POLYCHLORINATED DIBENZO-p-DIOXINS (PCDDs) AND DIBENZOFURANS (PCDFs) IN MUSCLE OF SOUTH BALTIC SEA FISH – PRELIMINARY STUDY

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Abstract

PCDD and PCDF congeners were analysed in 40 samples of four fish species: wild salmon, herring, cod, and flounder. The fish were collected during trading season in November-December 2002 at middle part of Polish Baltic coast (Koszalin region) by local fishermen. PCDD/PCDF concentration in fish muscle tissues was in range from 0.82 to 4.82 pg WHO-TEQ/g of fresh weight (f.w.) and only in two salmon samples slightly exceeded permitted the level (4 pg WHO-TEQ/g f.w.) set by the European Commission Scientific Committee for Food.

Key words: sea fish, dioxins, contamination, TEQ.

Dioxins and furans are known as by-products created during combustion of chlorine and chlorine containing materials. They are on the list of Stockholm Convention on Persistent Organic Pollutants as the twelve priority persistent organic pollutants (POPs) for special investigations. Although these chemicals are now being banned for manufacture and/or restricted for use in most countries, living organisms including human beings can still come in contact with them by breathing contaminated air, eating contaminated food, and drinking or washing in contaminated water. In most countries, the bulk of the dietary intake of dioxins and related compounds is due to the contamination of food. In general, food of animal origin contributes to about 90% of the overall human exposure (3, 5, 20, 22).

Dioxins accumulated in human body pass through cell membranes and combine with a natural receptor protein, aryl hydrocarbon receptor (Ah receptor), a ligand activated, bHLH-PAS transcription factor through which dioxin cause altered gene expression and toxicity. Dioxins also are turning on genes that control many biochemical reactions, such as the synthesis and metabolism of hormones, enzymes, growth factors, and others (21, 26).

The Baltic Sea has been exposed in the past century to heavy pollution, mostly from industrial activity (16, 30). This led to accumulation of dioxins, which are non-biodegradable and indestructible chemicals that accumulate in the muscle tissue of fish and enter the human food chain. Last thirty years study showed that Baltic contains a lot of persistent toxic organohalogen compounds and high contaminant level can be found in tissues of living in this area animals (eagles, seals, fish) (8, 9, 10). There are about 100 fish species living in the Baltic Sea. Baltic fisheries are dominated by cod, herring and sprat, which make up about 93 per cent of the total catches. No dioxin data are available for fish catches in Poland and in the Polish Baltic coastal waters.

Material and Methods

Fish samples. Four species of Baltic Sea fish were analysed for PCDD and PCDF levels: wild salmon, herrings, cod and flounder. The fish were collected during trading season in November-December 2002 at middle part of Polish Baltic coast by local fishermen (Kolobrzeg and Uniście, Koszalin region).
The samples were taken and processed for the analysis as described below. Ten samples of each fish species were prepared. Each sample weighed about 0.5 kg and consisted of muscles of 1-20 fish, depending on the species. The samples did not include skin and abdominal fat, which have a significant influence on the dioxin content. Fish meat samples were cooled down, then frozen, and stored at -20°C until analysis.

Chemicals and reagents. High purity solvents and other chemicals were purchased from J.T. Baker (Deventer, the Netherlands) and Merck (Darmstadt, Germany). Semipermeable membranes (SPM) of 25 mm x 300 mm of 80 µm polyethylene wall thicknesses were of EST, St. Joseph, MO, California USA, purchased from EXPOSMETER, Sweden. Native and isotopically labeled 13C-PCDDs/Fs/PCBs and 37Cl-TCDD standard solutions were from Wellington Laboratories, Guelph, Ontario, Canada. Spiking solution in acetone was prepared by dilution and mixing of concentrated 13C-PCDDs/Fs EPA 1613 LCS and coplanar 13C-PCB MBPC-CP standards to obtain final concentration of 1 ng/ml of the individual congener (except of 2 ng/ml of OCDD).

Sample preparation. Each sample was extracted to obtain fat, which was cleaned-up by the semipermeable membranes (SPM) and the carbon column (12, 13, 14, 15, 28).

Dioxin determination. Dioxin determination was performed using Thermo Quest GCQplus GC-MS/MS systems adjusted to double fragmentation mode. In this case, there were detected secondary ions which are formed by splitting out of COCl particle from PCDD/PCDF molecular ion M+ resulting in (M-COCl)+ ion. For nonortho-PCBs ((M-Cl)+ secondary ions were detected respectively. Gas chromatography conditions were as follows: helium flow set to 1.0 ml/min. DB-5MS or DB-17 Column temperature programs were as follows: 100°C for 1 min, 20°C/min to 200°C, 2°C/min to 280°C and hold for 15 min. Transfer line temperature was adjusted to 270°C. Because of different structures of cores of PCDD and PCDF molecules, optimum of collision energies with helium atoms in a MS/MS system was different (12, 13, 14, 15, 28).

Validation method. The described procedure was validated using fish samples fortified with natural PCDD/PCDF mixture up to the concentrations of 1–10 pg WHO-TEQ/g f.w. These samples were analysed using the same conditions, batch of solvents and reagents as well as calculation method. In this method the precision was better than 20% for concentration above 1 pg WHO-TEQ/g f.w. and better than 30% for lower concentrations.

Limit of detection (LOD) was calculated on the level of 0.01 pg-TEQ/g of fresh fish muscle. For individual PCDD and PCDF congeners the LOD was in the range of 0.001 pg/g for TCDD/TCDF and 0.05 pg/g for H-PCDD/F and OCDD/F. In the case, when the concentration of the individual congener was below the detection limit, LOD value was used for TEQ calculation, however, it was necessary in some of the analysed samples only.

Recovery of each of 17 PCDD/F congeners was in the acceptable range of 65-120%. All samples were analysed two times and average value was calculated.

Results

The results of analysis of PCDD and PCDF concentrations in fish meat samples are presented in Table 1 and Fig.1. The concentrations expressed as WHO-PCDD/F-TEQ was in range from 0.82 to 4.82 pg WHO-TEQ/g of fresh weight (f.w.) and slightly exceeded permitted by EU level set by the European Commission Scientific Committee for Food (SCF) only in two tested wild salmon samples (> 4 pg WHO-TEQ/g f.w.) (6).

Wild salmon with the average fat content of 8.5% generally had the highest dioxin concentration (2.01-4.82 pg WHO-TEQ/g f.w.). Other fish species, with average fat contents 4.6-8.2% showed dioxins concentration of 0.82-2.76 pg WHO-TEQ/g f.w. Moderate correlation (r=0.56) between fat and dioxin concentration was observed. The results are an average value from two independent analyses of each sample.

Presence of 2,3,7,8-TCDF was found in the most Baltic fish samples. Fig. 2 shows that 2,3,7,8-TCDF is the most abundant congener among the other TCDFs. The elevated concentration of 2,3,7,8-chlorosubstituted PCDD and PCDF congeners is probably caused by higher bioaccumulation of these compounds in fish adipose tissue.

Generally, the results of the present study of dioxins and dibenzofurans contamination of Baltic fish catches from Polish coast indicate that dioxins level was rather low and that in 95% of samples amount of PCDD/F congeners were below the level permitted by EU (4 pg WHO-TEQ/g f.w.).

Discussion

Dioxins are environmental contaminants which are detected in very low concentrations in many food products and aquatic biota. They are strongly lipophilic and as a result, they accumulate in the tissues of living organisms in elevated concentrations as they move up the food chain. PCDD and PCDF are particularly found in fatty foods, including fish.

The presented in this paper results indicate that dioxin concentration in Polish Baltic coast fish is low and does not exceed permitted by EU level (4 pg/g WHO-TEQ/g f.w.) which does not cause significant direct risk for consumers. However, the obtained results suggest that monitoring of food is needed.

Numerous surveys of dioxins in fish have revealed considerable variation in TDCC concentration. In general, concentrations of dioxins and PCBs in fish depend on fat content in fish organism, the extent to which fish migrate, the number of times they spawn, fish age, feeding habits, species, tissue and organs (2, 30).
Fig. 1. PCDD and PCDF level in individual fish samples.

Fig. 2. Typical MS/MS chromatogram of TCDF fraction in fish from the Baltic Sea.

Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of samples tested</th>
<th>Fat content average / range</th>
<th>PCDD/PCDF pg WHO-TEQ/g f.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>(x ± SD)</td>
</tr>
<tr>
<td>Salmon</td>
<td>10</td>
<td>8.46 ± 2.15, 6.0 - 11.9</td>
<td>3.2 ± 1.2, 2.01 - 4.82</td>
</tr>
<tr>
<td>Cod</td>
<td>10</td>
<td>6.43 ± 1.05, 4.5 - 7.9</td>
<td>1.6 ± 0.5, 0.82 - 2.43</td>
</tr>
<tr>
<td>Flounder</td>
<td>10</td>
<td>6.46 ± 1.15, 4.7 - 8.2</td>
<td>2.0 ± 0.4, 1.52 - 2.55</td>
</tr>
<tr>
<td>Herring</td>
<td>10</td>
<td>5.58 ± 1.33, 4.9 - 7.5</td>
<td>1.7 ± 0.5, 1.10 - 2.76</td>
</tr>
</tbody>
</table>
According to a recent study, the dioxin content in Baltic herring caught in Finnish waters and the Gulf of Bothnia can be much higher than required EU maximum level (4 pg WHO-TEQ/g f.w.). The Finnish Baltic herrings exceed this level by as much as four times, with concentrations for larger fish in the Gulf of Bothnia as high as 17.7 pg WHO-TEQ/g f.w. Large Baltic herring more than 17 centimeters in length, as well as salmon caught in the Baltic Sea – mainly in the Gulf of Finland – exceed permitted dioxin level and under the rules, such fish must not be exported to other EU countries, except Sweden. Dioxin concentrations found in Bothnia and Gulf of Finland fish were from 0.78 to 17.7 pg WHO-TEQ/g f.w. (3, 19). Only Finland and Sweden have obtained special EU permission until 2006 to continue to catch Baltic herring and sell them on local market. The large amounts of dioxin previously released from the Swedish and Finnish pulp industries seem to be the explanation, why the dioxin levels in fish are still high in the northern part of the Baltic Sea. Elevated concentrations were also observed for dioxins and dioxin-like PCBs in Baltic herring (Clupea harengus) collected in 1999 by German fishery from nine different fishing areas of the Baltic Sea. Concentration range was from 0.5 to 7 pg WHO-TEQ/g f.w. with the highest value at Bornholm and Latvia coast. Another German study has showed a clear interdependence of the fishing area and dioxin concentration. PCDD/PCDF contamination in the herring fillets ranged from 0.317 to 3.163 pg WHO-TEQ/g f.w. (18). The results from Latvian fish samples analysed in the UK in 2002 showed that in some of the samples dioxin levels only slightly exceeded levels allowed by EU regulations. In Isosari study, the average level in herring was 2.6 pg WHO-TEQ/g f.w. and was lower than predicted (17). Only the salmon caught in the northern Baltic Sea contained PCDD/F levels that exceeded the EU’s maximum permissible limit (17). In the Estonian data, only one sample (herring) from coastal waters was above the permitted level (23).

The Baltic Sea was exposed in the past century to heavy pollution, much of it deriving from industry in coastal cities. Since the middle of sixties this sea has been recognized as an aquatic system heavy contaminated by polychlorinated compounds such as pesticides, polychlorinated naphthalenes and PCBs. (16). The highest level of contamination was recorded in 1970s, however, the recent data show decrease in dioxins concentration. High contaminant level can be found in tissues collected from aquatic animals during last thirty years. Those contaminations have caused a number of environmental toxicological effects, e.g. reproduction problems of seals and sea eagles (8, 9). To express a comparable toxicity (Toxic Equivalency – TEQ) of samples contaminated with different concentrations of individual dioxin and furan congeners, the toxic equivalency factors (TEFs) have been introduced to facilitate regulatory control and risk assessment for total TEQ calculation (24, 25). Tolerable Daily Intakes (TDI) and Tolerable Weekly Intakes (TWI) are doses relate to the amount of a contaminant that can be ingested daily or weekly over a lifetime without appreciable risk. Based on risk assessment, the European Commission Scientific Committee for Food (SCF) has established a TWI of 14 picogram toxic equivalents (TEQ) per kilogram body weight for dioxins and dioxin-like PCBs. Dose TDI of 1-4 pg WHO-TEQ/kg body weight was established by the WHO. It has been stated also that the European average dietary intake is 1.2 to 3.0 pg WHO-TEQ/kg b.w. /day, which exceeds the Tolerable Daily Intake. The comparison of TDI dose (1-4 pg WHO-TEQ/kg) with average daily intake for Europe population (1-3 pg WHO-TEQ/kg) has shown that these unwanted compounds can have a broader impact on health problems than previously assumed. (3, 5, 20, 31).

It is now well established that fish and fish product constitute an important route of human exposure to polychlorinated dibenzo-p-dioxins, dibenzofurans, polychlorinated biphenyls, and other persistent organic chemicals (3, 4, 20, 22, 29). The dietary intake of dioxins and PCBs with bioaccumulation potential in aquatic organisms can be governed to a large extent of fish consumed by local societies – e.g. fishermen. Therefore, in some countries people are exposed to very high dioxin level. In Japan the total diet study revealed that about 60% of the dietary intake of dioxins and related compounds is likely to come from the intake of fish and shellfish (1). Finland study on 2,3,7,8-TCDD concentration in human fat have shown that the lifetime exposure in a population consuming mostly Baltic contaminated fatty fishes can reach the levels of exposures which occurred in patients after the Seveso accident in Italy in 1976 (19).

For identification and confirmation of dioxin congeners and dioxin-like PCBs in official food control, techniques of high resolution gas chromatography with high resolution mass spectrometry (HRGC/HRMS) is recommended by EU. Another recommended EU strategy is applying faster and cheaper screening methods (for example biotest, low resolution GS-MS) and dioxin confirmation by HRGC/HRM as the next step, because only HRGC/HRMS guaranties proper results at such low levels of concentrations. Recommended by EU method of dioxin determination (HRGC/HRMS) is not commonly available (7). The cost of dioxin analysis by high resolution mass spectrometry is prohibitively expensive and time consuming. Therefore, routine monitoring of foodstuffs or feedstuffs cannot be based only on congener-specific dioxin analysis using high resolution mass spectrometry. That is why interest in other alternative techniques and methods of PCDD/PCDF analysis has increased. Recently tandem mass spectrometry detection techniques have become more competitive for high resolution mass spectrometry and more frequently are used in residues analysis of biological samples. MS/MS technique requires more efficient sample clean-up than for HRMS but is simpler and less expensive and can be used for different application. Low detection limit, very high recovery and good repeatability make mass spectrometry with double fragmentation powerful tool for ultra trace analysis and in many cases may substitute...
high-resolution mass spectrometric systems (12, 13, 14, 15, 27).

Food safety is essential for public health issues for all countries. Although the industrial sources of dioxins are now in many countries strictly controlled, this group of chemicals is very persistent and will remain in the environment for many more years. Their toxic effects cause the long-term health consequences and will need to be monitored and followed up.

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