LEVELS OF DIOXINS, PCBs, BFRs, PFCs AND ORGANOTINS IN FISHERY PRODUCTS FROM LATVIA

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Introduction

The main objective of this study is to identify the concentrations of five groups of hazardous substances in selected fishery products from the Latvian market. All these hazardous substances cause concern because of their toxicity, persistence and bio-accumulative properties and 33 chemicals have been already identified as priority substances (2455/2001/EC). Dioxins (PCDD/Fs) are not intentionally produced, but are formed as by-products or impurities in several industrial processes as well as from most combustion processes. PCBs, brominated flame retardants (BFRs) like PBDEs and HBCD, perfluorinated chemicals (PFCs) e.g. PFOA and PFOS and organotin compounds (OTC) like TBT are four classes of persistent organic compounds (POPs) detected in the marine environment and food chain as a result of their various industrial and commercial applications. Certain POPs are hazardous because of their effects on hormone and immune systems, as well their toxicity and bio-accumulating properties in adipose tissue. Hence high concentrations are found in fatty fish of considerable commercial relevance such as herring (Clupea harengus), salmon (Salmo salar), and mackerel (Scomber scombrus), which are well recognised as important dietary sources for n-3 polyunsaturated fatty acids. However, it should be recognised that the comparison of contaminant levels in wild fish is made difficult because of the variation induced by the age of the fish, the season the fish is caught, and the geographic origin[1]. The Baltic Sea is one of the most thoroughly studied water bodies in the world. It is characterised by high levels of a number of pollutants in biota and sediments caused by industrial activities in the past and the long retention time of the water. One consequence of the serious dioxin contamination of Baltic fish was the banning of the trade of salmon caught in the Baltic Sea and the Gulf of Riga by the Latvian Food and Veterinary Service[2].

Due to serious ecotoxicological effects, some POPs are regulated and most of the technically applied hazardous substances are no longer in use. In order to encourage a proactive approach to reducing dioxins and dioxin-like PCBs (dl-PCBs) present in food and feed, action levels were set by the Commission Recommendation 2006/88/EC. The action levels are a tool for competent authorities to identify a source of contamination and to take measures to reduce or eliminate it. In the Commission Regulation (EC) 1881/2006 maximum levels for dioxins (4 pg WHO-PCDD/F-TEQ/g fw) and the sum of dioxins and dl-PCBs (8 pg WHO-TEQ-PCDD/F-PCB-TEQ/g fw) were established for muscle meat of fish and fish products. Although the PBDE contamination in marine fish was first detected in Sweden as early as 1979, and the use of certain PBDE formulations has been banned with the adoption of the Hazardous Substances Directive in 2004, the EU is still discussing regulations on PBDE levels in food. The organotin compound TBT which is at least immunotoxic has been used extensively as the active component in antifouling paints for ships and boats. In 2001 the International Maritime Organisation (IMO) adapted the International Convention on the Control of Harmful Anti-fouling Systems – a convention that prohibits the use of harmful organotins as antifoulants on large ships by 2008[3]. International regulatory authorities currently discuss adequate measures to control and reduce the presence of PFC related residues in the environment. Already in 2000, US EPA banned PFOS from the US market and currently, PFOA is also evaluated for regulatory actions. Due to harmful POP properties related to its continuous production and use, global action is warranted by the Review Committee of the Stockholm Convention to eliminate the pollution caused by PFOS[4].

Materials and methods

Recently Eurofins I GfA has performed an extensive dioxin monitoring on 140 food samples from the Latvian market, including 88 fish products. The samples were provided by the National Diagnostic Centre (NDC) in Riga and sent frozen in the autumn of 2007. The fish samples collected from producers and retailers in various Latvian cities were partly fresh and partly processed (smoked fish, canned fish), which may have an influence on the contaminant levels. In total 51 herring, salmon and mackerel samples were analysed for PCDD/F and dl-PCB (s. Table 1) and the monitoring of further POPs was applied on selected samples of these batches.

The sample preparation applied on all analytical procedures included fillet production (where necessary), homogenisation and freeze-drying. The analytical methodology for the determination of the seventeen toxic dioxin congeners and the twelve dl-PCB congeners is in compliance with the requirements for the HRGC/HRMS confirmatory analysis as laid down in the Commission Regulation (EC) 1883/2006. The analytical procedure has been specified in the literature[5,6].
Also twenty-four PBDE congeners (BDE-17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207 and 209) and HBCD (sum of α-, β-, and γ-HBCD) have been monitored in this study. Relevant aspects of the analytical operation have already been described before[5,7].
With regard to the PFC analysis the study`s emphasis was on PFOS and PFOA as these compounds have been discussed recently. After spiking with $^{13}C_4$-labelled PFOS and PFOA standards, an aliquot of about 0.5 g freeze-dried homogenised fish fillet was mixed with a sodium carbonate buffer, and tetrabutylammonium solution. The resulting solution was mixed, followed by the addition of methyl-t-butyl ether (MTBE) and a further mixing step. The MTBE was quantitatively transferred to another flask, the procedure was repeated and the combined MTBE extracts were then gently evaporated until dryness. 1 ml of methanol was added and finally the extract was filtered prior to LC/MS analysis. Main details of the LC/MS parameters were published last year[8]. The established LOQs were 0.2 ng/g fw for PFOS and 0.1 ng/g fw for PFOA respectively.
The butyltin compounds Monobutyltin (MBT), Dibutyltin (DBT) and TBT as well as Triphenyltin (TPhT) were also determined in this study. The clean-up procedure was based on method 10.00-9 laid down in § 64 of the German food law (LFGB). After addition of the internal standard mixture, approx. 3 g of the freeze-dried, homogenised tissue were treated with tetraethylammonium hydroxide followed by direct derivatisation and simultaneous multiple extraction of the analytes using sodium tetraethyl borate and n-hexane. The combined hexane extracts were cleaned-up by means of an alumina column and Tetrapentyltin chloride was added as recovery standard. GC/MS analysis was carried out by means of a HP6890 GC equipped with a HP5973 MS. The samples were routinely analysed on a 60 m x 0.25 mm x 0.25 µm DB-5MS column and the MS detector was operated in SIM mode. The recoveries were between 70 % and 110 % and the routine LOQ for the above-mentioned OTC in fish samples was 0.4 ng/g fw.

Results and discussion

Dioxin and dl-PCB results:

Table 1 presents summary information on the upper-bound levels of PCDD/Fs and dl-PCBs measured in herring, salmon and mackerel samples available in Latvia. Results are expressed as WHO-TEQ in pg/g fw for PCDD/Fs and dl-PCBs, additionally the sum (Σ) of PCDD/Fs and dl-PCBs (total TEQ) are reported.

Table 1: Upper-bound levels of PCDD/Fs, dl-PCBs and total TEQs in fishery products (pg WHO-TEQ/ g fw)

Fish species	N		Fat (%)	PCDD/F	dl-PCB	Σ PCDD/F+dl-PCB
Herring	19	Mean	13.8	1.91	1.49	3.40
		Min.	6.3	0.38	0.41	0.69
		Max.	24.1	4.83	3.08	7.38
Salmon	12	Mean	19.1	0.34	0.95	1.29
		Min.	11.2	0.25	0.69	0.96
		Max.	25.6	0.45	1.27	1.68
Mackerel	20	Mean	20.9	0.14	0.38	0.52
		Min.	8.1	0.02	0.10	0.11
		Max.	29.3	0.32	0.81	1.03

As reported in Table 1 the highest contamination was observed in herring samples at mean PCDD/F levels of 1.91 pg/WHO-TEQ g fw and 3.40 pg/WHO-TEQ g fw for the total TEQ. Distinctly higher PCDD/F and dl-PCB levels in Baltic herring and Baltic salmon were included in a comprehensive EFSA report[1].

However, PCDD/F concentrations between 1.4 and 2.9 pg WHO-TEQ/g fw were reported for 2 to 4 years old Baltic herring from the Gulf of Riga collected in the years 2002 to 2004[9].
Noticeable lower PCDD/F and PCB levels have been determined in the salmon and mackerel samples of this survey. Due to the afore mentioned banning of Baltic salmon most salmon sold in Latvia comes from fish farms in Norway and other European countries, which are less contaminated[5,10].

PBDE and HBCD results:
Altogether from the 24 PBDE congeners analysed, only 13 Tri- to HeptaBDEs (BDE-17, 28, 47, 49, 66, 77, 85, 99, 100, 119, 153, 154 and 183) could be determined in all fish samples. Octa- to DecaBDE were not detectable at all which is in accordance with literature data[11]. As illustrated in Figure 1 BDE-47 accounts for approx. 50 % of the total PBDEs found in the three fish species.

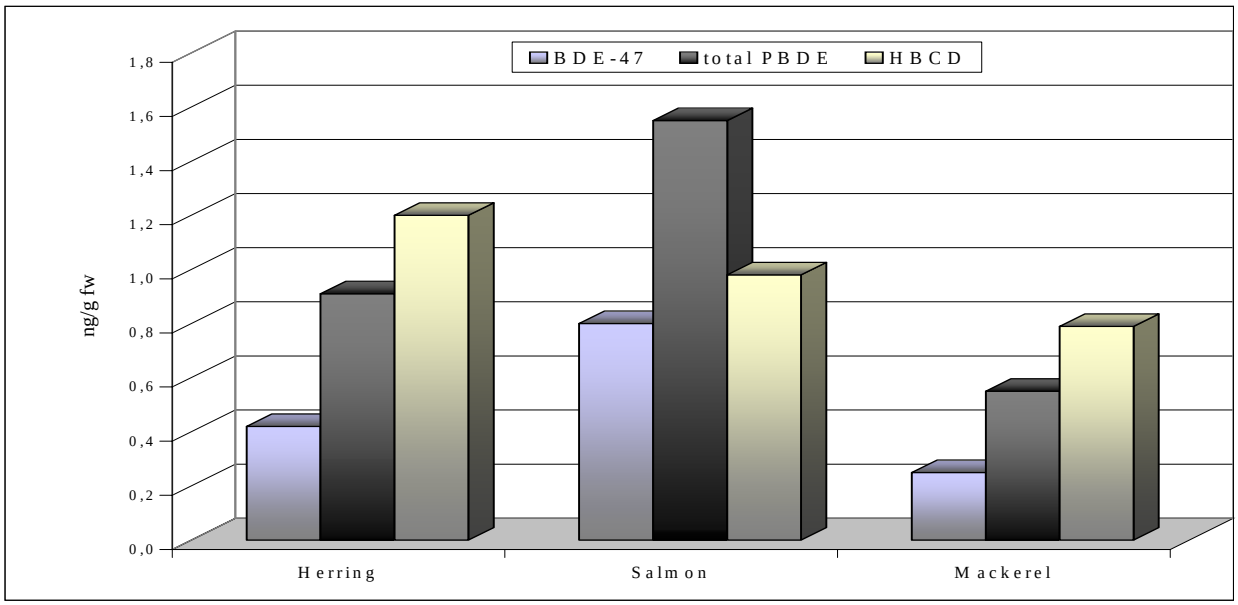

Figure 1: Mean concentration of BDE-47, sum of detectable PBDEs and HBCD in fish from Latvia (ng/g fw)

Regarding levels as well as congener patterns, the PBDE results reported here are comparable with findings in fish products from Norway and Ireland reported in previous studies[6,12]. Also, the average levels for HBCD (sum of α-, β- and γ-HBCD) observed in the herring, salmon and mackerel samples from the Latvian market were similar to those found in foregoing surveys[1,6].

Organotin and PFC results:
Increased levels of organic tin compounds in the aquatic environment have been observed particularly near ports, shipping lanes and marinas. OTC were also found in fish, but they are excreted rather quickly. In Baltic fish from contaminated areas in Finland organotin levels varied between 40 and 150 ng/g fw[13]. In contrast to these findings fish from Finnish lake areas (e. g. Baltic herring, salmon, sprat) showed relatively low average levels of TBT (ca. 4 ng/g fw) and TPhT (ca. 4 ng/g fw)[14].
The socio-economic and scientific interest in PFCs is increasing since this new group of contaminants has been found in biota from remote marine locations as well as in human blood. A recent screening study initiated by six Nordic countries indicated that PFC related chemicals are widely distributed in all environmental compartments. The patterns found in Nordic biota point towards both country specific release patterns and species dependent bio-accumulation properties. PFOS was the predominating PFC compound in freshwater fish (4.7 – 551 ng/g fw) and marine fish (0.9 to 62 ng/g fw) and PFOA levels in fish were significantly lower[4].

The butyltin and triphenyltin as well as the PFC levels in nearly all salmon and mackerel samples investigated here were negligible and only some of the analysed herring samples showed positive findings. In Figure 2 the TBT and TPhT as well as the PFOS and PFOA concentrations of five analysed herring samples are presented.

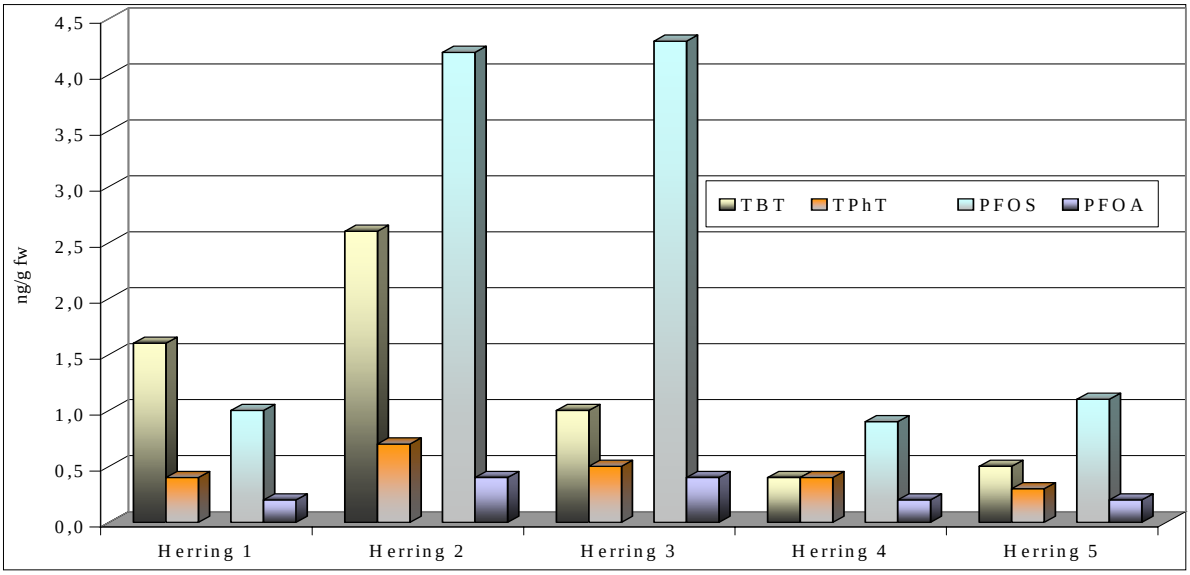

Figure 2: Upper-bound levels of TBT, TPhT, PFOS and PFOA in herring samples from Latvia (ng/g fw)

MBT and DBT contents were in the same range as the TBT levels, so that the total contamination of the analysed fish samples falls below the median organotin concentration estimated by EFSA (13.5 µg/kg fish). The low PFOS and PFOA levels in all fish species monitored here were in accordance with this. A comparably low PFOS level in herring muscle was also recognised in a current survey on PFCs in Dutch fish. By contrast to this finding distinctly higher PFC levels were determined in flatfish like flounder and sole[15].

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References
1. *The EFSA Journal 2005;* 236: 1.
2. *Global Agriculture Information Network (GAIN)* 2005; Report LG5002: 1.
3. www.imo.org/Conventions/mainframe.asp?topic_id=529
4. Nordic Council of Ministers, Tema Nord 2004:552, Copenhagen, Denmark , 2004.
5. Grümping R., Hamm S., Stegemann, D. and Maulshagen, A. *Organohalogen Comp* 2004; 66:1977.
6. Tlustos C., McHugh B., Pratt, I., Tyrell, L. and McGovern E. *Marine Environ & Health Series* 2006; No. 26.
7. Grümping R., Opel, M. and Petersen, M *Organohalogen Comp* 2007; 69:912.
8. Grümping R., Stegemann D., Homburg B., Petersen, M. and Opel M. *Organohalogen Comp* 2007; 69:2896.
9. Pandelova M., Henkelmann B., Roots O. Simm M., Benfenati E., Schramm K.-W. *Organohalogen Comp* 2006; 68:1932.
10. Hites R.A., Foran J.A., Carpenter D.O., Hamilton M., Knuth B.A. and Schwager S.J. *Science* 2004; 303: 226.
11. Herrmann T., Lohmann N. and Päpke O. *Organohalogen Comp* 2005; 67:640.
12. Bethune C., Nielsen J. and Julshamn K. *Organohalogen Comp* 2004; 66:3861.
13. www.fimr.fi/en/ajankohtaista/uutisia_muualta/2006/en_GB/1047/
14. www.evira.fi/portal/en/food/current_issues/?id=278
15. Leeuwen S.P.J. and de Boer J., RIVO Research Report nr. C034/06, IJmuiden, Netherlands, 2006.