$	0.76	-	-	-
	459.7277	$[M+4]^+$		-	-	
Br_1Cl_5B	403.7909	$[M+2]^+$	1.03	415.8312	$[M+2]^+$	1.03
	405.7889	$[M+4]^+$		417.8292	$[M+4]^+$	

Concentrations of PCDD/Fs and HBCDs in Baltic wild salmon samples calculated on f.w. basis

Sample Nr.	74505-1	74505-2	74505-3	74505-4	74505-5	74505-6	74505-7	74505-8	74505-9	74505-10	74505-11	74505-12
Sex*	F	M	F	M	F	F	M	M	M	F	M	F
Length, cm	74	57	59	63	83	83	71	65	56	81	58	84
Age, years	2	1	2	1	2	2	1	1	1	2	1	2
Weight, kg	4.4	2.5	3.5	3.0	5.8	5.8	3.8	3.6	2.0	5.3	2.6	5.6
Place of sampling	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River
Fat content, g/100 g of sample	2.4	3.5	7.9	6.3	2.9	6.1	3.5	5.8	5.3	5.5	4.8	5.2
PCDD/Fs (pg g^{-1})												
2,3,7,8-TetraCDF	9.34	7.30	8.98	5.01	6.39	9.79	8.23	5.63	4.99	8.83	4.58	7.92
1,2,3,7,8-PentaCDF	0.51	0.60	0.78	0.61	0.71	1.00	1.02	0.57	0.49	0.65	0.44	0.97
2,3,4,7,8-PentaCDF	4.74	4.66	6.29	4.02	4.96	6.03	5.78	3.50	3.20	5.20	3.26	5.67
1,2,3,4,7,8-HexaCDF	0.07	0.07	0.10	0.08	0.08	0.13	0.13	0.08	0.06	0.09	0.06	0.14
1,2,3,6,7,8-HexaCDF	0.12	0.13	0.16	0.13	0.14	0.22	0.23	0.13	0.09	0.14	0.10	0.22
2,3,4,6,7,8-HexaCDF	0.12	0.13	0.17	0.12	0.14	0.19	0.18	0.11	0.07	0.15	0.10	0.18
1,2,3,7,8,9-HexaCDF	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1,2,3,4,6,7,8-HeptaCDF	0.07	0.06	0.05	0.03	0.05	0.08	0.05	0.05	0.03	0.07	0.04	0.09
1,2,3,4,7,8,9-HeptaCDF	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OctaCDF	0.04	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.03	0.02	0.03
2,3,7,8-TetraCDD	0.34	0.36	0.44	0.28	0.37	0.55	0.46	0.28	0.32	0.42	0.23	0.47
12378-PentaCDD	0.57	0.56	0.74	0.58	0.69	0.89	0.83	0.48	0.43	0.69	0.42	0.83
1,2,3,4,7,8-HexaCDD	0.02	0.01	0.03	0.03	0.03	0.05	0.04	0.02	0.03	0.03	0.02	0.05
1,2,3,6,7,8-HexaCDD	0.30	0.26	0.34	0.22	0.23	0.32	0.31	0.20	0.09	0.25	0.18	0.31
1,2,3,7,8,9-HexaCDD	0.04	0.02	0.03	0.02	0.02	0.03	0.03	0.02	0.01	0.03	0.02	0.04
1,2,3,4,6,7,8-HeptaCDD	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.03
OctaCDD	0.05	0.04	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04
WHO_{2005}-PCDD/F-TEQ (LB)	3.35	3.13	4.07	2.64	3.27	4.36	3.97	2.45	2.25	3.64	2.15	3.91
WHO_{2005}-PCDD/F-TEQ (UB)	3.35	3.13	4.07	2.64	3.27	4.36	3.97	2.45	2.25	3.64	2.15	3.91
HBCDs (pg g^{-1})												
α-HBCD	720	900	870	970	1630	1800	1750	770	360	950	860	1700
β-HBCD	<10.0	<10.0	50.0	60.0	40.0	110	110	50.0	<10.0	40.0	50.0	80.0
γ-HBCD	20.0	30.0	40.0	30.0	70.0	80.0	70.0	40.0	20.0	50.0	150	80.0
Total-HBCD (LB)	750	940	960	1060	1740	1990	1930	860	390	1040	1060	1860
Total-HBCD (UB)	740	930	960	1060	1740	1990	1930	860	390	1040	1060	1860

* - F – female; M – male.

Annex 4 continues

Sample Nr.	74505-13	74505-14	74505-15	79050-1	79050-2	79050-3	79050-4	79050-5	79050-6	79050-7	79050-8	79050-9	79050-10
Sex*	F	F	M	M	F	M	F	M	M	M	F	F	M
Length, cm	86	78	69	62	76	77	81	76	93	78	83	83	68
Age, years	3	3	1	1	2	2	3	2	3	2	3	3	2
Weight, kg	7.1	6.6	3.5	3.5	5.1	6.3	6.4	5.8	11.3	6.5	6.2	10.2	5.0
Place of sampling	Daugava River	Daugava River	Daugava River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River
Fat content, g/100 g of sample	8.2	4.3	5.8	5.7	4.8	6.2	3.6	5.6	6.1	8.2	4.8	12.0	7.1
PCDD/Fs (pg g^{-1})													
2,3,7,8-TetraCDF	11.3	8.07	5.30	6.23	7.04	13.2	7.31	9.69	9.97	11.8	7.25	12.8	9.19
1,2,3,7,8-PentaCDF	1.41	1.01	0.41	0.59	0.69	1.56	0.82	0.90	1.20	1.42	0.81	1.50	1.15
2,3,4,7,8-PentaCDF	8.44	6.83	3.19	4.01	5.03	8.53	4.67	6.49	6.48	7.25	4.32	7.94	7.13
1,2,3,4,7,8-HexaCDF	0.19	0.13	0.04	0.07	0.08	0.22	0.10	0.12	0.17	0.22	0.13	0.24	0.15
1,2,3,6,7,8-HexaCDF	0.31	0.23	0.08	0.12	0.13	0.33	0.15	0.20	0.27	0.34	0.18	0.33	0.26
2,3,4,6,7,8-HexaCDF	0.28	0.19	0.08	0.11	0.12	0.26	0.13	0.17	0.22	0.26	0.17	0.28	0.22
1,2,3,7,8,9-HexaCDF	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1,2,3,4,6,7,8-HeptaCDF	0.05	0.07	0.04	0.05	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.06	0.07
1,2,3,4,7,8,9-HeptaCDF	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
OctaCDF	0.02	0.03	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.03
2,3,7,8-TetraCDD	0.63	0.50	0.23	0.30	0.38	0.73	0.43	0.46	0.58	0.66	0.36	0.67	0.57
12378-PentaCDD	1.19	0.96	0.37	0.56	0.66	1.31	0.68	0.86	0.99	1.13	0.68	1.19	1.06
1,2,3,4,7,8-HexaCDD	0.06	0.05	0.02	0.03	0.03	0.07	0.03	0.03	0.05	0.07	0.04	0.07	0.05
1,2,3,6,7,8-HexaCDD	0.44	0.33	0.15	0.23	0.24	0.48	0.22	0.34	0.40	0.44	0.28	0.46	0.41
1,2,3,7,8,9-HexaCDD	0.04	0.03	0.02	0.02	0.02	0.05	0.02	0.03	0.03	0.05	0.03	0.05	0.03
1,2,3,4,6,7,8-HeptaCDD	0.03	0.03	0.01	0.02	0.02	0.05	0.02	0.02	0.03	0.04	0.02	0.04	0.03
OctaCDD	0.04	0.04	0.03	0.03	0.04	0.04	0.04	0.03	0.04	0.05	0.02	0.04	0.04
WHO_{2005}-PCDD/F-TEQ (LB)	5.67	4.45	2.14	2.77	3.33	6.11	3.34	4.35	4.66	5.32	3.17	5.71	4.83
WHO_{2005}-PCDD/F-TEQ (UB)	5.67	4.45	2.14	2.77	3.33	6.11	3.34	4.35	4.66	5.32	3.17	5.71	4.83
HBCDs (pg g^{-1})													
α-HBCD	3670	2060	620	990	1170	2580	1500	1520	2000	1700	830	2720	1840
β-HBCD	<10.0	70.0	30.0	<10.0	60.0	180	<10. 0	60.0	160	110	50.0	180	80.0
γ-HBCD	150	80.0	30.0	40.0	50.0	120	80.0	60.0	90.0	90.0	50.0	140	70.0
Total-HBCD (LB)	3830	2210	680	1040	1280	2880	1590	1640	2250	1900	930	3040	1990
Total-HBCD (UB)	3820	2210	680	1030	1280	2880	1580	1640	2250	1900	930	3040	1990

* - F – female; M – male.

Concentrations of PBDD/Fs and PXDD/Fs in Baltic wild salmon samples calculated on f.w. basis

Sample Nr.	74505-1	74505-2	74505-3	74505-4	74505-5	74505-6	74505-7	74505-8	74505-9	74505-10	74505-11	74505-12
Sex*	F	M	F	M	F	F	M	M	M	F	M	F
Length, cm	74	57	59	63	83	83	71	65	56	81	58	84
Age, years	2	1	2	1	2	2	1	1	1	2	1	2
Weight, kg	4.4	2.5	3.5	3.0	5.8	5.8	3.8	3.6	2.0	5.3	2.6	5.6
Place of sampling	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River
Fat content, g/100 g of sample	2.4	3.5	7.9	6.3	2.9	6.1	3.5	5.8	5.3	5.5	4.8	5.2
PBDD/Fs (pg g^{-1})												
2,3,7,8-TetraBDF	0.92	0.42	0.29	0.45	0.29	0.28	0.65	0.63	0.47	0.34	0.39	0.71
2,4,6,8-TetraBDF	0.05	0.01	0.01	0.01	0.01	0.02	0.01	0.04	0.01	0.01	<0.01	0.02
1,2,3,7,8-PentaBDF	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2,3,4,7,8-PentaBDF	0.02	0.02	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.004	<0.01	0.01	0.01
1,2,3,4,7,8-HexaBDF	0.10	0.13	0.09	0.09	0.09	0.06	0.09	0.10	0.08	0.03	0.10	0.09
1,2,3,4,6,7,8-HeptaBDF	0.90	1.36	0.69	0.81	0.67	0.78	1.05	1.12	0.68	0.27	0.91	1.06
OctaBDF	1.06	1.39	0.75	0.84	0.74	0.75	1.12	1.06	0.81	<0.39	1.21	2.09
2,3,7,8-TetraBDD	0.01	0.003	0.004	<0.003	<0.004	<0.004	<0.003	0.01	<0.003	<0.01	<0.01	<0.004
1,2,3,7,8-PentaBDD	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1,2,3,4,7,8/1,2,3,6,7,8-HexaBDD	0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1,2,3,7,8,9-HexaBDD	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.01	<0.01
1,2,3,4,6,7,8-HeptaBDD	0.07	0.07	0.04	0.06	0.05	0.05	0.05	0.06	0.05	0.05	0.04	0.04
OctaBDD	<0.10	<0.07	<0.06	<0.10	<0.07	<0.08	<0.07	<0.06	<0.07	<0.25	<0.08	<0.07
WHO_{2005}-PBDD/F-TEQ (LB)	0.13	0.08	0.05	0.07	0.05	0.04	0.09	0.10	0.06	0.04	0.06	0.09
WHO_{2005}-PBDD/F-TEQ (UB)	0.14	0.09	0.06	0.08	0.06	0.06	0.10	0.11	0.08	0.06	0.08	0.11
PXDD/Fs (pg g^{-1})												
3-Br-2,7,8-TriClDF	0.02	0.02	0.03	0.03	0.04	0.05	0.05	0.02	0.02	0.03	0.02	0.04
2-Br-3,7,8-TriClDD	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2,3-DiBr-7,8-DiClDD	<0.004	<0.01	<0.003	<0.01	<0.01	<0.004	<0.01	<0.01	<0.002	<0.01	<0.003	<0.01
1-Br-2,3,7,8-TetraClDF	<0.004	<0.004	<0.002	<0.004	<0.004	<0.01	<0.01	<0.002	<0.004	<0.01	<0.003	<0.004
1-Br-2,3,7,8-TetraClDD	<0.004	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.004	<0.01
2-Br-1,3,7,8-TetraClDD	<0.004	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.004	<0.01
2-Br-3,6,7,8,9-PentaClDD	<0.004	<0.003	<0.002	<0.003	<0.003	<0.002	<0.002	<0.002	<0.003	<0.003	<0.002	<0.002
1-Br-2,3,6,7,8,9-HexaClDD	<0.01	<0.01	<0.004	<0.003	<0.004	<0.003	<0.004	<0.004	<0.01	<0.004	<0.003	<0.003
1-Br-2,3,4,6,7,8,9-HeptaClDD	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
WHO_{2005}-PXDD/F-TEQ (LB)	0.002	0.002	0.003	0.003	0.004	0.005	0.01	0.002	0.002	0.003	0.002	0.004
WHO_{2005}-PXDD/F-TEQ (UB)	0.02	0.03	0.02	0.04	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.03

* - F – female; M – male.

Sample Nr.	74505-13	74505-14	74505-15	79050-1	79050-2	79050-3	79050-4	79050-5	79050-6	79050-7	79050-8	79050-9	79050-10
Sex*	F	F	M	M	F	M	F	M	M	M	F	F	M
Length, cm	86	78	69	62	76	77	81	76	93	78	83	83	68
Age, years	3	3	1	1	2	2	3	2	3	2	3	3	2
Weight, kg	7.1	6.6	3.5	3.5	5.1	6.3	6.4	5.8	11.3	6.5	6.2	10.2	5.0
Place of sampling	Daugava River	Daugava River	Daugava River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River
Fat content, g/100 g of sample	8.2	4.3	5.8	5.7	4.8	6.2	3.6	5.6	6.1	8.2	4.8	12.0	7.1
PBDD/Fs (pg g^{-1})													
2,3,7,8-TetraBDF	0.49	0.60	0.42	0.16	0.29	0.22	0.33	0.22	0.17	0.26	0.28	0.56	0.36
2,4,6,8-TetraBDF	0.02	0.01	0.01	0.004	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1,2,3,7,8-PentaBDF	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2,3,4,7,8-PentaBDF	0.01	0.01	0.01	<0.01	0.01	<0.01	0.01	0.01	<0.01	0.01	0.01	<0.01	0.01
1,2,3,4,7,8-HexaBDF	0.06	0.07	0.05	0.03	0.09	0.05	0.12	0.08	0.03	0.08	0.08	0.05	0.09
1,2,3,4,6,7,8-HeptaBDF	0.69	0.82	0.66	0.32	0.93	0.44	1.03	0.65	0.40	0.59	0.63	0.44	1.01
OctaBDF	0.87	0.94	0.76	<1.06	0.91	0.47	1.10	0.68	0.32	0.59	0.70	0.38	1.05
2,3,7,8-TetraBDD	<0.004	<0.01	<0.003	<0.002	<0.01	<0.01	<0.003	<0.002	<0.004	<0.01	<0.004	<0.003	<0.003
1,2,3,7,8-PentaBDD	<0.01	<0.004	<0.003	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.004	<0.01	<0.01
1,2,3,4,7,8/1,2,3,6,7,8-HexaBDD	<0.01	<0.003	<0.01	<0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1,2,3,7,8,9-HexaBDD	<0.01	<0.003	<0.004	0.01	0.02	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01	<0.01
1,2,3,4,6,7,8-HeptaBDD	0.03	0.04	0.03	0.06	0.05	0.05	0.05	0.04	0.06	0.05	0.04	0.05	0.03
OctaBDD	<0.12	<0.06	<0.07	<0.47	<0.12	<0.21	<0.09	<0.08	<0.11	<0.14	<0.08	<0.09	<0.17
WHO_{2005}-PBDD/F-TEQ (LB)	0.07	0.08	0.06	0.02	0.05	0.03	0.06	0.04	0.03	0.04	0.04	0.07	0.06
WHO_{2005}-PBDD/F-TEQ (UB)	0.08	0.09	0.06	0.04	0.07	0.05	0.07	0.05	0.04	0.06	0.05	0.08	0.07
PXDD/Fs (pg g^{-1})													
3-Br-2,7,8-TriClDF	0.06	0.04	0.02	0.03	0.03	0.08	0.05	0.04	0.06	0.07	0.03	0.07	0.06
2-Br-3,7,8-TriClDD	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2,3-DiBr-7,8-DiClDD	<0.002	<0.01	<0.01	<0.00	<0.001	<0.004	<0.002	<0.01	<0.01	<0.002	<0.002	<0.004	<0.01
1-Br-2,3,7,8-TetraClDF	<0.01	<0.004	<0.004	<0.01	<0.003	<0.004	<0.003	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
1-Br-2,3,7,8-TetraClDD	<0.01	<0.004	<0.01	<0.004	<0.01	<0.01	<0.004	<0.01	<0.01	<0.01	<0.003	<0.01	<0.01
2-Br-1,3,7,8-TetraClDD	<0.01	<0.004	<0.01	<0.004	<0.01	<0.01	<0.004	<0.01	<0.01	<0.01	<0.003	<0.01	<0.01
2-Br-3,6,7,8,9-PentaClDD	<0.002	<0.001	<0.003	<0.003	<0.003	<0.003	<0.002	<0.003	<0.002	<0.002	<0.001	<0.003	<0.002
1-Br-2,3,6,7,8,9-HexaClDD	<0.004	<0.003	<0.003	<0.004	<0.004	<0.003	<0.004	<0.004	<0.01	<0.003	<0.004	<0.003	<0.01
1-Br-2,3,4,6,7,8,9-HeptaClDD	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02
WHO_{2005}-PXDD/F-TEQ (LB)	0.01	0.004	0.002	0.003	0.003	0.01	0.005	0.004	0.01	0.01	0.003	0.01	0.01
WHO_{2005}-PXDD/F-TEQ (UB)	0.03	0.03	0.03	0.02	0.03	0.03	0.02	0.03	0.04	0.03	0.02	0.03	0.04

* - F – female; M – male.

Concentrations of DL-PCBs and NDL-PCBs in Baltic wild salmon samples calculated on f.w. basis

Sample Nr.	74505-1	74505-2	74505-3	74505-4	74505-5	74505-6	74505-7	74505-8	74505-9	74505-10	74505-11	74505-12
Sex*	F	M	F	M	F	F	M	M	M	F	M	F
Length, cm	74	57	59	63	83	83	71	65	56	81	58	84
Age, years	2	1	2	1	2	2	1	1	1	2	1	2
Weight, kg	4.4	2.5	3.5	3.0	5.8	5.8	3.8	3.6	2.0	5.3	2.6	5.6
Place of sampling	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River
Fat content, g/100 g of sample	2.4	3.5	7.9	6.3	2.9	6.1	3.5	5.8	5.3	5.5	4.8	5.2
DL-PCBs (pg g^{-1})												
2',3,4,4',5-PentaCB (#123)	440	323	396	250	310	364	336	201	174	336	214	286
2,3',4,4',5-PentaCB (#118)	9110	8200	9750	4880	6390	7990	6450	4820	3900	8320	4600	6500
2,3,4,4',5-PentaCB (#114)	205	116	178	94.7	127	149	133	89.7	70.1	157	78.9	153
2,3,3,'4,4'-PentaCB (#105)	4030	3280	3820	1730	2250	2820	2410	1780	1250	3240	1770	2260
2,3',4,4',5,5'-HexaCB (#167)	659	491	587	354	464	604	456	335	284	566	314	488
2,3,3',4,4',5-HexaCB (#156)	1330	1080	1240	663	885	1060	918	632	510	1150	615	883
2,3,3',4,4',5'-HexaCB (#157)	363	283	327	173	232	290	249	167	141	294	164	239
2,3,3',4,4',5,5'-HeptaCB (#189)	104	98.6	113	69.8	103	118	92.4	61.8	54.9	115	60.1	99
3,4,4',5-TetraCB (#81)	4.95	1.98	2.61	1.90	2.80	5.31	2.92	1.87	1.98	3.77	1.54	3.84
3,3,'4,4'-TetraCB (#77)	157	139	153	87.9	92.6	142	134	91.2	57.9	155	81.7	120
3,3',4,4',5-PentaCB (#126)	37.0	38.7	42.7	29.5	36.3	51.8	47.3	27.8	20.2	42.7	24.5	45.3
3,3',4,4',5,5'-HexaCB (#169)	7.48	8.74	10.6	7.69	10.0	12.5	11.5	6.63	5.44	10.1	5.97	11.4
WHO_{2005}-PCB-TEQ (LB)	4.43	4.56	5.10	3.44	4.26	5.97	5.42	3.24	2.39	5.01	2.88	5.21
WHO_{2005}-PCB-TEQ (UB)	4.43	4.56	5.10	3.44	4.26	5.97	5.42	3.24	2.39	5.01	2.88	5.21
NDL-PCBs (ng g^{-1})												
2,2',5-TriCB (#18)	0.10	0.15	0.27	0.23	0.14	0.23	0.37	0.19	0.22	0.25	0.20	0.20
2,2,4'-TriCB (#28)	1.19	1.07	1.53	1.04	0.89	1.40	1.54	0.94	0.92	1.46	0.94	1.22
2',3,4-TriCB (#33)	0.11	0.12	0.18	0.14	0.07	0.14	0.22	0.13	0.11	0.12	0.11	0.11
2,2',4,4'-TetraCB (#47)	0.49	0.45	0.53	0.32	0.36	0.49	0.48	0.32	0.27	0.52	0.32	0.48
2,2',4,5'-TetraCB (#49)	0.85	0.67	0.78	0.53	0.52	0.83	0.68	0.50	0.37	0.82	0.49	0.68
2,2',4,6'-TetraCB (#51)	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01
2,2',5,5'-TetraCB (#52)	1.46	1.27	1.54	1.08	1.14	1.70	1.50	1.02	0.78	1.62	0.99	1.59
2,3,4,4'-TetraCB (#60)	0.52	0.53	0.66	0.37	0.38	0.50	0.44	0.37	0.27	0.53	0.32	0.40
2,3',4,4'-TetraCB (#66)	1.16	1.48	1.71	0.81	0.96	1.02	0.88	0.80	0.66	1.21	0.81	0.87
2,4,4',5-TetraCB (#74)	0.85	0.87	1.05	0.57	0.65	0.81	0.67	0.57	0.38	0.85	0.51	0.64
2,2',4,4',5-PentaCB (#99)	3.45	3.00	3.50	1.68	2.13	2.59	1.97	1.78	1.45	2.90	1.74	2.00
2,2',4,5,5'-PentaCB (#101)	4.88	5.01	5.57	3.24	3.99	4.96	4.08	3.30	1.94	4.83	3.06	3.98
2,3,3',4',6-PentaCB (#110)	4.28	4.15	4.73	2.42	2.80	3.37	3.18	2.48	1.90	3.83	2.50	2.92
2,2',3,4,4',5'-HexaCB (#138)	10.8	9.94	12.9	7.13	10.1	11.9	18.2	7.19	5.20	10.4	7.04	9.09
2,2',4,4',5,5'-HexaCB (#153)	12.0	15.1	19.5	10.4	14.3	16.3	16.7	10.6	8.05	15.9	10.1	13.8
2,2',3,4,4',5,5'-HeptaCB (#180)	5.27	5.45	6.12	3.46	4.93	5.81	4.56	3.06	2.93	5.71	2.96	4.63
∑PCB (LB)	47.5	49.2	60.5	33.5	43.3	52.1	55.4	33.3	25.4	51.0	32.1	42.6
∑PCB (UB)	47.5	49.2	60.5	33.5	43.3	52.1	55.4	33.3	25.4	51.0	32.1	42.6

* - F – female; M – male.

Annex 6 continues

Sample Nr.	74505-13	74505-14	74505-15	79050-1	79050-2	79050-3	79050-4	79050-5	79050-6	79050-7	79050-8	79050-9	79050-10
Sex*	F	F	M	M	F	M	F	M	M	M	F	F	M
Length, cm	86	78	69	62	76	77	81	76	93	78	83	83	68
Age, years	3	3	1	1	2	2	3	2	3	2	3	3	2
Weight, kg	7.1	6.6	3.5	3.5	5.1	6.3	6.4	5.8	11.3	6.5	6.2	10.2	5.0
Place of sampling	Daugava River	Daugava River	Daugava River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River
Fat content, g/100 g of sample	8.2	4.3	5.8	5.7	4.8	6.2	3.6	5.6	6.1	8.2	4.8	12.0	7.1
DL-PCBs (pg g^{-1})													
2',3,4,4',5-PentaCB (#123)	427	379	219	293	363	510	307	385	410	436	240	415	429
2,3',4,4',5-PentaCB (#118)	9230	7300	5350	6210	7080	1100	5870	9110	7730	9370	5320	8410	9500
2,3,4,4',5-PentaCB (#114)	223	160	103	129	145	244	109	171	164	203	118	196	176
2,3,3,'4,4'-PentaCB (#105)	3280	2570	2110	2350	2520	3920	1970	3460	2770	3300	1910	2970	3490
2,3',4,4',5,5'-HexaCB (#167)	723	547	327	402	480	764	465	619	598	740	417	658	684
2,3,3',4,4',5-HexaCB (#156)	1340	1040	681	815	920	1430	823	1257	1070	1280	735	1190	1330
2,3,3',4,4',5'-HexaCB (#157)	347	267	179	216	245	386	213	329	282	337	195	312	340
2,3,3',4,4',5,5'-HeptaCB (#189)	139	122	57.3	78.5	91.4	141	92	125	113	129	79.7	131	135
3,4,4',5-TetraCB (#81)	4.42	2.64	1.65	1.95	2.68	5.27	3.11	3.53	3.69	5.42	3.82	6.59	3.43
3,3,'4,4'-TetraCB (#77)	171	109	96.2	99.1	109	188	101	144	143	187	96.8	169	135
3,3',4,4',5-PentaCB (#126)	65.6	48.1	24.0	31.0	36.5	74.1	40.1	49.5	55.0	69.6	35.6	63.9	57.8
3,3',4,4',5,5'-HexaCB (#169)	16.8	14.1	5.13	7.91	9.57	17.5	9.69	12.8	13.7	14.2	8.35	14.8	14.5
WHO_{2005}-PCB-TEQ (LB)	7.55	5.62	2.83	3.67	4.31	8.48	4.60	5.81	6.32	7.88	4.10	7.28	6.71
WHO_{2005}-PCB-TEQ (UB)	7.55	5.62	2.83	3.67	4.31	8.48	4.60	5.81	6.32	7.88	4.10	7.28	6.71
NDL-PCBs (ng g^{-1})													
2,2',5-TriCB (#18)	0.39	0.21	0.21	0.22	0.30	0.34	0.20	0.25	0.37	0.45	0.19	0.34	0.27
2,2,4'-TriCB (#28)	1.96	1.24	0.94	1.16	1.21	1.96	0.95	1.52	1.51	1.85	0.98	1.82	1.61
2',3,4-TriCB (#33)	0.24	0.11	0.11	0.12	0.13	0.19	0.09	0.13	0.17	0.23	0.09	0.15	0.17
2,2',4,4'-TetraCB (#47)	0.66	0.50	0.32	0.40	0.40	0.75	0.34	0.57	0.52	0.50	0.37	0.64	0.57
2,2',4,5'-TetraCB (#49)	1.01	0.71	0.49	0.56	0.53	1.09	0.53	0.91	0.82	1.09	0.58	1.03	0.88
2,2',4,6'-TetraCB (#51)	0.02	0.01	0.01	0.01	0.01	0.03	0.01	0.02	0.02	0.03	0.01	0.02	0.02
2,2',5,5'-TetraCB (#52)	2.21	1.62	0.97	0.99	1.30	2.43	1.11	1.80	1.91	2.12	1.18	2.12	1.82
2,3,4,4'-TetraCB (#60)	0.62	0.47	0.37	0.42	0.37	0.70	0.33	0.55	0.41	0.53	0.32	0.56	0.68
2,3',4,4'-TetraCB (#66)	1.50	1.13	0.85	1.13	0.96	1.68	0.64	1.40	1.14	1.06	0.76	1.24	1.76
2,4,4',5-TetraCB (#74)	0.99	0.78	0.58	0.70	0.58	1.15	0.55	0.93	0.77	0.89	0.52	0.92	1.15
2,2',4,4',5-PentaCB (#99)	3.11	2.47	1.98	2.14	1.87	3.53	1.74	3.18	2.11	2.65	1.80	2.83	3.60
2,2',4,5,5'-PentaCB (#101)	6.17	4.92	3.26	3.66	2.98	6.86	3.59	5.64	3.99	5.37	3.40	5.59	6.84
2,3,3',4',6-PentaCB (#110)	4.59	3.51	2.91	2.94	2.80	5.45	2.53	4.36	3.54	4.40	2.47	3.96	5.09
2,2',3,4,4',5'-HexaCB (#138)	15.4	11.2	7.71	8.43	9.08	13.4	8.45	12.4	10.4	13.4	8.62	12.1	13.9
2,2',4,4',5,5'-HexaCB (#153)	18.8	15.2	9.74	12.1	9.64	17.0	11.0	15.9	10.8	14.4	12.1	16.2	18.4
2,2',3,4,4',5,5'-HeptaCB (#180)	6.82	5.66	3.24	4.15	3.86	6.63	4.41	6.41	4.80	5.35	3.91	7.21	6.56
∑PCB (LB)	64.5	49.8	33.7	39.2	36.0	63.2	36.4	56.0	43.3	54.3	37.3	56.8	63.3
∑PCB (UB)	64.5	49.8	33.7	39.2	36.0	63.2	36.4	56.0	43.3	54.3	37.3	56.8	63.3

* - F – female; M – male.

Concentrations of PBDEs in Baltic wild salmon samples calculated on f.w. basis

Sample Nr.	74505-1	74505-2	74505-3	74505-4	74505-5	74505-6	74505-7	74505-8	74505-9	74505-10	74505-11	74505-12
Sex*	F	M	F	M	F	F	M	M	M	F	M	F
Length, cm	74	57	59	63	83	83	71	65	56	81	58	84
Age, years	2	1	2	1	2	2	1	1	1	2	1	2
Weight, kg	4.4	2.5	3.5	3.0	5.8	5.8	3.8	3.6	2.0	5.3	2.6	5.6
Place of sampling	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River	Daugava River
Fat content, g/100 g of sample	2.4	3.5	7.9	6.3	2.9	6.1	3.5	5.8	5.3	5.5	4.8	5.2
PBDEs (ng g^{-1})												
2,4-DiBDE (#7)	0.001	0.001	0.001	0.001	0.0004	0.001	0.001	0.001	0.0003	0.001	0.0003	0.001
4,4-DiBDE (#15)	0.001	0.001	0.002	0.001	0.001	0.002	0.002	0.001	0.001	0.001	0.001	0.001
2,2',4-TriBDE (#17)	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.02	0.01	0.02	0.01	0.03
2,4,4'-TriBDE (#28)	0.03	0.06	0.05	0.04	0.04	0.06	0.07	0.03	0.02	0.05	0.02	0.05
2,2',4,4'-TetraBDE (#47)	0.89	1.19	1.15	0.84	1.12	1.41	1.41	0.83	0.47	1.14	0.47	1.31
2,2',4,5'-TetraBDE (#49)	0.28	0.40	0.43	0.38	0.40	0.58	0.80	0.37	0.15	0.39	0.15	0.54
2,3',4,4'-TetraBDE (#66)	0.03	0.04	0.05	0.05	0.06	0.08	0.08	0.04	0.02	0.05	0.02	0.08
2,3',4',6-TetraBDE (#71)	<0.0001	<0.0003	<0.0002	<0.0002	<0.0003	<0.0002	<0.0003	<0.0002	<0.0002	<0.0002	<0.0002	<0.0001
3,3',4,4'-TetraBDE (#77)	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.02
2,2',3,4,4'-PentaBDE (#85)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.003	0.01	0.004	0.01
2,2',4,4',5-PentaBDE (#99)	0.14	0.16	0.17	0.17	0.20	0.30	0.32	0.15	0.12	0.20	0.13	0.28
2,2',4,4',6-PentaBDE (#100)	0.26	0.30	0.32	0.20	0.35	0.43	0.25	0.24	0.14	0.34	0.21	0.37
2,3',4,4',6-PentaBDE (#119)	0.06	0.06	0.06	0.06	0.08	0.13	0.10	0.06	0.03	0.08	0.05	0.11
3,3',4,4',5-PentaBDE (#126)	<0.0001	<0.0002	0.001	0.001	0.001	0.0004	0.0003	0.0002	0.0001	0.0002	<0.0003	0.0003
2,2',3,4,4',5'-HexaBDE (#138)	0.0004	0.0002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.0003	0.0004
2,2',4,4',5,5'-HexaBDE (#153)	0.06	0.05	0.06	0.06	0.08	0.12	0.10	0.05	0.02	0.08	0.04	0.11
2,2',4,4',5,6'-HexaBDE (#154)	0.18	0.16	0.18	0.18	0.24	0.36	0.26	0.15	0.10	0.25	0.12	0.31
2,2',4,4',6,6'-HexaBDE (#155)	0.07	0.08	0.10	0.08	0.12	0.15	0.11	0.07	0.03	0.11	0.05	0.13
2,3,4,4',5,6-HexaBDE (#166)	0.0002	<0.0002	0.001	0.001	0.001	<0.001	<0.0003	<0.0004	0.0002	0.0004	<0.0002	<0.0003
2,2',3,4,4',5,6'-HeptaBDE (#181)	0.001	0.0004	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001
2,2',3,4,4',5',6-HeptaBDE (#183)	0.003	0.002	0.003	0.003	0.003	0.004	0.004	0.002	0.002	0.002	0.003	0.01
2,3,3',4,4',5,6-HeptaBDE (#190)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
2,2',3,4,4',5,5',6-OctaBDE (#203)	0.01	0.01	0.004	0.01	0.004	0.01	0.004	0.01	0.004	0.002	0.01	0.01
2,3,3',4,4',5,5',6-OctaBDE (#205)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.003	0.01	0.01
2,2',3,3',4,4',5,5',6-NonaBDE (#206)	0.01	0.01	0.01	0.01	0.004	0.003	0.01	0.004	0.01	0.004	0.01	0.01
2,2',3,3',4,4',5,6,6'-NonaBDE (#207)	0.04	0.04	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.04	0.04
2,2',3,3',4,4',5,5',6,6'-DecaBDE (#209)	0.31	0.18	0.10	0.28	0.17	0.14	0.18	0.15	0.16	0.13	0.20	0.45
∑PBDE (UB)	2.39	2.77	2.75	2.43	2.95	3.86	3.78	2.21	1.34	2.88	1.54	3.87
∑PBDE (LB)	2.39	2.77	2.75	2.43	2.95	3.86	3.78	2.21	1.34	2.88	1.54	3.87

* - F – female; M – male.

Sample Nr.	74505-13	74505-14	74505-15	79050-1	79050-2	79050-3	79050-4	79050-5	79050-6	79050-7	79050-8	79050-9	79050-10
Sex*	F	F	M	M	F	M	F	M	M	M	F	F	M
Length, cm	86	78	69	62	76	77	81	76	93	78	83	83	68
Age, years	3	3	1	1	2	2	3	2	3	2	3	3	2
Weight, kg	7.1	6.6	3.5	3.5	5.1	6.3	6.4	5.8	11.3	6.5	6.2	10.2	5.0
Place of sampling	Daugava River	Daugava River	Daugava River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River	Venta River
Fat content, g/100 g of sample	8.2	4.3	5.8	5.7	4.8	6.2	3.6	5.6	6.1	8.2	4.8	12.0	7.1
PBDEs (ng g^{-1})													
2,4-DiBDE (#7)	0.001	0.0004	0.0004	0.001	<0.0001	0.001	0.001	0.001	<0.00003	<0.0001	0.001	0.001	0.001
4,4-DiBDE (#15)	0.002	0.001	0.001	0.001	<0.00004	0.004	0.003	0.002	0.003	0.01	0.001	0.002	0.002
2,2',4-TriBDE (#17)	0.04	0.03	0.01	0.02	0.01	0.04	0.02	0.03	0.03	0.03	0.02	0.03	0.03
2,4,4'-TriBDE (#28)	0.08	0.06	0.03	0.04	0.05	0.10	0.05	0.06	0.08	0.11	0.03	0.07	0.08
2,2',4,4'-TetraBDE (#47)	1.79	1.51	0.65	0.92	1.10	2.14	1.21	1.47	1.62	2.08	0.98	1.67	1.70
2,2',4,5'-TetraBDE (#49)	0.75	0.57	0.26	0.34	0.49	0.90	0.44	0.51	0.66	0.94	0.37	0.67	0.69
2,3',4,4'-TetraBDE (#66)	0.11	0.10	0.03	0.04	0.05	0.14	0.07	0.08	0.09	0.13	0.05	0.09	0.10
2,3',4',6-TetraBDE (#71)	<0.0003	<0.0002	<0.0001	<0.0002	<0.0001	<0.0001	<0.0004	<0.0002	<0.0001	<0.0002	<0.0002	<0.0002	<0.0001
3,3',4,4'-TetraBDE (#77)	0.02	0.02	0.01	0.01	0.01	0.03	0.02	0.01	0.02	0.03	0.01	0.02	0.02
2,2',3,4,4'-PentaBDE (#85)	0.02	0.01	0.003	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.02	0.01
2,2',4,4',5-PentaBDE (#99)	0.41	0.28	0.10	0.16	0.20	0.48	0.29	0.26	0.39	0.51	0.20	0.36	0.36
2,2',4,4',6-PentaBDE (#100)	0.53	0.39	0.16	0.24	0.37	0.74	0.34	0.46	0.48	0.59	0.31	0.39	0.49
2,3',4,4',6-PentaBDE (#119)	0.15	0.11	0.04	0.06	0.08	0.18	0.10	0.10	0.13	0.17	0.09	0.15	0.13
3,3',4,4',5-PentaBDE (#126)	<0.001	<0.0002	<0.0001	<0.0003	<0.0002	<0.0002	<0.0002	<0.0003	<0.0003	<0.0003	<0.0001	0.0002	<0.0002
2,2',3,4,4',5'-HexaBDE (#138)	0.0004	<0.0003	0.0003	0.0002	0.0002	<0.0003	<0.0003	<0.0003	<0.0003	<0.0004	<0.0002	<0.0002	<0.0003
2,2',4,4',5,5'-HexaBDE (#153)	0.15	0.10	0.03	0.06	0.07	0.14	0.10	0.09	0.13	0.15	0.08	0.15	0.12
2,2',4,4',5,6'-HexaBDE (#154)	0.39	0.29	0.10	0.17	0.21	0.36	0.26	0.27	0.33	0.40	0.22	0.42	0.33
2,2',4,4',6,6'-HexaBDE (#155)	0.17	0.13	0.04	0.08	0.10	0.16	0.11	0.12	0.15	0.17	0.09	0.17	0.15
2,3,4,4',5,6-HexaBDE (#166)	<0.0003	<0.0004	<0.0002	<0.0002	<0.0003	<0.0003	<0.0003	<0.0003	<0.0003	<0.001	<0.0002	<0.0002	<0.0003
2,2',3,4,4',5,6'-HeptaBDE (#181)	0.001	0.001	0.001	<0.0002	0.0004	<0.0003	0.0004	0.0003	0.0002	0.001	<0.0003	0.0002	0.001
2,2',3,4,4',5',6-HeptaBDE (#183)	0.004	0.004	0.002	0.001	0.002	0.002	0.003	0.002	0.002	0.003	0.002	0.003	0.003
2,3,3',4,4',5,6-HeptaBDE (#190)	0.001	0.001	0.001	<0.0002	0.001	<0.0004	0.001	0.001	0.0003	<0.0004	0.0004	<0.001	0.001
2,2',3,4,4',5,5',6-OctaBDE (#203)	0.004	0.01	0.003	0.001	0.003	0.002	0.004	0.004	0.001	0.003	0.003	0.002	0.003
2,3,3',4,4',5,5',6-OctaBDE (#205)	0.01	0.01	0.004	0.002	0.004	0.003	0.01	0.004	0.002	0.004	0.004	0.002	0.01
2,2',3,3',4,4',5,5',6-NonaBDE (#206)	0.003	0.001	0.003	0.002	0.004	0.001	0.002	0.002	0.003	0.003	0.002	0.002	0.003
2,2',3,3',4,4',5,6,6'-NonaBDE (#207)	0.02	0.03	0.02	0.01	0.03	0.01	0.03	0.02	0.01	0.02	0.02	0.01	0.03
2,2',3,3',4,4',5,5',6,6'-DecaBDE (#209)	0.18	0.28	0.16	0.08	0.15	0.11	0.16	0.11	0.07	0.11	0.13	0.09	0.20
∑PBDE (UB)	4.85	3.93	1.65	2.22	2.94	5.56	3.22	3.60	4.20	5.47	2.62	4.34	4.45
∑PBDE (LB)	4.85	3.93	1.65	2.22	2.94	5.56	3.22	3.60	4.20	5.47	2.62	4.34	4.44

* - F – female; M – male.

Concentrations of PCDD/Fs and HBCDs in eel samples calculated on f.w. basis

Sample Nr.	1/14	2/14	3/14	4/14	5/14	6/14	7/14	8/14	9/14	12/14	13/14	14/14	15/14
Length, cm	81	83	95	78	74	82	76	88	76	70	78	85	71
Weight, kg	1.0	1.1	1.6	0.8	0.8	1.1	0.8	1.0	0.9	0.6	0.8	1.0	0.5
Place of sampling	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	28.8	20.3	29.1	23.8	28	26.6	35.3	21.2	24.4	21.2	22.0	26.9	21.8
PCDD/Fs (pg g^{-1})													
2,3,7,8-TetraCDF	0.04	0.03	0.05	0.02	0.03	0.04	0.05	0.01	0.04	0.24	0.05	0.05	0.30
1,2,3,7,8-PentaCDF	0.02	0.02	<0.01	0.01	0.02	0.04	0.03	0.01	0.01	0.11	<0.01	0.04	0.09
2,3,4,7,8-PentaCDF	0.44	0.53	0.71	0.28	0.63	0.51	0.63	0.50	0.45	0.91	0.71	<0.01	0.97
1,2,3,4,7,8-HexaCDF	0.06	0.08	0.10	0.06	0.11	0.08	0.09	0.11	0.06	0.50	0.10	0.18	0.41
1,2,3,6,7,8-HexaCDF	0.06	0.09	0.09	0.06	0.11	0.07	0.10	0.11	0.06	0.41	0.09	0.17	0.39
2,3,4,6,7,8-HexaCDF	0.06	0.08	0.10	0.05	0.11	0.08	0.10	0.12	0.07	0.31	0.10	0.15	0.29
1,2,3,7,8,9-HexaCDF	0.01	0.01	<0.01	0.01	0.01	0.05	0.02	0.01	0.01	0.06	<0.01	0.01	0.05
1,2,3,4,6,7,8-HeptaCDF	0.03	0.04	0.04	0.04	0.05	0.05	0.04	0.06	0.05	0.24	0.04	0.06	0.18
1,2,3,4,7,8,9-HeptaCDF	<0.01	0.01	<0.01	0.01	0.01	0.04	0.02	0.01	0.01	0.01	0.01	<0.01	0.01
OctaCDF	0.01	0.01	0.02	0.02	0.01	0.06	0.02	0.03	0.03	0.14	0.02	0.05	0.12
2,3,7,8-TetraCDD	0.10	0.09	0.17	0.17	0.11	0.13	0.11	0.13	0.09	0.31	0.17	0.14	0.20
12378-PentaCDD	0.15	0.18	0.27	0.13	0.23	0.22	0.19	0.27	0.15	0.80	0.27	0.43	0.64
1,2,3,4,7,8-HexaCDD	0.03	0.03	0.06	0.03	0.05	0.05	0.04	0.06	0.03	0.56	0.06	0.08	0.43
1,2,3,6,7,8-HexaCDD	0.12	0.12	0.19	0.10	0.17	0.12	0.12	0.20	0.11	2.76	0.19	0.34	1.75
1,2,3,7,8,9-HexaCDD	0.02	0.03	0.05	0.02	0.04	0.05	0.04	0.05	0.03	0.15	0.05	0.05	0.12
1,2,3,4,6,7,8-HeptaCDD	0.04	0.05	0.07	0.05	0.07	0.07	0.06	0.09	0.04	0.47	0.07	0.09	0.35
OctaCDD	0.09	0.08	0.14	0.06	0.06	0.13	0.08	0.09	0.06	0.23	0.14	0.10	0.20
WHO_{2005}-PCDD/F-TEQ (LB)	0.42	0.48	0.71	0.42	0.59	0.56	0.55	0.62	0.42	1.89	0.71	0.68	1.52
WHO_{2005}-PCDD/F-TEQ (UB)	0.42	0.48	0.71	0.42	0.59	0.56	0.55	0.62	0.42	1.89	0.71	0.68	1.52
WHO_{1998}-PCDD/F-TEQ (UB)	0.51	0.58	0.86	0.48	0.72	0.66	0.67	0.72	0.51	2.07	0.86	0.68	1.71
HBCDs (pg g^{-1})													
α-HBCD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
β-HBCD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
γ-HBCD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total-HBCD (LB)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total-HBCD (UB)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

NA - not analyzed

Annex 8 continues

Sample Nr.	16/14	17/14	18/14	19/14	20/14	21/14	22/14	23/14	24/14	25/14	26/14	27/14	28/14	29/14	30/14
Length, cm	68	64	71	53	68	58	84	96	86	84	99	93	90	89	84
Weight, kg	0.5	0.4	0.5	0.2	0.5	0.3	1.0	1.9	1.1	0.9	1.5	1.4	1.2	1.3	1.0
Place of sampling	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake
Fat content, g/100 g of sample	24.3	21.9	22.1	27.6	24.0	22.4	25.2	26.8	23.9	26.0	22.9	23.8	25.5	25.9	27.1
PCDD/Fs (pg g^{-1})															
2,3,7,8-TetraCDF	0.02	0.03	0.01	0.26	0.02	0.03	0.89	0.27	0.73	0.23	0.35	0.38	0.35	0.16	0.25
1,2,3,7,8-PentaCDF	0.01	0.02	0.01	0.10	0.02	0.02	0.31	0.10	0.73	0.08	0.15	0.08	<0.04	0.08	0.10
2,3,4,7,8-PentaCDF	0.70	0.74	0.85	0.61	0.49	0.29	0.93	0.81	1.23	0.81	0.92	0.67	0.89	2.02	0.59
1,2,3,4,7,8-HexaCDF	0.38	0.20	0.35	0.16	0.26	0.10	0.31	0.18	0.59	0.16	0.20	0.15	0.18	0.25	0.16
1,2,3,6,7,8-HexaCDF	0.34	0.19	0.32	0.16	0.22	0.10	0.37	0.18	0.60	0.14	0.20	0.15	0.16	0.25	0.17
2,3,4,6,7,8-HexaCDF	0.22	0.25	0.30	0.19	0.28	0.11	0.30	0.19	0.56	0.13	0.19	0.15	0.17	0.26	0.17
1,2,3,7,8,9-HexaCDF	0.01	0.02	0.01	0.04	0.02	0.01	0.17	0.08	0.33	0.04	0.10	0.04	0.04	0.04	0.08
1,2,3,4,6,7,8-HeptaCDF	0.19	0.11	0.14	0.11	0.14	0.06	0.30	0.10	0.43	0.07	0.12	0.07	0.08	0.08	0.13
1,2,3,4,7,8,9-HeptaCDF	0.02	0.02	0.02	<0.01	0.02	0.01	<0.03	<0.01	<0.05	<0.01	0.01	<0.01	<0.01	0.01	<0.01
OctaCDF	0.03	0.02	0.02	0.12	0.02	0.03	0.50	0.12	0.99	0.08	0.22	0.11	0.16	0.09	0.16
2,3,7,8-TetraCDD	0.09	0.07	0.10	0.12	0.05	0.04	0.30	0.20	0.62	<0.07	0.21	0.10	0.43	0.26	0.15
12378-PentaCDD	0.66	0.35	0.60	0.26	0.25	0.11	0.56	0.38	0.53	0.28	0.37	0.32	0.08	0.61	0.29
1,2,3,4,7,8-HexaCDD	0.43	0.09	0.30	0.11	0.10	0.04	0.40	0.11	0.88	0.11	0.15	0.09	0.19	0.12	0.12
1,2,3,6,7,8-HexaCDD	2.17	0.36	1.46	0.28	0.46	0.14	0.91	0.23	1.50	0.33	0.32	0.26	0.36	0.48	0.26
1,2,3,7,8,9-HexaCDD	0.08	0.07	0.11	0.10	0.12	0.04	0.35	0.11	0.65	0.07	0.14	0.08	0.09	0.09	0.11
1,2,3,4,6,7,8-HeptaCDD	0.38	0.12	0.29	0.13	0.21	0.21	0.34	0.12	0.60	0.12	0.15	0.11	0.14	0.11	0.14
OctaCDD	0.13	0.12	0.14	0.14	0.14	0.10	0.48	0.17	1.14	0.14	0.23	0.15	0.14	0.11	0.18
WHO_{2005}-PCDD/F-TEQ (LB)	1.33	0.76	1.24	0.70	0.60	0.29	1.52	0.97	2.14	0.65	1.03	0.76	0.94	1.65	0.76
WHO_{2005}-PCDD/F-TEQ (UB)	1.33	0.76	1.24	0.70	0.60	0.29	1.52	0.97	2.14	0.72	1.03	0.76	0.94	1.65	0.76
WHO_{1998}-PCDD/F-TEQ (UB)	1.47	0.91	1.41	0.82	0.70	0.35	1.71	1.13	2.40	0.88	1.22	0.89	1.12	2.06	0.88
HBCDs (pg g^{-1})															
α-HBCD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
β-HBCD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
γ-HBCD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total-HBCD (LB)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total-HBCD (UB)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

NA - not analyzed

Annex 8 continues

Sample Nr.	31/14	32/14	33/14	34/14	35/14	36/14	37/14	38/14	39/14	40/14	41/14	42/14	43/14	1	2
Length, cm	89	93	72	82	75	94	101	95	94	92	95	83	83	55	50
Weight, kg	1.1	1.3	0.6	0.9	0.6	1.6	1.8	1.6	1.4	1.5	1.3	0.9	1.0	0.8	1.0
Place of sampling	Sivers Lake	Sivers Lake	Liepajas Lake	Liepajas Lake	Liepajas Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Liepajas Lake	Liepajas Lake	Liepajas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	23.0	25.5	28.9	41.3	33.2	37.0	36.9	34.7	27.2	32.2	27.2	32.1	34.2	24.4	20.6
PCDD/Fs (pg g^{-1})															
2,3,7,8-TetraCDF	<0.08	<0.29	0.23	0.63	0.36	0.33	0.58	0.28	0.50	0.61	0.68	0.26	0.49	0.05	0.03
1,2,3,7,8-PentaCDF	0.06	0.04	0.05	0.18	0.06	0.07	0.16	0.06	0.07	0.16	0.09	0.05	0.07	0.03	0.03
2,3,4,7,8-PentaCDF	0.78	1.20	1.57	3.68	0.87	1.03	1.57	1.97	1.63	1.36	1.35	1.69	1.59	1.00	0.56
1,2,3,4,7,8-HexaCDF	0.16	0.15	0.38	0.30	0.46	0.16	0.31	0.23	0.14	0.19	0.17	0.28	0.17	0.21	0.10
1,2,3,6,7,8-HexaCDF	0.17	0.16	0.20	0.25	0.21	0.15	0.32	0.17	0.14	0.17	0.14	0.23	0.12	0.21	0.11
2,3,4,6,7,8-HexaCDF	0.18	0.15	0.18	0.22	0.15	0.17	0.31	0.18	0.15	0.16	0.12	0.21	0.14	0.20	0.07
1,2,3,7,8,9-HexaCDF	0.05	0.02	0.06	0.04	<0.01	0.04	0.12	0.02	<0.02	0.07	0.03	0.01	<0.01	<0.01	<0.01
1,2,3,4,6,7,8-HeptaCDF	0.08	0.05	0.07	0.13	0.09	0.09	0.20	0.07	0.06	0.10	0.05	0.09	0.04	0.13	0.07
1,2,3,4,7,8,9-HeptaCDF	<0.01	<0.01	0.01	0.01	<0.02	0.01	<0.03	<0.01	<0.01	0.01	<0.01	0.01	<0.01	<0.03	<0.02
OctaCDF	0.11	0.31	0.10	0.08	0.10	0.09	0.28	0.08	0.06	0.17	0.07	0.04	0.03	0.04	0.02
2,3,7,8-TetraCDD	0.11	0.30	0.17	0.55	1.00	0.24	0.67	0.37	0.41	0.43	0.47	0.85	1.29	0.10	0.06
12378-PentaCDD	0.36	0.48	0.46	0.87	0.45	0.32	0.41	0.47	0.32	0.43	0.44	0.54	0.58	0.31	0.21
1,2,3,4,7,8-HexaCDD	0.09	0.09	0.15	0.13	0.09	0.09	0.24	0.11	0.06	0.12	0.06	0.10	0.07	0.09	0.05
1,2,3,6,7,8-HexaCDD	0.24	0.26	0.33	0.39	0.30	0.26	0.44	0.38	0.24	0.25	0.19	0.32	0.22	0.31	0.17
1,2,3,7,8,9-HexaCDD	0.08	0.07	0.11	0.10	0.06	0.07	0.20	0.07	<0.02	0.12	0.06	0.09	0.08	0.07	0.03
1,2,3,4,6,7,8-HeptaCDD	0.11	0.08	0.18	0.10	0.17	0.10	0.20	0.11	0.09	0.13	0.10	0.16	0.10	0.12	0.08
OctaCDD	0.14	0.52	0.33	0.13	0.53	0.16	0.39	0.19	0.14	0.21	0.13	0.13	0.21	0.10	0.08
WHO_{2005}-PCDD/F-TEQ (LB)	0.80	1.24	1.27	2.74	1.87	1.01	1.82	1.57	1.34	1.44	1.47	2.05	2.48	0.83	0.50
WHO_{2005}-PCDD/F-TEQ (UB)	0.81	1.27	1.27	2.74	1.88	1.01	1.82	1.57	1.35	1.44	1.47	2.05	2.48	0.83	0.50
WHO_{1998}-PCDD/F-TEQ (UB)	0.97	1.51	1.58	3.48	2.05	1.21	2.13	1.97	1.68	1.72	1.74	2.39	2.80	1.03	0.61
HBCDs (pg g^{-1})															
α-HBCD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	168	188
β-HBCD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	60.0	34.8
γ-HBCD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	18.0	17.2
Total-HBCD (LB)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	246	240
Total-HBCD (UB)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	246	240

NA - not analyzed

Annex 8 continues

Sample Nr.	3	4	5	6	7	8	1a	2a	3a	4a	5a	6a	7a	8a	9a
Length, cm	55	67	55	55	55	50	87	40	77	81	79	75	39	42	42
Weight, kg	0.9	1.7	0.9	1.1	0.9	0.9	1.2	0.1	0.9	1.0	0.9	0.9	1.0	0.1	0.1
Place of sampling	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	26.0	29.4	22.8	24.4	24.2	23.7	21.2	31.5	29.2	30.0	28.0	23.1	30.1	35.2	31.7
PCDD/Fs (pg g^{-1})															
2,3,7,8-TetraCDF	0.04	0.10	0.05	0.08	0.04	0.06	0.05	0.05	0.03	<0.03	0.04	0.02	0.02	0.04	0.04
1,2,3,7,8-PentaCDF	0.02	0.05	<0.01	0.05	0.03	0.04	0.03	0.05	0.02	<0.02	0.03	0.02	0.02	0.02	0.02
2,3,4,7,8-PentaCDF	0.69	1.42	0.88	1.27	1.26	1.80	1.08	2.35	1.82	1.32	1.32	1.50	0.68	1.38	0.56
1,2,3,4,7,8-HexaCDF	0.12	0.37	0.23	0.41	0.29	0.33	0.21	0.95	0.30	0.37	0.28	0.37	0.38	0.58	0.07
1,2,3,6,7,8-HexaCDF	0.13	0.34	0.19	0.44	0.28	0.29	0.17	0.89	0.31	0.33	0.23	0.33	0.33	0.54	0.07
2,3,4,6,7,8-HexaCDF	0.12	0.33	0.20	0.36	0.24	0.29	0.14	0.80	0.29	0.34	0.26	0.35	0.40	0.51	0.08
1,2,3,7,8,9-HexaCDF	<0.01	<0.01	0.23	0.32	<0.01	<0.01	0.01	0.03	0.01	<0.02	0.02	0.01	0.01	0.02	0.01
1,2,3,4,6,7,8-HeptaCDF	0.08	0.12	0.10	0.12	0.10	0.12	0.05	0.37	0.11	0.20	0.10	0.12	0.20	0.29	0.05
1,2,3,4,7,8,9-HeptaCDF	<0.05	<0.02	<0.03	<0.03	<0.03	<0.02	0.01	0.03	0.02	0.04	0.01	0.02	0.03	0.02	0.004
OctaCDF	0.04	0.03	0.03	0.02	0.02	0.03	0.01	0.06	<0.01	0.05	0.03	0.02	0.03	0.02	0.02
2,3,7,8-TetraCDD	0.08	0.18	0.09	0.14	0.14	0.17	0.10	0.22	0.16	0.12	0.14	0.13	0.06	0.14	0.14
12378-PentaCDD	0.37	0.52	0.43	0.50	0.66	0.73	0.32	1.31	0.56	0.64	0.51	0.51	0.27	0.78	0.22
1,2,3,4,7,8-HexaCDD	0.07	0.08	0.10	0.15	0.13	0.14	0.07	0.76	0.11	0.14	0.14	0.13	0.13	0.38	0.04
1,2,3,6,7,8-HexaCDD	0.22	0.53	0.37	0.34	0.63	0.66	0.29	2.89	0.52	0.55	0.48	0.62	0.58	1.36	0.10
1,2,3,7,8,9-HexaCDD	0.05	0.10	0.06	<0.08	0.06	0.09	0.04	0.40	0.06	0.10	0.10	0.09	0.17	0.20	0.02
1,2,3,4,6,7,8-HeptaCDD	0.09	0.15	0.14	0.14	0.14	0.14	0.14	1.02	0.13	0.39	0.15	0.18	0.33	0.52	0.05
OctaCDD	0.12	0.09	0.11	0.11	0.11	0.11	0.06	0.32	0.08	0.19	0.09	0.13	0.16	0.22	0.07
WHO_{2005}-PCDD/F-TEQ (LB)	0.73	1.31	0.93	1.23	1.35	1.63	0.84	2.93	1.43	1.34	1.20	1.29	0.74	1.71	0.58
WHO_{2005}-PCDD/F-TEQ (UB)	0.74	1.31	0.93	1.24	1.35	1.63	0.84	2.93	1.43	1.35	1.20	1.29	0.74	1.71	0.58
WHO_{1998}-PCDD/F-TEQ (UB)	0.87	1.59	1.11	1.50	1.60	1.99	1.06	3.40	1.79	1.61	1.46	1.59	0.87	1.98	0.69
HBCDs (pg g^{-1})															
α-HBCD	168	168	188	269	360	562	NA	NA	NA	NA	NA	NA	NA	NA	NA
β-HBCD	64.0	35.0	30.0	34.8	<13.0	22.0	NA	NA	NA	NA	NA	NA	NA	NA	NA
γ-HBCD	24.0	21.0	13.2	16.0	12.0	12.8	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total-HBCD (LB)	256	224	231	320	372	597	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total-HBCD (UB)	256	224	231	320	385	597	NA	NA	NA	NA	NA	NA	NA	NA	NA

NA - not analyzed

Concentrations of PBDD/Fs, PXDD/Fs and PXBs in eel samples calculated on f.w. basis

Sample Nr.	1/14	2/14	3/14	4/14	5/14	6/14	7/14	8/14	9/14	12/14	13/14	14/14	15/14
Length, cm	81	83	95	78	74	82	76	88	76	70	78	85	71
Weight, kg	1.0	1.1	1.6	0.8	0.8	1.1	0.8	1.0	0.9	0.6	0.8	1.0	0.5
Place of sampling	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	28.8	20.3	29.1	23.8	28	26.6	35.3	21.2	24.4	21.2	22.0	26.9	21.8
PBDD/Fs (pg g^{-1})													
2,3,7,8-TetraBDF	<0.01	<0.02	<0.01	0.02	0.02	<0.02	0.03	0.02	<0.01	<0.01	0.02	0.03	<0.004
1,2,3,7,8-PentaBDF	<0.02	0.06	<0.01	<0.01	<0.02	<0.04	<0.01	<0.02	<0.01	<0.01	<0.02	<0.01	<0.01
2,3,4,7,8-PentaBDF	<0.02	<0.03	<0.01	0.02	<0.02	<0.04	<0.01	<0.02	<0.01	<0.01	<0.02	0.03	<0.01
1,2,3,4,7,8-HexaBDF	<0.08	<0.14	<0.08	0.04	<0.08	<0.09	<0.05	<0.05	<0.05	<0.03	0.10	0.13	<0.04
1,2,3,4,6,7,8-HeptaBDF	0.28	0.36	0.17	0.18	0.24	0.27	0.46	0.18	0.11	0.07	0.32	0.41	0.05
2,3,7,8-TetraBDD	<0.01	<0.01	<0.004	<0.002	<0.004	<0.02	<0.004	<0.01	<0.004	<0.003	<0.004	<0.004	<0.003
1,2,3,7,8-PentaBDD	<0.02	<0.02	<0.01	<0.01	<0.02	<0.05	<0.02	<0.02	<0.01	<0.02	<0.02	<0.02	<0.01
1,2,3,4,7,8/1,2,3,6,7,8-HexaBDD	<0.07	<0.08	<0.04	<0.02	<0.04	<0.10	<0.05	<0.06	<0.04	<0.04	<0.03	<0.02	<0.02
1,2,3,7,8,9-HexaBDD	<0.04	<0.05	<0.03	<0.02	<0.03	<0.06	<0.03	<0.04	<0.02	<0.03	<0.02	<0.01	<0.02
WHO_{2005}-PBDD/F-TEQ (LB)	0.003	0.01	0.002	0.01	0.004	0.003	0.01	0.003	0.001	0.001	0.02	0.03	0.001
WHO_{2005}-PBDD/F-TEQ (UB)	0.06	0.07	0.04	0.03	0.05	0.10	0.05	0.05	0.03	0.04	0.05	0.06	0.03
PXDD/Fs (pg g^{-1})													
3-Br-2,7,8-TriClDF	<0.03	<0.04	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.002	<0.01	<0.02	<0.004
2-Br-3,7,8-TriClDD	<0.07	<0.18	<0.01	<0.02	<0.04	<0.07	<0.04	<0.03	<0.02	<0.01	<0.02	<0.004	<0.01
2,3-DiBr-7,8-DiClDD	<0.03	<0.06	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.05	<0.01	<0.02	<0.06
1-Br-2,3,7,8-TetraClDF	<0.06	<0.06	<0.01	<0.01	<0.01	<0.04	<0.01	<0.01	<0.01	<0.003	<0.01	<0.03	<0.01
1-Br-2,3,7,8-TetraClDD	<0.03	<0.07	<0.01	<0.01	<0.01	<0.03	<0.02	<0.01	<0.01	<0.003	<0.02	<0.03	<0.01
2-Br-1,3,7,8-TetraClDD	<0.03	<0.06	<0.01	<0.01	<0.01	<0.03	<0.02	<0.01	<0.004	<0.003	<0.01	<0.03	<0.004
2-Br-3,6,7,8,9-PentaClDD	<0.02	<0.04	<0.003	<0.003	<0.01	<0.02	<0.01	<0.01	<0.003	<0.002	<0.004	0.01	<0.003
1-Br-2,3,6,7,8,9-HexaClDD	<0.08	<0.18	<0.01	<0.02	<0.03	<0.06	<0.03	<0.02	<0.01	<0.01	<0.02	<0.05	<0.01
1-Br-2,3,4,6,7,8,9-HeptaClDD	<0.14	<0.15	<0.02	<0.04	<0.05	<0.05	<0.03	<0.02	<0.02	<0.01	<0.03	<0.04	<0.02
WHO_{2005}-PXDD/F-TEQ (LB)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.001	ND
WHO_{2005}-PXDD/F-TEQ (UB)	0.16	0.39	0.04	0.04	0.08	0.15	0.08	0.05	0.03	0.07	0.06	0.13	0.08
PXBs (pg g^{-1})													
4'-Br-2,3',4,5-Cl-XB (#118 mono-Br)	0.22	0.16	0.43	NA	0.29	0.32	0.23	0.41	0.32	NA	0.30	0.34	NA
4'-Br-2,3,3',4-Cl-XB (#105 mono-Br)	0.08	0.05	0.14	NA	0.09	0.11	0.09	0.15	0.13	NA	0.12	0.15	NA
4'-Br-2,3,3',4,5-Cl-XB (#156 mono-Br)	0.09	0.05	0.12	NA	0.06	0.08	<0.06	0.11	0.06	NA	0.13	0.13	NA
4'-Br-3,3',4,5-Cl-XB (#126 mono-Br)	0.01	0.02	0.02	NA	<0.01	0.02	0.02	<0.01	0.02	NA	0.01	0.02	NA
3,4-Br-3',4',5'-Cl-XB (#126 di-Br)	<0.004	<0.01	<0.01	NA	<0.01	<0.01	<0.01	<0.01	<0.04	NA	<0.01	<0.004	NA
3',4',5-Br-3,4-Cl-XB (#126 tri-Br)	<0.03	<0.06	<0.03	NA	<0.03	<0.04	<0.05	<0.04	<0.04	NA	<0.04	<0.03	NA
WHO_{2005}-PXB-TEQ (LB)	0.001	0.002	0.002	NA	0.00001	0.002	0.002	0.00002	0.002	NA	0.001	0.002	NA
WHO_{2005}-PXB-TEQ (UB)	0.004	0.01	0.01	NA	0.01	0.01	0.01	0.01	0.01	NA	0.01	0.01	NA

ND - not detected since none of the analyzed congeners show the levels above the LOQ
NA - not analyzed

Annex 9 continues

Sample Nr.	16/14	17/14	18/14	19/14	20/14	21/14	22/14	23/14	24/14	25/14	26/14	27/14	28/14	29/14	30/14
Length, cm	68	64	71	53	68	58	84	96	86	84	99	93	90	89	84
Weight, kg	0.5	0.4	0.5	0.2	0.5	0.3	1.0	1.9	1.1	0.9	1.5	1.4	1.2	1.3	1.0
Place of sampling	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake
Fat content, g/100 g of sample	24.3	21.9	22.1	27.6	24.0	22.4	25.2	26.8	23.9	26.0	22.9	23.8	25.5	25.9	27.1
PBDD/Fs (pg g^{-1})															
2,3,7,8-TetraBDF	0.02	0.02	0.02	<0.02	<0.01	0.03	0.05	0.10	<0.01	<0.01	0.25	<0.01	<0.01	<0.01	0.14
1,2,3,7,8-PentaBDF	<0.02	<0.01	0.03	<0.02	<0.01	<0.02	0.02	<0.02	<0.02	<0.02	<0.05	<0.01	<0.01	<0.01	<0.01
2,3,4,7,8-PentaBDF	0.02	<0.01	<0.01	<0.02	<0.01	0.03	<0.01	<0.02	<0.02	<0.02	<0.04	<0.01	<0.01	<0.01	<0.01
1,2,3,4,7,8-HexaBDF	0.20	<0.02	<0.06	<0.09	<0.06	0.14	<0.04	<0.11	<0.11	<0.08	<0.07	<0.07	<0.07	<0.02	<0.07
1,2,3,4,6,7,8-HeptaBDF	1.05	0.16	0.22	0.16	0.31	0.84	0.12	0.21	0.03	0.11	0.17	0.09	0.16	0.05	0.06
2,3,7,8-TetraBDD	<0.01	<0.004	<0.003	<0.004	<0.01	0.01	<0.004	<0.004	<0.01	<0.01	<0.004	<0.002	<0.002	<0.07	<0.002
1,2,3,7,8-PentaBDD	<0.01	<0.01	<0.02	<0.05	<0.02	<0.02	<0.01	<0.01	<0.02	<0.02	<0.05	<0.01	<0.01	<0.02	<0.01
1,2,3,4,7,8/1,2,3,6,7,8-HexaBDD	<0.04	<0.02	<0.05	<0.07	<0.06	<0.03	<0.03	<0.03	<0.06	<0.03	<0.07	<0.05	<0.05	<0.04	<0.05
1,2,3,7,8,9-HexaBDD	<0.02	<0.01	<0.03	<0.04	<0.04	<0.02	<0.02	<0.02	<0.04	<0.02	<0.04	<0.03	<0.03	<0.02	<0.03
WHO_{2005}-PBDD/F-TEQ (LB)	0.04	0.004	0.01	0.002	0.003	0.04	0.01	0.01	0.0003	0.001	0.03	0.001	0.002	0.001	0.01
WHO_{2005}-PBDD/F-TEQ (UB)	0.06	0.02	0.04	0.09	0.05	0.06	0.04	0.05	0.06	0.04	0.12	0.04	0.04	0.10	0.05
PXDD/Fs (pg g^{-1})															
3-Br-2,7,8-TriClDF	<0.01	0.01	<0.004	<0.01	<0.01	<0.01	<0.02	<0.004	<0.003	<0.003	<0.003	<0.01	<0.003	<0.003	<0.01
2-Br-3,7,8-TriClDD	<0.01	<0.01	<0.01	<0.01	<0.03	<0.03	<0.03	<0.01	<0.01	<0.01	<0.01	<0.03	<0.01	<0.01	<0.01
2,3-DiBr-7,8-DiClDD	<0.01	<0.01	<0.01	<0.08	<0.01	<0.02	<0.11	<0.05	<0.05	<0.05	<0.05	<0.12	<0.03	<0.08	<0.07
1-Br-2,3,7,8-TetraClDF	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.04	<0.003	<0.004	<0.003	<0.004	<0.03	<0.01	<0.004	<0.01
1-Br-2,3,7,8-TetraClDD	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.04	<0.01	<0.01	<0.01	<0.01	<0.04	<0.004	<0.01	<0.01
2-Br-1,3,7,8-TetraClDD	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.03	<0.004	<0.01	<0.01	<0.004	<0.04	<0.003	<0.01	<0.01
2-Br-3,6,7,8,9-PentaClDD	0.02	<0.04	0.01	<0.003	<0.004	<0.01	<0.01	<0.002	<0.002	<0.002	<0.002	<0.01	<0.002	<0.002	<0.004
1-Br-2,3,6,7,8,9-HexaClDD	<0.01	<0.02	<0.02	<0.01	<0.03	<0.03	<0.02	<0.01	<0.01	<0.01	<0.02	<0.03	<0.01	<0.01	<0.02
1-Br-2,3,4,6,7,8,9-HeptaClDD	<0.03	<0.02	<0.03	<0.03	<0.04	<0.04	<0.06	<0.02	<0.03	<0.02	<0.01	<0.05	<0.02	<0.01	<0.03
WHO_{2005}-PXDD/F-TEQ (LB)	0.002	0.001	0.001	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
WHO_{2005}-PXDD/F-TEQ (UB)	0.04	0.04	0.05	0.13	0.06	0.06	0.21	0.07	0.07	0.08	0.07	0.23	0.05	0.10	0.11
PXBs (pg g^{-1})															
4'-Br-2,3',4,5-Cl-XB (#118 mono-Br)	NA	NA	0.15	NA	0.18	0.07	NA	0.66	NA	NA	0.47	0.37	NA	NA	0.23
4'-Br-2,3,3',4-Cl-XB (#105 mono-Br)	NA	NA	0.07	NA	0.08	0.03	NA	0.27	NA	NA	0.21	0.16	NA	NA	0.10
4'-Br-2,3,3',4,5-Cl-XB (#156 mono-Br)	NA	NA	0.08	NA	<0.05	<0.04	NA	<0.18	NA	NA	<0.28	0.10	NA	NA	<0.11
4'-Br-3,3',4,5-Cl-XB (#126 mono-Br)	NA	NA	0.01	NA	0.01	0.01	NA	0.05	NA	NA	0.03	0.01	NA	NA	0.02
3,4-Br-3',4',5'-Cl-XB (#126 di-Br)	NA	NA	<0.01	NA	<0.01	<0.003	NA	<0.02	NA	NA	<0.02	<0.01	NA	NA	<0.01
3',4',5-Br-3,4-Cl-XB (#126 tri-Br)	NA	NA	<0.04	NA	<0.04	<0.02	NA	<0.05	NA	NA	<0.08	<0.02	NA	NA	<0.02
WHO_{2005}-PXB-TEQ (LB)	NA	NA	0.001	NA	0.001	0.001	NA	0.002	NA	NA	0.001	0.001	NA	NA	0.002
WHO_{2005}-PXB-TEQ (UB)	NA	NA	0.01	NA	0.01	0.003	NA	0.01	NA	NA	0.01	0.004	NA	NA	0.004

ND - not detected since none of the analyzed congeners show the levels above the LOQ
NA - not analyzed

Annex 9 continues

Sample Nr.	31/14	32/14	33/14	34/14	35/14	36/14	37/14	38/14	39/14	40/14	41/14	42/14	43/14	1	2
Length, cm	89	93	72	82	75	94	101	95	94	92	95	83	83	55	50
Weight, kg	1.1	1.3	0.6	0.9	0.6	1.6	1.8	1.6	1.4	1.5	1.3	0.9	1.0	0.8	1.0
Place of sampling	Sivers Lake	Sivers Lake	Liepajas Lake	Liepajas Lake	Liepajas Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Liepajas Lake	Liepajas Lake	Liepajas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	23.0	25.5	28.9	41.3	33.2	37.0	36.9	34.7	27.2	32.2	27.2	32.1	34.2	24.4	20.6
PBDD/Fs (pg g^{-1})															
2,3,7,8-TetraBDF	0.07	<0.01	<0.01	<0.01	<0.01	0.17	0.12	<0.01	<0.01	<0.05	<0.02	0.21	<0.01	0.02	0.03
1,2,3,7,8-PentaBDF	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	0.02	<0.01	<0.02	<0.01	<0.02	<0.01	<0.01	<0.01	0.02
2,3,4,7,8-PentaBDF	<0.01	0.02	<0.04	<0.01	<0.01	<0.01	<0.02	<0.01	<0.02	0.02	<0.02	<0.01	<0.01	<0.01	<0.01
1,2,3,4,7,8-HexaBDF	<0.03	<0.03	<0.07	0.14	<0.07	<0.03	<0.09	<0.03	<0.07	<0.05	<0.09	<0.05	<0.07	<0.05	0.12
1,2,3,4,6,7,8-HeptaBDF	0.09	0.19	0.13	0.28	0.26	0.22	0.20	0.15	0.13	0.09	0.14	0.16	0.12	0.13	0.08
2,3,7,8-TetraBDD	<0.003	<0.004	<0.002	<0.003	<0.002	<0.004	<0.004	<0.003	<0.01	<0.09	<0.01	<0.09	<0.002	<0.002	0.01
1,2,3,7,8-PentaBDD	0.04	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01	<0.03	<0.02	<0.03	<0.01	<0.01	<0.01	<0.02
1,2,3,4,7,8/1,2,3,6,7,8-HexaBDD	<0.03	0.05	<0.05	<0.02	<0.05	<0.03	<0.03	<0.08	<0.06	<0.05	<0.09	<0.05	<0.07	<0.03	<0.04
1,2,3,7,8,9-HexaBDD	<0.02	<0.02	0.05	<0.01	<0.03	<0.02	<0.02	<0.05	<0.04	<0.03	0.14	<0.03	<0.04	<0.02	<0.03
WHO_{2005}-PBDD/F-TEQ (LB)	0.04	0.01	0.01	0.02	0.003	0.02	0.01	0.002	0.001	0.01	0.02	0.02	0.001	0.003	0.02
WHO_{2005}-PBDD/F-TEQ (UB)	0.06	0.03	0.05	0.04	0.04	0.05	0.05	0.04	0.06	0.13	0.08	0.14	0.04	0.03	0.05
PXDD/Fs (pg g^{-1})															
3-Br-2,7,8-TriClDF	<0.002	<0.01	<0.003	<0.002	<0.01	<0.003	<0.01	<0.003	<0.004	<0.002	0.01	<0.003	<0.003	<0.003	0.01
2-Br-3,7,8-TriClDD	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.03	<0.01	<0.02	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
2,3-DiBr-7,8-DiClDD	<0.02	<0.04	<0.05	<0.09	<0.12	<0.07	<0.08	<0.04	<0.06	<0.08	<0.13	<0.03	<0.08	<0.003	<0.003
1-Br-2,3,7,8-TetraClDF	<0.004	<0.01	<0.004	<0.01	<0.01	<0.01	<0.01	<0.002	<0.004	<0.003	<0.01	<0.01	<0.04	<0.004	0.01
1-Br-2,3,7,8-TetraClDD	<0.003	<0.02	<0.01	<0.004	<0.01	<0.004	<0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.004	<0.01
2-Br-1,3,7,8-TetraClDD	<0.003	<0.01	<0.004	<0.004	<0.01	<0.003	<0.02	<0.01	<0.01	<0.01	<0.01	<0.004	<0.004	<0.003	<0.01
2-Br-3,6,7,8,9-PentaClDD	<0.002	<0.003	<0.002	<0.002	<0.003	<0.002	<0.01	<0.003	<0.003	<0.002	<0.01	<0.002	<0.003	<0.002	0.01
1-Br-2,3,6,7,8,9-HexaClDD	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.02	<0.01
1-Br-2,3,4,6,7,8,9-HeptaClDD	<0.01	<0.02	<0.02	<0.02	<0.02	<0.02	<0.05	<0.01	<0.02	<0.01	<0.02	<0.01	<0.02	<0.02	<0.02
WHO_{2005}-PXDD/F-TEQ (LB)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.001	ND	ND	ND	0.002
WHO_{2005}-PXDD/F-TEQ (UB)	0.04	0.08	0.07	0.11	0.15	0.08	0.15	0.07	0.09	0.10	0.17	0.05	0.10	0.02	0.02
PXBs (pg g^{-1})															
4'-Br-2,3',4,5-Cl-XB (#118 mono-Br)	NA	NA	0.58	NA	3.18	NA	NA	NA	NA	0.87	0.84	1.45	NA	NA	NA
4'-Br-2,3,3',4-Cl-XB (#105 mono-Br)	NA	NA	0.22	NA	0.77	NA	NA	NA	NA	0.26	0.24	0.52	NA	NA	NA
4'-Br-2,3,3',4,5-Cl-XB (#156 mono-Br)	NA	NA	<0.16	NA	<0.30	NA	NA	NA	NA	<0.21	<0.11	<0.26	NA	NA	NA
4'-Br-3,3',4,5-Cl-XB (#126 mono-Br)	NA	NA	0.03	NA	0.01	NA	NA	NA	NA	0.02	0.02	0.03	NA	NA	NA
3,4-Br-3',4',5'-Cl-XB (#126 di-Br)	NA	NA	<0.01	NA	<0.01	NA	NA	NA	NA	<0.01	<0.01	<0.01	NA	NA	NA
3',4',5-Br-3,4-Cl-XB (#126 tri-Br)	NA	NA	<0.02	NA	<0.01	NA	NA	NA	NA	<0.02	<0.01	<0.02	NA	NA	NA
WHO_{2005}-PXB-TEQ (LB)	NA	NA	0.003	NA	0.001	NA	NA	NA	NA	0.002	0.002	0.003	NA	NA	NA
WHO_{2005}-PXB-TEQ (UB)	NA	NA	0.01	NA	0.003	NA	NA	NA	NA	0.01	0.003	0.01	NA	NA	NA

ND - not detected since none of the analyzed congeners show the levels above the LOQ
NA - not analyzed

Annex 9 continues

Sample Nr.	3	4	5	6	7	8	1a	2a	3a	4a	5a	6a	7a	8a	9a
Length, cm	55	67	55	55	55	50	87	40	77	81	79	75	39	42	42
Weight, kg	0.9	1.7	0.9	1.1	0.9	0.9	1.2	0.1	0.9	1.0	0.9	0.9	1.0	0.1	0.1
Place of sampling	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	26.0	29.4	22.8	24.4	24.2	23.7	21.2	31.5	29.2	30.0	28.0	23.1	30.1	35.2	31.7
PBDD/Fs (pg g^{-1})															
2,3,7,8-TetraBDF	0.03	0.02	0.04	<0.01	0.02	<0.004	0.03	0.03	<0.01	<0.02	0.03	0.03	0.03	0.02	<0.01
1,2,3,7,8-PentaBDF	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.02	<0.02	<0.05	0.02	<0.02	<0.02	<0.01
2,3,4,7,8-PentaBDF	<0.01	<0.02	0.03	<0.01	<0.01	<0.01	0.02	<0.01	<0.02	<0.02	<0.04	<0.01	<0.02	0.02	<0.01
1,2,3,4,7,8-HexaBDF	0.10	<0.04	0.08	0.10	<0.04	<0.03	<0.06	0.06	<0.08	<0.09	<0.07	<0.07	<0.07	<0.11	<0.03
1,2,3,4,6,7,8-HeptaBDF	0.43	0.15	0.33	0.45	0.21	0.23	0.52	0.24	0.28	0.58	0.15	0.11	0.20	0.37	0.15
2,3,7,8-TetraBDD	<0.003	<0.002	<0.003	<0.002	0.01	<0.003	<0.01	<0.004	<0.01	<0.01	<0.004	<0.002	<0.01	<0.01	<0.004
1,2,3,7,8-PentaBDD	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.03	<0.05	<0.01	<0.03	<0.02	<0.01
1,2,3,4,7,8/1,2,3,6,7,8-HexaBDD	<0.02	<0.03	<0.02	<0.03	<0.03	<0.02	<0.03	<0.03	<0.08	<0.09	<0.07	<0.05	<0.06	<0.06	<0.03
1,2,3,7,8,9-HexaBDD	<0.01	<0.02	<0.02	<0.02	<0.02	<0.01	<0.02	<0.02	<0.05	<0.06	<0.04	<0.03	<0.04	<0.04	<0.02
WHO_{2005}-PBDD/F-TEQ (LB)	0.02	0.003	0.02	0.01	0.01	0.002	0.01	0.01	0.003	0.01	0.01	0.01	0.01	0.01	0.001
WHO_{2005}-PBDD/F-TEQ (UB)	0.04	0.04	0.04	0.04	0.04	0.02	0.05	0.04	0.05	0.08	0.09	0.04	0.06	0.06	0.03
PXDD/Fs (pg g^{-1})															
3-Br-2,7,8-TriClDF	<0.01	<0.004	<0.003	0.004	0.01	0.003	0.01	<0.01	<0.01	<0.03	<0.01	<0.01	<0.004	<0.004	<0.01
2-Br-3,7,8-TriClDD	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.04	<0.05	<0.02	<0.02	<0.01	<0.01	<0.02
2,3-DiBr-7,8-DiClDD	<0.01	<0.01	<0.003	<0.003	<0.01	<0.01	<0.004	<0.01	<0.01	<0.01	<0.004	<0.004	<0.004	<0.01	<0.01
1-Br-2,3,7,8-TetraClDF	<0.02	<0.003	<0.01	<0.01	<0.01	<0.004	<0.01	<0.01	<0.01	<0.06	<0.01	<0.01	<0.01	<0.01	<0.01
1-Br-2,3,7,8-TetraClDD	<0.02	<0.01	<0.003	<0.01	<0.01	<0.004	<0.01	<0.01	<0.02	<0.10	<0.01	<0.01	<0.01	<0.01	<0.01
2-Br-1,3,7,8-TetraClDD	<0.02	<0.01	<0.003	<0.04	<0.01	<0.003	<0.01	<0.01	<0.02	<0.09	<0.01	<0.01	<0.01	<0.01	<0.01
2-Br-3,6,7,8,9-PentaClDD	<0.01	<0.002	<0.001	<0.002	<0.003	<0.003	<0.003	<0.002	<0.01	<0.03	<0.01	<0.01	<0.003	0.01	<0.004
1-Br-2,3,6,7,8,9-HexaClDD	<0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.06	<0.04	<0.01	<0.01	<0.02	<0.02
1-Br-2,3,4,6,7,8,9-HeptaClDD	<0.04	<0.02	<0.02	<0.01	<0.02	<0.01	<0.03	<0.03	<0.02	<0.07	<0.03	<0.02	<0.02	<0.03	<0.02
WHO_{2005}-PXDD/F-TEQ (LB)	ND	ND	ND	0.0004	0.001	0.0003	0.001	ND	ND	ND	ND	ND	ND	0.001	ND
WHO_{2005}-PXDD/F-TEQ (UB)	0.06	0.04	0.02	0.02	0.03	0.02	0.03	0.03	0.09	0.25	0.04	0.05	0.02	0.03	0.05
PXBs (pg g^{-1})															
4'-Br-2,3',4,5-Cl-XB (#118 mono-Br)	NA	0.31	NA	NA	0.43	0.56	0.20	NA	NA	NA	NA	NA	NA	NA	NA
4'-Br-2,3,3',4-Cl-XB (#105 mono-Br)	NA	0.15	NA	NA	0.18	0.22	0.10	NA	NA	NA	NA	NA	NA	NA	NA
4'-Br-2,3,3',4,5-Cl-XB (#156 mono-Br)	NA	0.10	NA	NA	0.18	0.24	0.08	NA	NA	NA	NA	NA	NA	NA	NA
4'-Br-3,3',4,5-Cl-XB (#126 mono-Br)	NA	0.03	NA	NA	0.02	0.03	0.02	NA	NA	NA	NA	NA	NA	NA	NA
3,4-Br-3',4',5'-Cl-XB (#126 di-Br)	NA	<0.004	NA	NA	<0.01	<0.01	<0.004	NA	NA	NA	NA	NA	NA	NA	NA
3',4',5-Br-3,4-Cl-XB (#126 tri-Br)	NA	<0.03	NA	NA	<0.03	<0.03	<0.03	NA	NA	NA	NA	NA	NA	NA	NA
WHO_{2005}-PXB-TEQ (LB)	NA	0.003	NA	NA	0.002	0.003	0.002	NA	NA	NA	NA	NA	NA	NA	NA
WHO_{2005}-PXB-TEQ (UB)	NA	0.01	NA	NA	0.01	0.01	0.01	NA	NA	NA	NA	NA	NA	NA	NA

ND - not detected since none of the analyzed congeners show the levels above the LOQ
NA - not analyzed

Concentrations of DL-PCBs and NDL-PCBs in eel samples calculated on f.w. basis

Sample Nr.	1/14	2/14	3/14	4/14	5/14	6/14	7/14	8/14	9/14	12/14	13/14	14/14	15/14
Length, cm	81	83	95	78	74	82	76	88	76	70	78	85	71
Weight, kg	1.0	1.1	1.6	0.8	0.8	1.1	0.8	1.0	0.9	0.6	0.8	1.0	0.5
Place of sampling	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	28.8	20.3	29.1	23.8	28.0	26.6	35.3	21.2	24.4	21.2	22.0	26.9	21.8
DL-PCBs (pg g^{-1})													
2',3,4,4',5-PentaCB (#123)	141	87.9	240	188	169	145	82.7	170	124	146	31.4	76.0	78.2
2,3',4,4',5-PentaCB (#118)	6150	4990	7180	6620	6520	6570	4880	6830	6100	8030	3670	3660	4430
2,3,4,4',5-PentaCB (#114)	202	134	379	388	236	230	149	315	232	182	104	116	102
2,3,3,'4,4'-PentaCB (#105)	2580	1840	4490	4410	2850	2980	1750	3960	3140	2430	1350	1500	1480
2,3',4,4',5,5'-HexaCB (#167)	486	318	931	695	568	535	321	769	543	387	444	433	267
2,3,3',4,4',5-HexaCB (#156)	1010	655	1740	1530	1150	1090	645	1580	1150	777	844	848	608
2,3,3',4,4',5'-HexaCB (#157)	203	141	351	304	240	227	137	318	236	143	158	162	119
2,3,3',4,4',5,5'-HeptaCB (#189)	58.5	34.7	99.3	67.9	70.6	61.6	36.6	100	60.9	91.8	121	102	75.7
3,4,4',5-TetraCB (#81)	0.51	0.27	0.55	0.14	0.23	0.45	0.40	<0.04	0.31	0.11	0.10	0.17	0.10
3,3,'4,4'-TetraCB (#77)	2.01	0.99	2.38	1.01	1.23	2.13	1.85	0.97	1.87	0.62	0.54	0.88	0.71
3,3',4,4',5-PentaCB (#126)	35.1	26.7	47.1	25.8	39.1	38.2	26.7	46.7	36.8	11.5	18.9	23.3	11.6
3,3',4,4',5,5'-HexaCB (#169)	3.17	2.62	4.67	2.35	3.99	3.19	2.46	5.29	3.38	7.08	8.86	7.00	6.13
WHO_{2005}-PCB-TEQ (LB)	3.93	2.99	5.31	3.07	4.39	4.27	2.99	5.25	4.13	1.73	2.36	2.75	1.56
WHO_{2005}-PCB-TEQ (UB)	3.93	2.99	5.31	3.07	4.39	4.27	2.99	5.25	4.13	1.73	2.36	2.75	1.56
WHO_{1998}-PCB-TEQ (UB)	5.14	3.86	7.20	4.85	5.73	5.61	3.84	6.95	5.47	2.85	3.05	3.50	2.24
NDL-PCBs (ng g^{-1})													
2,2',5-TriCB (#18)	<0.0002	0.01	0.03	0.01	0.04	0.09	0.06	0.03	0.03	0.02	0.03	0.03	0.04
2,2,4'-TriCB (#28)	0.50	0.33	0.76	0.52	0.48	0.76	0.59	0.48	0.48	0.43	0.76	0.27	0.58
2',3,4-TriCB (#33)	0.02	0.00	0.02	<0.0001	0.02	0.08	0.04	0.02	0.01	0.01	0.02	0.02	0.03
2,2',4,4'-TetraCB (#47)	0.58	0.26	0.58	0.85	0.38	0.47	0.31	0.59	0.38	0.98	0.58	0.19	0.49
2,2',4,5'-TetraCB (#49)	0.08	0.02	0.07	0.15	0.05	0.11	0.06	0.05	0.04	0.25	0.07	0.04	0.10
2,2',4,6'-TetraCB (#51)	0.003	0.003	0.002	0.002	0.002	0.01	0.004	0.002	0.002	0.003	0.002	0.001	0.01
2,2',5,5'-TetraCB (#52)	1.43	0.60	1.25	1.98	1.13	1.37	0.82	1.46	0.83	1.41	1.25	0.52	1.05
2,3,4,4'-TetraCB (#60)	0.19	0.15	0.35	0.42	0.22	0.26	0.17	0.29	0.23	0.22	0.35	0.15	0.23
2,3',4,4'-TetraCB (#66)	0.70	0.77	1.84	1.78	0.46	1.25	0.83	1.27	0.89	1.26	1.84	0.25	0.76
2,4,4',5-TetraCB (#74)	0.74	0.59	1.41	1.56	0.86	0.97	0.66	1.13	0.85	0.77	1.41	0.36	0.53
2,2',4,4',5-PentaCB (#99)	4.62	3.40	7.25	7.12	4.79	5.43	3.35	7.16	4.98	4.33	7.25	1.81	2.12
2,2',4,5,5'-PentaCB (#101)	2.13	1.39	4.50	3.15	3.29	2.79	2.37	4.37	1.97	1.94	4.50	1.24	1.14
2,3,3',4',6-PentaCB (#110)	3.99	2.76	5.63	5.25	4.34	4.67	2.84	5.72	4.14	2.97	5.63	2.33	2.25
2,2',3,4,4',5'-HexaCB (#138)	9.38	6.70	14.2	10.5	8.95	9.96	6.49	10.7	9.59	9.32	14.2	7.97	6.32
2,2',4,4',5,5'-HexaCB (#153)	8.23	6.96	12.0	8.53	9.30	9.84	5.55	9.93	8.34	10.7	12.0	7.48	7.92
2,2',3,4,4',5,5'-HeptaCB (#180)	7.24	3.02	8.00	7.35	7.21	3.37	2.83	9.28	6.10	3.65	8.00	11.4	2.90
∑PCB (UB)	39.8	27.0	58.0	49.1	41.5	41.4	27.0	52.4	38.8	38.3	58.0	34.1	26.5
∑PCB (LB)	39.8	27.0	58.0	49.1	41.5	41.4	27.0	52.4	38.8	38.3	58.0	34.1	26.5

Annex 10 continues

Sample Nr.	16/14	17/14	18/14	19/14	20/14	21/14	22/14	23/14	24/14	25/14	26/14	27/14	28/14	29/14	30/14
Length, cm	68	64	71	53	68	58	84	96	86	84	99	93	90	89	84
Weight, kg	0.5	0.4	0.5	0.2	0.5	0.3	1.0	1.9	1.1	0.9	1.5	1.4	1.2	1.3	1.0
Place of sampling	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake
Fat content, g/100 g of sample	24.3	21.9	22.1	27.6	24.0	22.4	25.2	26.8	23.9	26.0	22.9	23.8	25.5	25.9	27.1
DL-PCBs (pg g^{-1})															
2',3,4,4',5-PentaCB (#123)	36.9	27.8	26.4	54.1	29.0	12.2	34.0	50.3	19.0	56.4	52.8	86.1	34.1	161	66.6
2,3',4,4',5-PentaCB (#118)	2180	1840	2060	2920	1970	924	2050	3340	1630	3220	3010	5480	2130	7820	3870
2,3,4,4',5-PentaCB (#114)	66.2	47.4	57.3	51.8	47.9	26.8	50.7	78.8	43.2	78.4	67.7	124	50.7	182	94.7
2,3,3,'4,4'-PentaCB (#105)	830	775	770	843	779	376	645	1120	457	988	965	1710	681	2370	1250
2,3',4,4',5,5'-HexaCB (#167)	262	170	235	239	146	57.6	133	193	123	192	179	342	125	474	222
2,3,3',4,4',5-HexaCB (#156)	508	345	485	474	306	132	263	398	221	400	371	696	252	967	476
2,3,3',4,4',5'-HexaCB (#157)	79.7	77.2	85.5	77.7	65.1	27.1	48.5	82.4	35.0	77.2	75.2	144	50.1	190	98.8
2,3,3',4,4',5,5'-HeptaCB (#189)	93.1	40.6	73.8	43.3	30.5	10.5	35.7	40.7	37.5	46.3	40.6	76.7	29.1	119	48.6
3,4,4',5-TetraCB (#81)	<0.04	0.08	0.09	0.16	0.07	0.14	0.11	0.17	0.11	0.21	0.23	0.12	0.20	0.13	0.17
3,3,'4,4'-TetraCB (#77)	0.49	0.55	0.49	0.84	0.66	0.77	0.70	0.88	0.68	0.82	1.24	0.69	1.01	0.70	0.96
3,3',4,4',5-PentaCB (#126)	14.3	11.2	16.4	8.63	10.2	4.69	9.64	16.5	6.59	16.2	15.3	22.5	10.9	40.1	17.8
3,3',4,4',5,5'-HexaCB (#169)	7.25	4.48	6.26	2.39	4.16	1.40	3.77	4.20	3.40	4.45	4.21	6.69	3.14	10.8	5.10
WHO_{2005}-PCB-TEQ (LB)	1.77	1.35	1.94	1.08	1.24	0.56	1.17	1.94	0.84	1.90	1.80	2.71	1.28	4.70	2.12
WHO_{2005}-PCB-TEQ (UB)	1.77	1.35	1.94	1.08	1.24	0.56	1.17	1.94	0.84	1.90	1.80	2.71	1.28	4.70	2.12
WHO_{1998}-PCB-TEQ (UB)	2.15	1.66	2.31	1.58	1.55	0.71	1.46	2.43	1.06	2.37	2.24	3.54	1.58	5.84	2.69
NDL-PCBs (ng g^{-1})															
2,2',5-TriCB (#18)	<0.0001	0.01	0.01	0.05	0.05	0.04	0.04	0.05	0.03	0.03	0.11	0.03	0.04	0.02	0.04
2,2,4'-TriCB (#28)	0.19	0.12	0.22	0.29	0.31	0.25	0.32	0.47	0.33	0.38	0.52	0.36	0.39	0.79	0.40
2',3,4-TriCB (#33)	<0.0001	<0.00002	<0.0001	0.04	0.03	0.02	0.04	0.05	0.03	0.02	0.09	0.03	0.03	0.02	0.05
2,2',4,4'-TetraCB (#47)	0.19	0.12	0.16	0.16	0.17	0.09	0.19	0.25	0.18	0.22	0.19	0.28	0.20	0.54	0.26
2,2',4,5'-TetraCB (#49)	0.02	0.01	0.04	0.06	0.04	0.05	0.04	0.07	0.08	0.04	0.10	0.04	0.06	0.04	0.07
2,2',4,6'-TetraCB (#51)	0.003	0.003	0.002	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.01	0.01	0.01	0.003	0.01
2,2',5,5'-TetraCB (#52)	0.46	0.22	0.37	0.65	0.60	0.32	0.48	0.65	0.59	0.60	0.80	0.67	0.58	1.64	0.81
2,3,4,4'-TetraCB (#60)	0.11	0.08	0.10	0.10	0.11	0.06	0.10	0.13	0.08	0.13	0.13	0.17	0.11	0.33	0.14
2,3',4,4'-TetraCB (#66)	0.33	0.12	0.37	0.25	0.33	0.18	0.28	0.42	0.23	0.43	0.33	0.35	0.38	1.00	0.49
2,4,4',5-TetraCB (#74)	0.27	0.19	0.26	0.23	0.26	0.14	0.26	0.33	0.21	0.36	0.34	0.46	0.28	0.87	0.38
2,2',4,4',5-PentaCB (#99)	1.25	0.72	1.12	1.02	0.99	0.49	0.99	1.40	0.83	1.47	1.35	2.21	1.03	3.59	1.75
2,2',4,5,5'-PentaCB (#101)	0.86	0.42	0.73	1.11	0.75	0.35	0.48	0.67	0.79	0.91	0.86	1.31	1.07	2.51	1.38
2,3,3',4',6-PentaCB (#110)	1.39	1.15	1.26	1.75	1.07	0.51	1.00	1.16	0.80	1.49	1.46	2.31	1.11	3.89	1.85
2,2',3,4,4',5'-HexaCB (#138)	5.38	3.60	3.86	5.20	3.02	1.33	2.67	3.86	2.21	3.92	3.63	7.15	2.55	9.73	4.61
2,2',4,4',5,5'-HexaCB (#153)	5.37	3.76	3.67	6.13	3.04	1.41	3.30	4.86	2.87	4.86	4.85	9.11	3.53	12.6	5.92
2,2',3,4,4',5,5'-HeptaCB (#180)	5.82	3.93	6.40	1.95	1.96	1.12	1.24	1.52	1.24	1.72	1.56	3.11	1.14	4.60	1.92
∑PCB (UB)	21.6	14.4	18.6	19.0	12.7	6.37	11.4	15.9	10.5	16.6	16.3	27.6	12.5	42.2	20.1
∑PCB (LB)	21.6	14.4	18.6	19.0	12.7	6.37	11.4	15.9	10.5	16.6	16.3	27.6	12.5	42.2	20.1

Annex 10 continues

Sample Nr.	31/14	32/14	33/14	34/14	35/14	36/14	37/14	38/14	39/14	40/14	41/14	42/14	43/14	1	2
Length, cm	89	93	72	82	75	94	101	95	94	92	95	83	83	55	50
Weight, kg	1.1	1.3	0.6	0.9	0.6	1.6	1.8	1.6	1.4	1.5	1.3	0.9	1.0	0.8	1.0
Place of sampling	Sivers Lake	Sivers Lake	Liepajas Lake	Liepajas Lake	Liepajas Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Liepajas Lake	Liepajas Lake	Liepajas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	23.0	25.5	28.9	41.3	33.2	37.0	36.9	34.7	27.2	32.2	27.2	32.1	34.2	24.4	20.6
DL-PCBs (pg g^{-1})															
2',3,4,4',5-PentaCB (#123)	78.8	37.5	78.1	819	2100	271	420	611	463	519	372	781	659	49.2	35.3
2,3',4,4',5-PentaCB (#118)	3740	2470	4470	27300	39300	11500	17000	21500	20100	22800	28500	34800	26400	2280	1710
2,3,4,4',5-PentaCB (#114)	88.2	58.9	96.0	1480	3540	232	409	430	388	494	581	983	747	56.3	41.4
2,3,3,'4,4'-PentaCB (#105)	1250	859	1090	17300	30500	3860	5770	7630	6300	7560	8920	14400	10400	839	610
2,3',4,4',5,5'-HexaCB (#167)	224	133	330	2770	4650	617	797	1320	1020	1200	1270	2140	1610	151	158
2,3,3',4,4',5-HexaCB (#156)	472	283	639	7260	10500	1150	1730	2480	1950	2280	2440	3980	3010	310	314
2,3,3',4,4',5'-HexaCB (#157)	98.2	59.2	137	1760	2220	289	388	613	464	558	601	966	713	65.1	58.6
2,3,3',4,4',5,5'-HeptaCB (#189)	49.8	28.2	74.3	360	269	70.1	84.6	151	104	113	103	179	135	36.7	43.4
3,4,4',5-TetraCB (#81)	0.22	0.14	0.50	2.24	1.21	0.66	1.49	0.87	2.05	1.07	1.48	2.72	2.35	0.22	0.11
3,3,'4,4'-TetraCB (#77)	1.04	0.98	2.82	18.9	11.9	3.83	9.48	4.59	14.1	11.7	7.82	13.0	13.5	0.62	0.34
3,3',4,4',5-PentaCB (#126)	20.2	14.6	23.6	109	81.1	25.5	44.0	49.8	42.7	79.9	50.1	86.9	64.6	12.4	9.71
3,3',4,4',5,5'-HexaCB (#169)	4.79	3.78	5.95	13.9	4.72	3.81	4.25	5.29	3.74	4.84	4.14	7.06	5.26	3.38	2.97
WHO_{2005}-PCB-TEQ (LB)	2.35	1.69	2.75	13.1	11.0	3.21	5.33	6.18	5.31	9.20	6.42	10.7	7.93	1.45	1.15
WHO_{2005}-PCB-TEQ (UB)	2.35	1.69	2.75	13.1	11.0	3.21	5.33	6.18	5.31	9.20	6.42	10.7	7.93	1.45	1.15
WHO_{1998}-PCB-TEQ (UB)	2.92	2.03	3.44	20.9	23.6	5.00	8.04	9.79	8.42	12.8	10.7	26.8	12.5	1.81	1.45
NDL-PCBs (ng g^{-1})															
2,2',5-TriCB (#18)	0.04	0.04	0.10	0.08	0.22	0.07	0.12	0.05	0.09	0.10	0.06	0.15	0.05	0.01	0.01
2,2,4'-TriCB (#28)	0.36	0.41	1.14	8.82	6.05	2.13	2.74	4.32	4.44	5.10	5.11	8.59	6.27	0.31	0.13
2',3,4-TriCB (#33)	0.03	0.05	0.08	0.06	0.15	0.05	0.11	0.03	0.06	0.07	0.06	0.15	0.04	0.01	0.003
2,2',4,4'-TetraCB (#47)	0.23	0.20	0.64	8.60	24.0	1.21	1.66	2.72	2.17	2.47	4.13	6.70	4.69	0.18	0.09
2,2',4,5'-TetraCB (#49)	0.04	0.06	0.23	1.39	6.65	0.33	1.20	0.95	1.26	1.03	0.71	0.76	1.20	0.05	0.01
2,2',4,6'-TetraCB (#51)	0.004	0.01	0.01	0.01	0.07	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.002	0.001
2,2',5,5'-TetraCB (#52)	0.68	0.60	1.71	14.6	27.0	4.27	7.81	10.7	7.74	9.28	7.44	10.9	7.70	0.49	0.20
2,3,4,4'-TetraCB (#60)	0.14	0.11	0.31	3.69	3.02	0.61	0.76	1.22	1.10	1.36	1.56	2.52	1.76	0.12	0.07
2,3',4,4'-TetraCB (#66)	0.40	0.39	1.08	12.5	21.8	2.15	3.38	4.68	4.14	3.73	4.71	9.23	7.12	0.44	0.25
2,4,4',5-TetraCB (#74)	0.38	0.29	0.70	8.52	13.7	1.40	1.97	2.70	2.35	3.03	3.98	6.46	4.59	0.30	0.17
2,2',4,4',5-PentaCB (#99)	1.59	1.08	2.00	20.5	36.6	6.23	9.80	13.8	10.1	12.4	14.9	23.4	17.0	1.07	0.75
2,2',4,5,5'-PentaCB (#101)	1.32	0.96	1.94	14.8	27.4	4.38	10.5	13.4	10.5	12.4	3.76	12.3	10.8	1.35	0.50
2,3,3',4',6-PentaCB (#110)	1.79	1.24	2.76	18.0	26.5	7.32	10.3	15.6	11.3	14.4	10.4	15.5	12.9	1.37	0.83
2,2',3,4,4',5'-HexaCB (#138)	4.90	2.66	7.24	37.1	56.9	13.3	19.7	26.7	23.0	28.1	26.5	38.1	28.7	3.50	3.32
2,2',4,4',5,5'-HexaCB (#153)	5.87	3.40	10.9	31.6	41.9	16.6	22.2	26.2	25.7	27.9	28.2	34.8	26.8	4.65	4.40
2,2',3,4,4',5,5'-HeptaCB (#180)	2.09	0.99	5.53	22.8	28.1	3.96	5.27	9.37	6.78	8.17	6.69	12.4	11.1	1.58	2.08
∑PCB (UB)	19.9	12.5	36.4	203	320	64.0	97.5	132	111	130	118	182	141	15.4	12.8
∑PCB (LB)	19.9	12.5	36.4	203	320	64.0	97.5	132	111	130	118	182	141	15.4	12.8

Annex 10 continues

Sample Nr.	3	4	5	6	7	8	1a	2a	3a	4a	5a	6a	7a	8a	9a
Length, cm	55	67	55	55	55	50	87	40	77	81	79	75	39	42	42
Weight, kg	0.9	1.7	0.9	1.1	0.9	0.9	1.2	0.1	0.9	1.0	0.9	0.9	1.0	0.1	0.1
Place of sampling	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	26.0	29.4	22.8	24.4	24.2	23.7	21.2	31.5	29.2	30.0	28.0	23.1	30.1	35.2	31.7
DL-PCBs (pg g^{-1})															
2',3,4,4',5-PentaCB (#123)	45.6	58.7	56.8	99.9	91.5	117	39.4	63.9	63.8	88.2	54.2	74.5	76.3	39.8	206
2,3',4,4',5-PentaCB (#118)	2570	3150	3560	4830	6100	7190	2280	4400	4090	5570	2600	4790	8650	2980	8810
2,3,4,4',5-PentaCB (#114)	55.8	73.3	84.8	116	142	174	69.6	137	103	153	75.1	136	321	91.0	285
2,3,3,'4,4'-PentaCB (#105)	945	1120	1230	1680	2040	2380	972	1940	1600	2070	1030	1870	4230	1130	3710
2,3',4,4',5,5'-HexaCB (#167)	217	243	307	457	641	761	224	324	447	671	280	531	702	231	682
2,3,3',4,4',5-HexaCB (#156)	412	532	641	918	1260	1490	453	746	885	1190	558	1070	1640	487	1360
2,3,3',4,4',5'-HexaCB (#157)	81.6	107	125	175	241	287	92.4	148	176	216	108	212	329	87.3	280
2,3,3',4,4',5,5'-HeptaCB (#189)	55.3	65.7	80.9	112	167	194	58.1	85.7	118	160	75.8	137	135	57.0	76.2
3,4,4',5-TetraCB (#81)	<0.22	0.49	<0.29	0.33	<0.25	0.36	0.26	0.21	0.22	<0.06	0.12	0.10	0.10	0.17	0.54
3,3,'4,4'-TetraCB (#77)	0.60	0.93	0.42	1.07	0.31	0.65	1.02	0.77	0.47	0.58	0.71	0.45	0.75	0.66	1.74
3,3',4,4',5-PentaCB (#126)	12.2	18.5	17.2	23.7	31.2	38.2	17.3	25.5	27.8	25.0	16.2	25.9	23.4	18.8	46.0
3,3',4,4',5,5'-HexaCB (#169)	4.03	5.00	5.80	7.86	11.9	13.8	4.37	7.91	8.38	9.20	5.85	9.20	7.89	5.47	3.78
WHO_{2005}-PCB-TEQ (LB)	1.47	2.16	2.08	2.85	3.80	4.62	1.99	3.02	3.26	3.08	1.94	3.13	3.06	2.20	5.18
WHO_{2005}-PCB-TEQ (UB)	1.47	2.16	2.08	2.85	3.80	4.62	1.99	3.02	3.26	3.08	1.94	3.13	3.06	2.20	5.18
WHO_{1998}-PCB-TEQ (UB)	1.89	2.69	2.70	3.73	4.90	5.93	2.42	3.79	4.04	4.17	2.43	4.09	4.88	2.69	6.89
NDL-PCBs (ng g^{-1})															
2,2',5-TriCB (#18)	0.01	0.02	0.02	0.02	0.03	0.02	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.01	<0.0001
2,2,4'-TriCB (#28)	0.19	0.49	0.21	0.30	0.24	0.34	0.27	0.27	0.23	0.26	0.26	0.18	0.23	0.30	0.57
2',3,4-TriCB (#33)	0.01	0.01	0.01	0.01	0.01	0.01	0.02	<0.0001	0.01	0.002	0.02	<0.0001	<0.0001	<0.0001	<0.0001
2,2',4,4'-TetraCB (#47)	0.14	0.19	0.13	0.16	0.18	0.21	0.25	0.37	0.30	0.37	0.38	0.31	0.28	0.29	0.43
2,2',4,5'-TetraCB (#49)	0.02	0.06	0.02	0.03	0.02	0.03	0.08	0.10	0.05	0.05	0.07	0.02	0.03	0.06	0.04
2,2',4,6'-TetraCB (#51)	0.002	0.002	0.001	0.001	0.001	0.001	0.01	0.003	0.01	0.01	0.01	0.01	0.001	0.004	0.002
2,2',5,5'-TetraCB (#52)	0.36	0.48	0.32	0.42	0.47	0.61	0.86	1.03	0.74	1.07	0.63	0.83	0.63	0.73	1.12
2,3,4,4'-TetraCB (#60)	0.10	0.14	0.12	0.17	0.18	0.21	0.10	0.17	0.16	0.20	0.13	0.17	0.20	0.14	0.30
2,3',4,4'-TetraCB (#66)	0.30	0.47	0.28	0.48	0.48	0.60	0.21	0.49	0.18	0.48	0.41	0.30	0.62	0.42	1.42
2,4,4',5-TetraCB (#74)	0.22	0.34	0.32	0.41	0.42	0.49	0.24	0.49	0.41	0.49	0.31	0.40	0.59	0.38	1.11
2,2',4,4',5-PentaCB (#99)	1.01	1.25	1.25	1.71	2.27	2.65	1.68	3.44	2.94	4.28	2.24	3.65	3.78	2.28	6.31
2,2',4,5,5'-PentaCB (#101)	1.10	1.18	1.06	1.08	1.27	2.41	1.39	2.99	2.24	3.33	1.18	1.38	1.08	1.89	3.16
2,3,3',4',6-PentaCB (#110)	1.38	1.25	1.59	2.13	2.85	3.26	2.06	3.69	4.00	5.33	2.65	4.12	2.82	2.15	5.35
2,2',3,4,4',5'-HexaCB (#138)	4.77	5.27	6.40	9.03	13.5	16.5	6.81	9.54	13.2	18.7	8.55	15.2	12.2	6.44	14.4
2,2',4,4',5,5'-HexaCB (#153)	6.13	7.00	7.95	12.5	18.0	22.0	8.10	9.86	14.6	16.9	10.3	14.9	11.7	6.92	13.2
2,2',3,4,4',5,5'-HeptaCB (#180)	2.67	2.95	3.82	5.61	8.16	10.2	4.80	3.54	6.81	9.09	4.04	7.36	6.65	2.32	3.77
∑PCB (UB)	18.4	21.1	23.5	34.1	48.0	59.5	26.9	36.0	45.9	60.6	31.2	48.8	40.8	24.4	51.3
∑PCB (LB)	18.4	21.1	23.5	34.1	48.0	59.5	26.9	36.0	45.9	60.6	31.2	48.8	40.8	24.4	51.3

Concentrations of PBDEs in eel samples calculated on f.w. basis

Sample Nr.	1/14	2/14	3/14	4/14	5/14	6/14	7/14	8/14	9/14	12/14	13/14	14/14
Length, cm	81	83	95	78	74	82	76	88	76	70	78	85
Weight, kg	1.0	1.1	1.6	0.8	0.8	1.1	0.8	1.0	0.9	0.6	0.8	1.0
Place of sampling	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Aluksnes Lake	Usmas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	28.8	20.3	29.1	23.8	28	26.6	35.3	21.2	24.4	21.2	22.0	26.9
PBDEs (ng g^{-1})												
2,4-DiBDE (#7)	<0.0001	<0.0001	<0.002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.00004	<0.001	<0.0001	<0.0001
4,4-DiBDE (#15)	<0.0001	<0.00004	<0.001	<0.00003	<0.0001	<0.0001	<0.00004	<0.00004	<0.00002	0.01	<0.0001	<0.00004
2,2',4-TriBDE (#17)	0.001	0.001	0.001	0.0004	0.002	0.002	0.002	0.002	0.001	0.02	0.001	0.001
2,4,4'-TriBDE (#28)	0.004	0.004	0.01	0.004	0.01	0.01	0.004	0.010	0.004	0.04	0.004	0.01
2,2',4,4'-TetraBDE (#47)	0.48	0.38	0.88	0.51	0.58	0.56	0.39	0.69	0.60	2.39	0.50	0.69
2,2',4,5'-TetraBDE (#49)	0.16	0.10	0.20	0.08	0.15	0.15	0.12	0.17	0.17	1.06	0.15	0.22
2,3',4,4'-TetraBDE (#66)	0.01	0.004	0.004	0.010	0.02	0.01	0.01	0.02	0.01	0.05	0.01	0.01
2,3',4',6-TetraBDE (#71)	<0.0001	<0.0001	0.02	<0.0001	<0.0002	<0.0001	<0.0001	<0.0002	0.02	<0.002	<0.0001	<0.0001
3,3',4,4'-TetraBDE (#77)	0.001	0.0004	0.001	<0.0001	0.001	0.001	0.001	0.001	0.002	0.004	0.0004	0.001
2,2',3,4,4'-PentaBDE (#85)	0.002	0.002	0.002	0.001	0.002	0.002	0.002	0.002	0.002	0.04	0.003	0.003
2,2',4,4',5-PentaBDE (#99)	0.003	0.004	0.002	0.01	0.01	0.004	0.01	0.003	0.01	0.04	0.003	0.02
2,2',4,4',6-PentaBDE (#100)	0.08	0.06	0.19	0.09	0.12	0.12	0.06	0.15	0.19	0.70	0.20	0.18
2,3',4,4',6-PentaBDE (#119)	0.02	0.02	0.04	0.02	0.04	0.04	0.02	0.04	0.03	0.14	0.03	0.04
3,3',4,4',5-PentaBDE (#126)	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0001	<0.0002	<0.0002	<0.0002	<0.002	<0.0002	<0.0003
2,2',3,4,4',5'-HexaBDE (#138)	<0.0002	<0.0002	<0.0002	<0.0002	0.0004	<0.0004	<0.0003	<0.0003	<0.0002	<0.002	<0.001	<0.0003
2,2',4,4',5,5'-HexaBDE (#153)	0.02	0.02	0.03	0.02	0.04	0.04	0.03	0.05	0.03	0.23	0.03	0.05
2,2',4,4',5,6'-HexaBDE (#154)	0.10	0.07	0.11	0.07	0.12	0.13	0.07	0.14	0.12	0.75	0.09	0.17
2,2',4,4',6,6'-HexaBDE (#155)	0.02	0.02	0.03	0.01	0.02	0.02	0.01	0.02	0.02	0.24	0.03	0.03
2,3,4,4',5,6-HexaBDE (#166)	<0.0002	<0.0003	<0.0002	<0.0003	<0.0003	<0.0004	<0.0003	<0.0003	<0.0002	<0.003	<0.001	<0.0003
2,2',3,4,4',5,6'-HeptaBDE (#181)	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0001	<0.0001	<0.0002	<0.0004	<0.01	<0.001	<0.0003
2,2',3,4,4',5',6-HeptaBDE (#183)	0.001	0.001	0.001	0.001	0.002	0.001	0.002	0.001	0.001	0.02	0.001	0.001
2,3,3',4,4',5,6-HeptaBDE (#190)	<0.0002	<0.0003	<0.0002	<0.0003	<0.0004	<0.0002	<0.0002	<0.0002	<0.001	<0.01	<0.001	<0.0004
2,2',3,4,4',5,5',6-OctaBDE (#203)	0.004	0.003	0.003	<0.01	0.003	<0.0004	0.002	<0.001	<0.01	<0.01	<0.01	0.004
2,3,3',4,4',5,5',6-OctaBDE (#205)	<0.004	<0.003	<0.004	<0.01	<0.004	<0.001	<0.003	<0.001	<0.01	<0.01	<0.01	<0.01
2,2',3,3',4,4',5,5',6-NonaBDE (#206)	<0.001	<0.001	<0.0004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.002	<0.001	<0.001	<0.001
2,2',3,3',4,4',5,6,6'-NonaBDE (#207)	0.001	0.002	0.0003	0.01	0.001	0.002	0.001	0.001	0.003	0.01	0.002	0.002
2,2',3,3',4,4',5,5',6,6'-DecaBDE (#209)	0.04	0.04	0.01	0.09	0.03	0.03	0.038	0.01	<0.01	0.07	0.05	0.05
∑PBDE (UB)	0.96	0.72	1.55	0.95	1.16	1.12	0.777	1.30	1.26	5.88	1.12	1.48
∑PBDE (LB)	0.95	0.71	1.54	0.93	1.16	1.12	0.772	1.30	1.22	5.82	1.11	1.47

Annex 11 continues

Sample Nr.	16/14	17/14	18/14	19/14	20/14	21/14	22/14	23/14	24/14	25/14	26/14	27/14
Length, cm	68	64	71	53	68	58	84	96	86	84	99	93
Weight, kg	0.5	0.4	0.5	0.2	0.5	0.3	1.0	1.9	1.1	0.9	1.5	1.4
Place of sampling	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake	Sivers Lake
Fat content, g/100 g of sample	24.3	21.9	22.1	27.6	24.0	22.4	25.2	26.8	23.9	26.0	22.9	23.8
PBDEs (ng g^{-1})												
2,4-DiBDE (#7)	<0.0001	<0.0001	<0.0001	<0.0004	<0.0001	<0.0001	<0.0004	<0.0004	<0.001	<0.001	<0.0004	<0.001
4,4-DiBDE (#15)	<0.00004	<0.00003	<0.00004	0.002	<0.0001	<0.0001	0.004	0.01	0.01	0.01	0.01	0.002
2,2',4-TriBDE (#17)	0.001	0.0004	0.0003	0.01	0.001	0.001	0.03	0.04	0.03	0.04	0.04	0.04
2,4,4'-TriBDE (#28)	0.01	0.002	0.01	0.02	0.003	0.002	0.02	0.06	0.04	0.04	0.04	0.02
2,2',4,4'-TetraBDE (#47)	0.31	0.24	0.32	2.26	0.25	0.14	1.28	2.78	1.00	2.55	2.12	3.36
2,2',4,5'-TetraBDE (#49)	0.13	0.07	0.13	0.46	0.09	0.03	0.42	0.62	0.49	1.08	0.83	1.02
2,3',4,4'-TetraBDE (#66)	0.01	0.002	0.003	0.02	0.01	0.003	0.04	0.08	0.04	0.08	0.03	0.02
2,3',4',6-TetraBDE (#71)	<0.0002	<0.0002	<0.0002	<0.002	<0.0001	<0.0001	<0.001	<0.001	<0.001	<0.002	<0.001	<0.001
3,3',4,4'-TetraBDE (#77)	0.001	<0.0001	<0.0001	<0.001	0.001	0.0003	0.01	<0.02	0.003	0.01	0.004	0.001
2,2',3,4,4'-PentaBDE (#85)	0.01	0.002	0.004	0.01	0.001	0.0002	<0.002	0.01	0.01	0.003	0.004	0.01
2,2',4,4',5-PentaBDE (#99)	0.01	0.004	0.003	0.03	0.01	0.01	0.01	0.01	0.004	0.01	0.01	0.01
2,2',4,4',6-PentaBDE (#100)	0.11	0.08	0.08	0.47	0.09	0.02	0.09	0.07	0.03	0.13	0.03	0.07
2,3',4,4',6-PentaBDE (#119)	0.03	0.01	0.02	0.06	0.01	0.004	0.03	0.02	0.01	0.04	0.01	0.01
3,3',4,4',5-PentaBDE (#126)	<0.0003	<0.0002	<0.0003	<0.001	<0.0002	<0.0001	<0.001	<0.003	<0.001	<0.002	<0.002	<0.001
2,2',3,4,4',5'-HexaBDE (#138)	<0.0003	0.0003	<0.0003	<0.002	<0.0003	<0.0002	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2,2',4,4',5,5'-HexaBDE (#153)	0.06	0.02	0.03	0.11	0.01	0.01	0.10	0.13	0.11	0.16	0.11	0.17
2,2',4,4',5,6'-HexaBDE (#154)	0.19	0.05	0.12	0.24	0.04	0.01	0.30	0.41	0.51	0.52	0.43	0.66
2,2',4,4',6,6'-HexaBDE (#155)	0.05	0.01	0.04	0.07	0.01	0.002	0.10	0.11	0.20	0.10	0.11	0.15
2,3,4,4',5,6-HexaBDE (#166)	<0.0004	<0.0003	<0.0004	<0.002	<0.0004	<0.0002	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2,2',3,4,4',5,6'-HeptaBDE (#181)	<0.0003	<0.0002	<0.0004	<0.01	<0.0003	<0.0001	<0.003	<0.004	<0.003	<0.004	<0.01	<0.01
2,2',3,4,4',5',6-HeptaBDE (#183)	0.01	0.001	0.003	0.01	0.001	0.001	0.02	0.02	0.03	<0.003	0.01	0.01
2,3,3',4,4',5,6-HeptaBDE (#190)	<0.001	<0.0003	<0.001	<0.02	<0.001	<0.0002	<0.004	<0.01	<0.004	<0.01	<0.01	<0.01
2,2',3,4,4',5,5',6-OctaBDE (#203)	0.01	0.01	0.003	<0.01	<0.004	0.01	<0.004	<0.003	<0.01	<0.01	<0.01	<0.004
2,3,3',4,4',5,5',6-OctaBDE (#205)	<0.01	<0.01	<0.004	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2,2',3,3',4,4',5,5',6-NonaBDE (#206)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2,2',3,3',4,4',5,6,6'-NonaBDE (#207)	0.003	0.002	0.002	0.01	0.002	0.002	0.01	0.01	0.01	0.01	0.01	0.004
2,2',3,3',4,4',5,5',6,6'-DecaBDE (#209)	0.02	0.02	0.05	0.11	0.04	0.04	0.08	0.10	0.08	0.07	0.12	0.06
∑PBDE (UB)	0.96	0.53	0.81	3.96	0.58	0.28	2.55	4.51	2.65	4.89	3.97	5.66
∑PBDE (LB)	0.95	0.52	0.80	3.90	0.56	0.27	2.52	4.48	2.61	4.85	3.92	5.61

Annex 11 continues.,

Sample Nr.	31/14	32/14	33/14	34/14	35/14	36/14	37/14	38/14	39/14	40/14	41/14	42/14
Length, cm	89	93	72	82	75	94	101	95	94	92	95	83
Weight, kg	1.1	1.3	0.6	0.9	0.6	1.6	1.8	1.6	1.4	1.5	1.3	0.9
Place of sampling	Sivers Lake	Sivers Lake	Liepajas Lake	Liepajas Lake	Liepajas Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Kisezers Lake	Liepajas Lake	Liepajas Lake
Fat content, g/100 g of sample	23.0	25.5	28.9	41.3	33.2	37.0	36.9	34.7	27.2	32.2	27.2	32.1
PBDEs (ng g^{-1})												
2,4-DiBDE (#7)	<0.0002	<0.0003	<0.001	<0.001	<0.0004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.0001
4,4-DiBDE (#15)	0.003	0.01	0.02	0.03	0.004	0.02	0.06	0.07	0.06	0.10	0.01	0.002
2,2',4-TriBDE (#17)	0.03	0.03	0.04	0.09	0.03	0.04	0.05	0.07	0.05	0.05	0.02	0.01
2,4,4'-TriBDE (#28)	0.05	0.03	0.20	0.19	0.02	0.05	0.31	0.36	0.43	0.43	0.05	0.01
2,2',4,4'-TetraBDE (#47)	2.40	1.67	11.5	15.2	1.28	4.13	5.25	13.8	12.8	14.6	2.46	0.78
2,2',4,5'-TetraBDE (#49)	0.81	0.63	1.69	2.61	0.42	1.13	0.76	1.31	0.90	1.36	0.47	0.22
2,3',4,4'-TetraBDE (#66)	0.04	0.05	0.15	0.36	0.04	0.04	0.13	0.17	0.14	0.19	0.03	0.01
2,3',4',6-TetraBDE (#71)	<0.001	<0.001	<0.002	<0.002	<0.001	<0.001	<0.002	<0.003	<0.002	<0.002	<0.001	<0.0001
3,3',4,4'-TetraBDE (#77)	0.004	0.01	0.01	0.01	0.01	0.01	<0.002	<0.002	<0.002	<0.002	<0.001	0.001
2,2',3,4,4'-PentaBDE (#85)	0.02	0.01	0.02	0.16	<0.002	0.05	0.03	0.03	0.03	0.04	0.02	0.01
2,2',4,4',5-PentaBDE (#99)	0.03	0.02	0.08	0.58	0.01	0.13	0.34	0.45	0.61	0.32	0.08	0.02
2,2',4,4',6-PentaBDE (#100)	0.55	0.31	0.23	3.71	0.09	0.81	0.86	1.66	1.55	1.80	0.56	0.22
2,3',4,4',6-PentaBDE (#119)	0.14	0.10	0.03	0.34	0.03	0.15	0.11	0.130	0.15	0.12	0.08	0.04
3,3',4,4',5-PentaBDE (#126)	<0.001	<0.001	<0.004	<0.01	<0.001	<0.01	<0.01	<0.004	<0.003	<0.01	<0.002	<0.001
2,2',3,4,4',5'-HexaBDE (#138)	<0.001	<0.002	<0.02	<0.002	<0.01	<0.002	<0.001	<0.002	<0.002	<0.002	<0.001	<0.0003
2,2',4,4',5,5'-HexaBDE (#153)	0.15	0.11	0.44	0.82	0.10	0.29	0.26	0.33	0.36	0.32	0.14	0.05
2,2',4,4',5,6'-HexaBDE (#154)	0.44	0.28	1.00	1.67	0.30	0.74	0.41	0.50	0.68	0.69	0.38	0.14
2,2',4,4',6,6'-HexaBDE (#155)	0.08	0.06	0.34	0.69	0.10	0.25	0.10	0.11	0.15	0.18	0.12	0.05
2,3,4,4',5,6-HexaBDE (#166)	<0.001	<0.002	<0.02	<0.002	<0.01	<0.002	0.002	<0.002	<0.003	<0.002	<0.001	<0.0003
2,2',3,4,4',5,6'-HeptaBDE (#181)	<0.003	<0.001	<0.01	<0.01	<0.003	<0.01	0.01	<0.02	<0.02	<0.01	<0.004	<0.001
2,2',3,4,4',5',6-HeptaBDE (#183)	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	<0.001
2,3,3',4,4',5,6-HeptaBDE (#190)	<0.004	<0.002	<0.02	0.02	<0.004	<0.01	<0.02	<0.02	<0.03	<0.01	<0.01	<0.001
2,2',3,4,4',5,5',6-OctaBDE (#203)	<0.004	0.002	<0.01	0.004	<0.01	0.003	<0.01	<0.01	<0.01	<0.01	0.002	<0.01
2,3,3',4,4',5,5',6-OctaBDE (#205)	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
2,2',3,3',4,4',5,5',6-NonaBDE (#206)	<0.001	<0.001	<0.002	<0.002	<0.003	<0.002	<0.002	<0.003	<0.001	<0.003	<0.001	<0.0004
2,2',3,3',4,4',5,6,6'-NonaBDE (#207)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.001
2,2',3,3',4,4',5,5',6,6'-DecaBDE (#209)	0.06	0.08	0.10	0.19	0.14	0.12	0.14	0.12	0.09	0.08	0.08	0.02
∑PBDE (UB)	4.84	3.43	16.0	26.7	2.64	8.04	8.88	19.2	18.1	20.3	4.55	1.58
∑PBDE (LB)	4.82	3.41	15.9	26.7	2.59	7.99	8.83	19.1	18.0	20.3	4.52	1.56

Annex 11 continues

Sample Nr.	3	4	5	6	7	8	1a	2a	3a	4a	5a	6a
Length, cm	55	67	55	55	55	50	87	40	77	81	79	75
Weight, kg	0.9	1.7	0.9	1.1	0.9	0.9	1.2	0.1	0.9	1.0	0.9	0.9
Place of sampling	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	26.0	29.4	22.8	24.4	24.2	23.7	21.2	31.5	29.2	30.0	28.0	23.1
PBDEs (ng g^{-1})												
2,4-DiBDE (#7)	<0.0001	<0.00003	<0.00003	<0.0001	<0.00003	<0.00003	<0.0001	<0.00003	<0.00003	<0.00003	<0.00004	<0.00003
4,4-DiBDE (#15)	<0.00004	0.001	0.0002	<0.00004	<0.00002	<0.00001	<0.00004	0.0002	<0.00001	<0.00002	0.0001	<0.00001
2,2',4-TriBDE (#17)	0.002	0.003	0.001	0.001	0.002	0.003	0.0003	0.002	0.001	0.002	0.004	0.0004
2,4,4'-TriBDE (#28)	0.004	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.004
2,2',4,4'-TetraBDE (#47)	0.34	0.66	0.53	0.79	0.77	1.14	0.47	0.38	0.84	0.79	0.50	0.72
2,2',4,5'-TetraBDE (#49)	0.14	0.20	0.20	0.26	0.31	0.49	0.15	0.17	0.33	0.32	0.18	0.29
2,3',4,4'-TetraBDE (#66)	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.01	0.004
2,3',4',6-TetraBDE (#71)	<0.0001	<0.0001	<0.0002	<0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
3,3',4,4'-TetraBDE (#77)	0.001	0.002	0.001	0.0001	0.001	0.001	<0.0001	0.001	<0.0001	0.0002	0.0003	<0.0001
2,2',3,4,4'-PentaBDE (#85)	0.002	0.01	0.003	0.003	0.01	0.01	0.002	0.003	0.01	0.01	0.004	0.01
2,2',4,4',5-PentaBDE (#99)	0.01	0.03	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.004	0.01	0.01
2,2',4,4',6-PentaBDE (#100)	0.10	0.13	0.20	0.33	0.30	0.43	0.10	0.12	0.23	0.34	0.14	0.32
2,3',4,4',6-PentaBDE (#119)	0.02	0.03	0.03	0.04	0.06	0.07	0.02	0.03	0.05	0.03	0.02	0.04
3,3',4,4',5-PentaBDE (#126)	<0.0003	<0.0003	<0.0001	<0.0001	<0.0002	<0.0003	<0.0004	<0.0001	<0.0002	<0.0004	<0.0003	<0.0002
2,2',3,4,4',5'-HexaBDE (#138)	<0.0002	<0.0002	<0.0004	<0.0002	<0.0001	<0.0001	<0.0003	<0.001	<0.0001	<0.0002	0.0003	<0.0002
2,2',4,4',5,5'-HexaBDE (#153)	0.03	0.05	0.04	0.04	0.08	0.07	0.04	0.03	0.07	0.03	0.03	0.04
2,2',4,4',5,6'-HexaBDE (#154)	0.11	0.13	0.08	0.16	0.24	0.29	0.10	0.13	0.21	0.20	0.10	0.19
2,2',4,4',6,6'-HexaBDE (#155)	0.02	0.02	0.03	0.03	0.02	0.04	0.01	0.04	0.02	0.03	<0.00003	0.02
2,3,4,4',5,6-HexaBDE (#166)	<0.0002	<0.0002	<0.0004	<0.0002	<0.0002	<0.0001	<0.0003	<0.001	<0.0001	<0.0002	<0.0001	<0.0002
2,2',3,4,4',5,6'-HeptaBDE (#181)	<0.0004	<0.001	<0.001	<0.001	<0.001	<0.0004	<0.0002	<0.002	<0.001	<0.001	<0.0004	<0.0004
2,2',3,4,4',5',6-HeptaBDE (#183)	0.001	0.01	0.001	0.002	0.003	0.001	0.002	0.01	0.001	0.001	0.001	0.001
2,3,3',4,4',5,6-HeptaBDE (#190)	<0.001	<0.002	<0.002	<0.001	<0.002	<0.001	<0.0002	<0.002	<0.001	<0.001	<0.001	<0.001
2,2',3,4,4',5,5',6-OctaBDE (#203)	<0.001	<0.001	<0.004	<0.004	<0.001	<0.001	0.004	<0.004	<0.001	<0.002	<0.001	<0.001
2,3,3',4,4',5,5',6-OctaBDE (#205)	<0.001	<0.001	<0.01	<0.01	<0.001	<0.001	<0.004	<0.01	<0.001	<0.003	<0.001	<0.001
2,2',3,3',4,4',5,5',6-NonaBDE (#206)	0.0003	<0.0001	<0.001	<0.001	<0.0001	<0.0001	<0.001	<0.0002	<0.0002	<0.0001	<0.0002	<0.0002
2,2',3,3',4,4',5,6,6'-NonaBDE (#207)	0.001	0.001	<0.001	0.001	0.0003	0.001	0.002	0.001	0.0004	0.001	0.0002	0.0003
2,2',3,3',4,4',5,5',6,6'-DecaBDE (#209)	0.02	0.01	0.04	0.02	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01
∑PBDE (UB)	0.81	1.33	1.19	1.72	1.85	2.60	0.97	0.95	1.78	1.79	1.00	1.66
∑PBDE (LB)	0.81	1.32	1.17	1.70	1.84	2.59	0.97	0.94	1.77	1.78	1.00	1.66

Annex 11 continues

Sample Nr.	15/14	28/14	29/14	30/14	43/14	1	2	7a	8a	9a
Length, cm	71	90	89	84	83	55	50	39	42	42
Weight, kg	0.5	1.2	1.3	1.0	1.0	0.8	1.0	1.0	0.1	0.1
Place of sampling	Usmas Lake	Sivers Lake	Sivers Lake	Sivers Lake	Liepajas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake	Usmas Lake
Fat content, g/100 g of sample	21.8	25.5	25.9	27.1	34.2	24.4	20.6	30.1	35.2	31.7
PBDEs (ng g^{-1})										
2,4-DiBDE (#7)	<0.001	<0.001	<0.0004	<0.0004	<0.001	<0.0001	0.0001	<0.00004	<0.00002	<0.00004
4,4-DiBDE (#15)	0.01	0.01	0.01	0.01	0.02	0.00002	<0.00001	<0.00003	<0.00001	<0.00003
2,2',4-TriBDE (#17)	0.02	0.04	0.11	0.04	0.04	0.002	0.001	0.002	0.002	0.003
2,4,4'-TriBDE (#28)	0.04	0.05	0.06	0.04	0.05	0.01	0.01	0.01	0.01	0.01
2,2',4,4'-TetraBDE (#47)	2.39	1.92	7.44	2.39	4.13	0.42	0.52	0.55	0.37	0.67
2,2',4,5'-TetraBDE (#49)	1.06	0.60	3.34	0.90	1.13	0.15	0.20	0.07	0.15	0.22
2,3',4,4'-TetraBDE (#66)	0.05	0.06	0.27	0.06	0.04	0.01	0.01	0.01	0.01	0.01
2,3',4',6-TetraBDE (#71)	<0.002	<0.002	<0.003	<0.002	<0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0002
3,3',4,4'-TetraBDE (#77)	0.004	0.01	0.01	0.01	0.01	0.001	0.0003	0.001	0.0004	0.002
2,2',3,4,4'-PentaBDE (#85)	0.04	0.004	0.07	0.004	0.05	0.001	0.003	0.001	0.003	0.003
2,2',4,4',5-PentaBDE (#99)	0.04	0.01	0.05	0.01	0.13	0.01	0.01	0.01	0.004	0.01
2,2',4,4',6-PentaBDE (#100)	0.70	0.09	1.64	0.33	0.81	0.11	0.12	0.33	0.08	0.16
2,3',4,4',6-PentaBDE (#119)	0.14	0.02	0.49	0.09	0.15	0.02	0.02	0.02	0.02	0.05
3,3',4,4',5-PentaBDE (#126)	<0.002	<0.001	<0.003	<0.003	<0.01	<0.0002	<0.0001	<0.0001	<0.0002	<0.0002
2,2',3,4,4',5'-HexaBDE (#138)	<0.002	<0.02	<0.001	<0.003	<0.002	<0.0002	<0.0001	<0.0003	<0.0002	<0.0002
2,2',4,4',5,5'-HexaBDE (#153)	0.23	0.08	0.65	0.13	0.29	0.03	0.02	0.03	0.03	0.04
2,2',4,4',5,6'-HexaBDE (#154)	0.75	0.34	1.97	0.38	0.74	0.07	0.11	0.05	0.07	0.15
2,2',4,4',6,6'-HexaBDE (#155)	0.24	0.09	0.30	0.07	0.25	0.01	0.02	0.01	0.01	0.02
2,3,4,4',5,6-HexaBDE (#166)	<0.003	<0.02	<0.002	<0.003	<0.002	<0.0003	<0.0001	<0.0003	<0.0002	<0.0002
2,2',3,4,4',5,6'-HeptaBDE (#181)	<0.01	<0.01	<0.01	<0.003	<0.01	<0.001	<0.001	<0.0004	<0.001	<0.0003
2,2',3,4,4',5',6-HeptaBDE (#183)	0.02	0.01	0.02	0.01	0.01	0.002	0.001	0.002	0.01	0.001
2,3,3',4,4',5,6-HeptaBDE (#190)	<0.01	<0.01	<0.01	<0.004	<0.01	<0.001	<0.001	<0.001	<0.002	<0.001
2,2',3,4,4',5,5',6-OctaBDE (#203)	<0.004	<0.01	<0.003	<0.003	<0.01	<0.004	<0.004	<0.001	<0.001	<0.003
2,3,3',4,4',5,5',6-OctaBDE (#205)	<0.01	<0.01	<0.004	<0.004	<0.01	<0.01	<0.01	<0.001	<0.001	<0.004
2,2',3,3',4,4',5,5',6-NonaBDE (#206)	<0.002	<0.002	<0.001	0.0002	<0.002	<0.0002	<0.0002	<0.0002	<0.0002	<0.001
2,2',3,3',4,4',5,6,6'-NonaBDE (#207)	0.01	0.01	0.004	0.01	0.01	0.001	0.001	0.001	0.001	0.0003
2,2',3,3',4,4',5,5',6,6'-DecaBDE (#209)	0.11	0.11	0.07	0.08	0.09	0.01	0.01	0.01	0.01	0.01
∑PBDE (UB)	5.91	3.51	16.6	4.58	7.99	0.86	1.07	1.10	0.78	1.36
∑PBDE (LB)	5.86	3.43	16.5	4.55	7.95	0.85	1.06	1.10	0.77	1.35

The research for doctoral thesis "**Development of complex analytical scheme for determination of priority persistent organic pollutants in fish using high resolution mass specrometry**" was carried out at the Faculty of Chemistry, University of Latvia and at the Institute of Food Safety, Animal Health and Environment "BIOR" from 2012 to 2015.

I hereby confirm that I have written the doctoral thesis independently, that I have not used other sources or facilities that the ones mentioned and that the submitted electronic copy of the work is identical to printed version.

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May 11th, 2015

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The thesis was defended at the session of the Doctoral Committee of Chemistry:

_____ protocol Nr _____

Secretary of the Doctoral Committee::_____ / _____ /