Research Articles

Chlorinated Dibenzo-*p*-Dioxins and Dibenzofurans in the Baltic Herring and Sprat of Estonian Coastal Waters

Ott Roots[1]* and Vladimir Zitko[2]

[1] Estonian Environmental Research Centre Ltd., Marja 4D, 10617, Tallinn, Estonia
[2] Consultant, 114 Reed Ave, St. Andrews, NB E5B 1A1, Canada

* **Corresponding author** (Ott.Roots@klab.ee)

DOI: http://dx.doi.org/10.1065/espr2004.04.198

Abstract

Background, Aims and Scope. The concentration of chlorinated dibenzo-*p*-dioxins and dibenzofurans in many fish from the Baltic requires monitoring, since it approaches or exceeds the European Union threshold limit value of 4 pg TEQ/g wet weight of fish for human consumption. The concentrations, expressed in TEQs, are important for toxicology and regulations, but hide the concentrations of the individual congeners, which are important for other environmental sciences, source allocation, and for the detection of measurement errors. This report evaluates the results of a survey reported earlier only in the terms of the TEQ concentrations.

Methods. Principal Component Analysis (PCA) was used to reduce the dimensions of the data (17 = 7 chlorinated dibenzo-*p*-dioxin and 10 chlorinated dibenzofuran congeners) to three principal components. This facilitated the interpretation of the congener profiles. Slopes of the congener concentrations as a function of age of the fish were estimated by least squares regression. The results were compared with a large set of data for lake trout from Lake Ontario.

Results and Discussion. The congener profiles of Baltic herring are less scattered than those of sprat. The profiles of herring from the central Baltic differ from those of herring from the Gulf of Riga and both appear to be affected relatively minimally by the age of the fish. The congener profiles of herring from the western Gulf of Finland are similar to those from the central Baltic, those from middle Gulf of Finland are similar to those from the Gulf of Riga. Both seem to be more affected by age of the fish than the profiles of the first two groups. The concentrations of several pentachloro- and hexachloro-dibenzo-*p*-dioxins and dibenzofurans increase with the age of the fish

Conclusion. PCA is a good technique for the evaluation of the congener profiles. The resulting loading and score plots provide a good graphic summary of the multidimensional data. Additional analyses are needed to confirm the observed profile patterns. A comparison with the results of a long-term monitoring from another area shows that the age of the fish is a more important factor than the year of capture.

Recommendation and Outlook. The surveys should continue for a number of years and the results should be presented and evaluated both in terms of the TEQs as well as in terms of weight concentrations. Since the concentrations do not appear to change very much from year to year, it would be better to carry out surveys only every 3–4 years and, instead, stratify the sampling according to age and gender of the fish, and to analyze replicate extracts by replicate measurements. The inclusion of unmarked replicate samples would be a good quality assurance measure. It would be desirable to analyze additional parts of the food chain in order to understand the fate of the compounds in the ecosystem.

Keywords: Baltic Sea; chemometrics; chlorinated dibenzofurans (CDF); chlorinated dibenzo-*p*-dioxins (CDD); dioxins; Estonian coastal waters; herring; principal component analysis (PCA); sprat

Introduction

There are large regional differences in the concentrations of chlorinated dibenzo-*p*-dioxins (CDD) and dibenzofurans (CDF) in fish from the Baltic (Vartiainen et al. 1997, Olsson et al. 2002, Vuorinen et al. 2002). The European Union (EU) Council Regulation 2375/2001 established a threshold limit value for 4 pg TEQ/g fresh weight. Some of the fish caught in the Baltic Sea exceed the EU limits on concentrations of dioxin in food (The Baltic Marine Environment 1999–2002, 2003). The highest concentrations were found in fatty old specimens from the Bothnian Sea (The Baltic Marine Environment 2003) and the Gulf of Finland. According to Vartiainen et al. (1997) and Vuorinen et al. (2002), the highest CDD and CDF concentrations, 2.9–24 pg TEQ/g wet weight, were found in fish from the inner part of the Gulf of Finland. The concentration of CDD and CDF in Baltic herring from the northern part of the Baltic Sea increases with the age of the fish (Hallikainen and Kiviranta 2002).

In 1995, the German Ministry of Consumer Protection, Food and Agriculture carried out a comprehensive survey of dioxin contamination of important food groups, including fish. Samples of fish and fish products with a market share of over 1% (184 samples) were analyzed. The highest values, 2.9 pg TEQ/g wet weight, were found in herring from the Baltic Sea. This study confirmed the EU report of June 2000 which dealt with the CDD and CDF contamination in ten European countries. The report concluded that the contamination in most seafood species is below 1 pg TEQ/g product

© ecomed publishers, D-86899 Landsberg, Germany and Ft. Worth/TX • Tokyo • Mumbai • Seoul • Melbourne • Paris

weight, with the exception of shrimp, eel and some Baltic fish species (Klinkhard 2001).

The EU regulation may ban the human consumption of herring and salmon from the Baltic. However, Sweden and Finland were granted a transition period, during which an extensive monitoring and reporting of CDD and CDF levels is required.

In the Estonian program, the TEQ concentrations of CDD and CDF in Baltic herring (*Clupea harengus*) and sprat (*Spratus spratus*) collected in the fall of 2002, and the biological characteristics of the fish have been reported by Otsa et al. (2003). In all nine Baltic herring and eight Baltic sprat samples taken from Estonian coastal waters during the fall of 2002, the CDD and CDF concentration in pg TEQ/g wet weight was below the EU threshold. Accordingly, Baltic herring and sprat from the sampled areas of Estonian coastal waters are suitable for human consumption. Of the three Baltic herring samples taken from the Central Baltic Sea the CDD and CDF concentration of only one fish, 5–6 years old and > 17 cm long, was above the EU threshold (Otsa et al. 2003). A preliminary assessment of the data in terms of CDD and CDF congener profiles was given by Roots et al. (2003). This paper evaluates the profiles of the CDD and CDF congeners by chemometrics.

1 Materials and Methods

1.1 Samples

Fish were caught by industrial trawlers in the Central Baltic (samples B), in the western and middle Gulf of Finland (samples T and K, respectively) and in the Gulf of Riga (samples R). Only edible parts of the fish were submitted for analysis.

1.2 Chemical analyses

The analyses were carried out at the Institute of Ecological Chemistry of the National Research Centre for Environment and Health in Neuherberg, Germany. The laboratory has been accredited in Germany for the determination of dioxins and polychlorinated biphenyls (accreditation license No. DAC-P-0141-01-00 valid through 21. Nov. 2006). Briefly, the fish were freeze-dried, homogenized, and extracted by an accelerated solvent extraction. The extracts were cleaned by percolations through a column of silica coated with H_2SO_4 and NaOH, followed by chromatography on a column of aluminum oxide and Florisil. The identification and quantification of CDD and CDF congeners was achieved by ^{13}C-labeled standards and HRGC-HRMS measurements (Henkelmann et al. 1996).

1.3 Chemometrics

The profiles (concentrations scaled to a sum of 100) of CDD and CDF were evaluated by Principal Component Analysis (PCA, see for example Zitko 1994). PCA reduces the high dimensionality of the profiles (7 CDD and 10 CDF) by introducing new variables, the 'principal components'. These are linear combinations of the CDD and CDF concentrations. The combinations are selected in a manner that retains most of the information contained in the original data. The number of the principal components that represents most of the original information is considerably lower than the number of the original concentrations. In most cases this allows the viewing of the data in two or three dimensions in 'score plots'. In these plots, samples with similar profiles are located in proximity. The effect of the original concentrations on the principal components can be seen in the 'loading plots'. In these, the proximity indicates correlation of the concentrations of the congeners. In one dimension, PCA is similar to the calculation of TEQ: TEQ is the 'score', and the TEFs are the 'loadings'.

The results of PCA depend on the pre-processing of the data. In this work, the concentrations were centered (mean = 0) and scaled (std = 1). PCA was performed by PLS_Toolbox 2 (Wise and Gallagher 1998), running in Matlab 5.0 (The MathWorks Inc., Natick, MA 01760, USA). Graphs were prepared in Excel (Microsoft). The points in the graphs were labeled by Labeler2 (Billo 2001). Excel was also used to calculate the slopes of the time trends of the concentrations by the linear least squares method.

2 Results and Discussion

2.1 Biological parameters

The biological characteristics of the samples are given in **Table 1**. As can be seen, fish in the samples were only partially separated according to age and not separated according to their gender. This does not matter for the determination of the suitability of the fish for human consumption. However, from an environmental point of view, it would have been desirable to obtain CDD and CDF concentrations separately according to age and gender of the fish.

2.2 CDD and CDF concentrations

The concentrations of CDD and CDF in terms of TEQ are important for toxicological and regulatory purposes. Much additional information can be obtained from the weight concentrations of the individual congeners (see, for example, Naito et al. 2003, Zitko 2004). It is easily noticeable from Tables 2 and 3 that the sample K5 differs from the others in the CDD and CDF concentrations as well as in the profile (percent composition) of the congeners.

2.3 PCA

As the loading plot (**Fig. 1**) shows, the principal component pc-1 (ev-1) separates the CDD and CDF congeners according to the degree of chlorination. The highly chlorinated congeners are on the left-hand side, and the less chlorinated congeners on the right-hand side of the loading plot. The principal component pc-2 (ev-2) separates 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (66d), 2,3,7,8-tetrachlorodibenzofuran (66f), and 2,3,4,7,8 pentachlorodibenzofuran (E6f) from 1,2,3,6,7,8-hexachlorodibenzofuran (77f), 2,3,4,6,7,8-hexachlorodibenzofuran (E7f) and 1,2,3,4,7,8-hexachlorodibenzofuran (F6f).

Table 1: Gender, number (N), and median size (L cm, W g), age (Y, years), and maturity (M) of the fish, percent female fish in the sample (%F), and weighted median age (WA), (Otsa et al. 2003). Samples B – central Baltic, T, K – western and middle Gulf of Finland, respectively, R – Gulf of Riga

Herring												
	Female					Male						
Samples	N	L	W	Y	M	N	L	W	Y	M	% F	WA
B1	6	17.6	37.5	5.0	2.8	3	17.8	39.3	7.0	3.0	67	5.7
B2	10	16.0	28.6	4.5	2.5	2	16.2	30.0	5.5	3.0	83	4.7
B3	8	16.0	30.3	3.5	2.5	4	14.6	22.6	3.0	3.0	67	3.3
T1	7	14.9	23.0	3.0	2.0	7	15.3	23.4	3.0	2.0	50	3.0
T2	15	14.1	17.6	2.0	2.0	5	14.0	19.9	2.0	2.0	75	2.0
T3	10	13.8	17.3	1.5	2.0	10	13.5	17.1	1.5	2.0	50	1.5
K1	2	15.4	23.2	5.0	2.3	12	15.1	23.1	4.0	2.5	14	4.1
K2	7	14.3	19.5	3.0	2.5	13	14.6	20.4	3.0	3.0	35	3.0
K3	7	14.0	19.9	3.0	2.5	13	14.4	18.4	2.0	3.0	35	2.4
R1	8	16.1	27.5	4.0	2.3	4	16.2	29.0	4.5	3.0	67	4.2
R2	8	14.2	19.3	2.0	2.5	10	14.7	22.5	2.0	3.0	44	2.0
R3	7	14.6	21.2	2.0	2.0	11	14.4	20.8	2.0	3.0	39	2.0

Sprat												
	Female					Male						
Samples	N	L	W	Y	M	N	L	W	Y	M	% F	WA
B4	19	12.1	11.8	3.0	2.5	11	11.5	10.4	2.0	2.5	63	2.6
B5	11	12.2	12.2	3.0	2.5	19	11.7	10.3	2.0	3.0	37	2.4
T4	21	12.5	12.7	3.0	2.0	5	12.6	12.5	4.0	2.0	81	3.2
T5	14	12.2	12.1	3.0	2.0	12	11.8	11.1	2.0	2.0	54	2.5
T6	18	12.3	12.1	3.0	2.0	11	11.8	11.0	3.0	2.0	62	3.0
T7	19	12.1	11.9	3.0	2.0	16	11.45	10.8	2.0	2.0	54	2.5
K4	13	11.8	11.2	2.0	2.5	10	11.5	9.8	2.0	2.0	57	2.0
K5	8	12.1	11.1	3.0	2.3	27	11.4	9.8	2.0	2.0	23	2.2

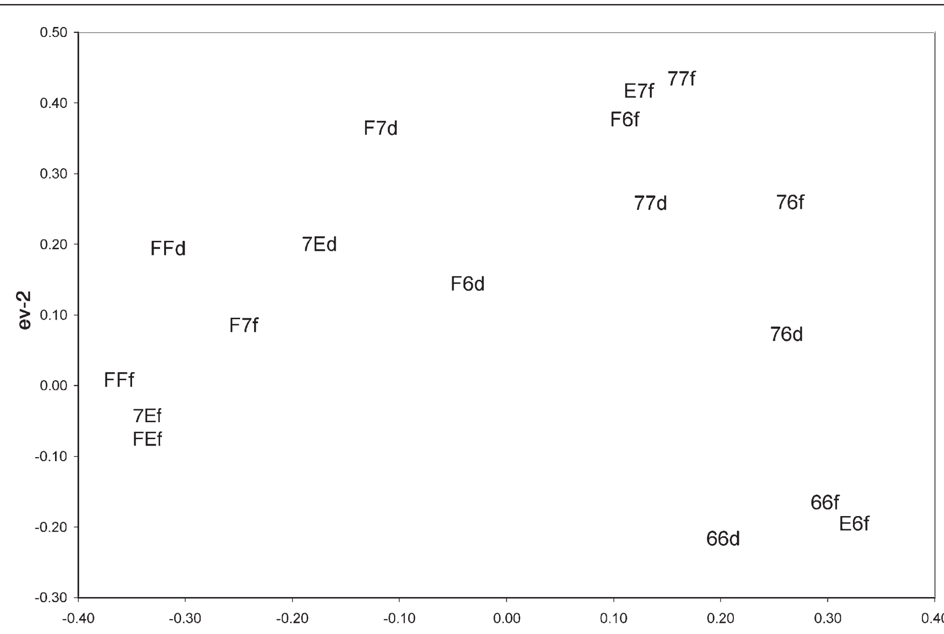

Fig. 1: The loading plot shows the effect of the original variables (CDD and CDF congener concentrations) on the principal component pc-1 (ev-1) and pc-2 (ev-2). For congener codes, see Table 2 or Table 3. The codes may also be easily interpreted: '6' describes substitutions in positions 2,3 or 7, 8; '7' in positions 1,2,3 or 6,7,8; 'E' in positions 2,3,4 or 7,8,9. 'F' means that chlorine atoms are in all positions, and 'd' and 'f' indicate dioxin and furan, respectively (Zitko 1985). It can be seen that most of the less chlorinated congeners have a high loading on the pc-1. Profiles with high concentrations of these congeners will be located at higher values of pc-1 in the score plots. Similarly, the loadings on the pc-2 distinguish between the congeners 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (66d), dibenzofuran (66f), 2,3,4,7,8-tetrachlorodibenzofuran (E6f), and 1,2,3,6,7,8-hexachloro-(77f), 2,3,4,6,7,8-hexachloro-(E7f), 1,2,3,4,7,8-hexachlorodibenzofuran (F6f) and 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (F7d). The loadings on the principal component pc-3 (ev-3) are not shown in a separate loading plot, to save space. The congeners 1,2,3,7,8,9-hexachloro-(7Ed) and 1,2,3,4,7,8-heptachloro-dibenzo-*p*-dioxin (F6d) have high positive and the congeners 2,3,4,6,7,8-hexachloro-(E7f) and 1,2,3,4,6,7,8-heptachlorodibenzofurna (F7f) have high negative loadings on the pc-3

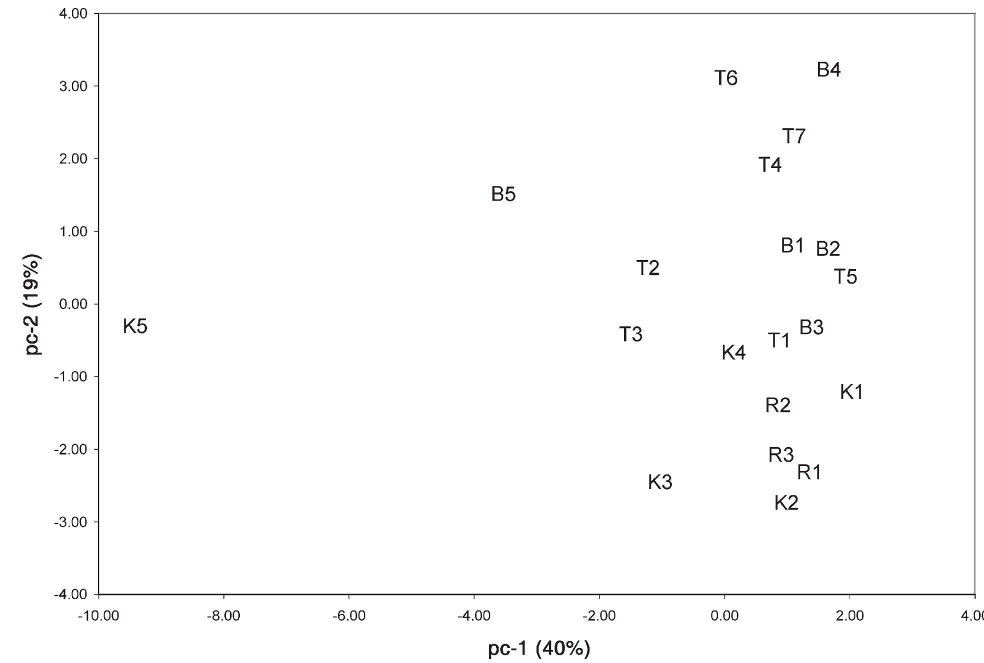

Fig. 2: The score plot shows the projection of the CDD and CDF congener profiles on the plane of the principal components pc-1 and pc-2. The plot retains 59% (40+19) of the variation of the original data. The projections of samples with similar congener profiles are close together. For sample codes, see Tables 1–3

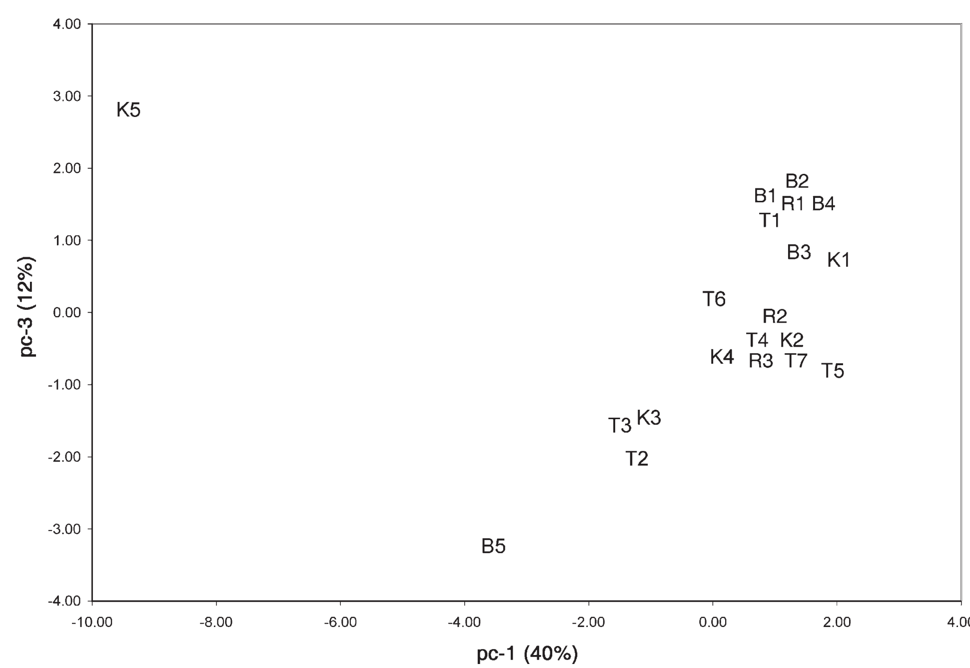

Fig. 3: The score plot shows the projection of the CDD and CDF congener profiles on the plane of the principal components pc-1 and pc-3. The principal component pc-3 accounts for an additional 12% of the original variability. For sample codes, see Tables 1–3

As can be seen from the score plots (**Figs. 2, 3**), the CDD and CDF profiles of herring from the central Baltic (samples B1–B3) form a 'cluster' in the planes of the principal components pc-1 & pc-2 and pc1 & pc-3. The cluster shows that the congener profile does not change much with the age of the fish (see Table 1). On the other hand, the congener profiles of sprat from the same area (B4, B5) differ from those of the herring, as well as from each other. These sprat are smaller and younger than the herring and this could account for the lower CDD and CDF concentrations in the sample B4 (**Table 2**). Sample B5 is an 'outlier', primarily because of the unusually high concentrations of 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin (FFd)

Table 2: Concentration of CDD and CDF congeners (pg/g lipid) in edible tissues of Baltic herring and sprat. For the origin of the samples, see Table 1

Herring												
Congener	**B1**	**B2**	**B3**	**K1**	**K2**	**K3**	**T1**	**T2**	**T3**	**R1**	**R2**	**R3**
2378D	2.40	2.20	2.30	0.82	2.10	1.20	2.50	0.57	1.10	1.80	1.50	0.97
12378D	7.10	5.00	4.10	5.80	2.20	2.70	5.50	2.00	1.50	2.60	1.50	1.20
123478D	1.30	0.61	0.07	0.15	0.11	0.08	0.94	0.11	0.11	0.73	0.28	0.05
123678D	4.20	3.00	2.60	4.20	2.00	1.70	3.60	2.90	2.00	1.10	1.10	0.60
123789D	0.36	0.44	0.42	0.15	0.10	0.08	0.10	0.10	0.11	0.08	0.07	0.05
1234678D	2.10	1.20	1.70	0.17	0.47	1.20	1.60	2.30	1.70	0.91	1.10	0.92
12346789D	10.10	6.10	6.30	2.20	2.60	5.10	8.50	11.30	7.90	2.40	3.20	1.90
2378F	32.20	33.70	30.20	24.30	21.80	23.00	21.90	19.20	16.00	22.60	19.10	18.60
12378F	7.10	6.40	4.90	2.90	1.80	1.90	2.70	1.80	1.10	3.80	2.20	1.90
23478F	45.10	31.60	25.50	38.10	18.30	19.00	29.40	17.40	10.60	30.90	15.20	16.80
123478F	2.10	2.10	1.10	0.91	0.49	0.55	0.90	0.83	0.35	0.75	0.84	0.58
123678F	3.30	2.60	1.60	1.60	0.56	0.45	1.60	0.70	0.82	0.95	0.54	0.74
123789F	0.02	0.03	0.02	0.04	0.04	0.04	0.04	0.04	0.03	0.02	0.03	0.02
234678F	2.90	2.10	1.80	1.80	1.20	1.20	1.80	1.70	1.10	1.10	0.93	0.91
1234678F	2.10	1.30	0.66	0.82	1.10	2.60	1.30	1.80	1.60	0.53	0.55	0.49
1234789F	0.06	0.05	0.11	0.14	0.22	0.13	0.08	0.08	0.07	0.05	0.05	0.04
12346789F	8.50	3.10	0.46	0.60	0.85	10.00	4.30	3.20	5.20	1.60	1.70	1.40

Sprat									
Congener	**Code in figures**	**B4**	**B5**	**K4**	**K5**	**T4**	**T5**	**T6**	**T7**
2378D	66d	1.60	1.10	0.61	1.50	0.60	1.50	0.86	0.89
12378D	76d	2.70	2.90	1.30	3.30	3.40	2.60	3.40	2.20
123478D	F6d	0.38	0.45	0.31	2.30	0.48	0.06	0.51	0.36
123678D	77d	2.60	2.60	1.60	5.40	2.80	2.50	3.10	2.60
123789D	7Ed	0.38	0.07	0.06	2.40	0.23	0.05	0.23	0.13
1234678D	F7d	1.70	3.30	0.88	7.60	2.10	1.30	3.10	2.10
12346789D	FFd	5.10	20.80	2.60	60.00	6.50	2.60	10.50	3.30
2378F	66f	20.10	21.20	16.30	20.40	21.60	21.80	18.80	18.90
12378F	76f	4.70	4.40	1.60	2.80	3.90	4.60	3.70	3.00
23478F	E6f	17.20	19.40	13.10	13.60	22.70	18.40	17.70	14.20
123478F	F6f	1.30	2.60	0.35	2.40	0.95	1.00	1.20	0.83
123678F	77f	2.20	2.20	0.83	2.40	1.80	1.00	2.10	1.20
123789F	7Ef	0.02	0.05	0.03	1.80	0.02	0.03	0.03	0.03
234678F	E7f	2.10	4.80	0.94	2.80	2.50	1.90	1.90	1.90
1234678F	F7f	1.00	10.20	1.50	9.60	1.40	1.00	1.40	1.10
1234789F	FEf	0.07	0.30	0.11	4.90	0.05	0.05	0.07	0.05
12346789F	FFf	0.37	46.00	0.32	128.00	2.50	2.20	2.50	1.00

and 1,2,3,4,6,7,8,9-octachlorodibenzofuran (F7f) (**Table 3**). On the whole, the CDD and CDF congener pattern in sprat from the central Baltic is very different from that in the herring from the same area.

The CDD and CDF congener profiles of herring from the western Gulf of Finland (samples T1–T3) are similar to those of the B1–B3 herring. The herring in the samples T2 and T3 are younger than herring in the samples T1 and B1–B3. It may be that younger fish contain a relatively lower concentration of 2,3,4,7,8-pentachloro-dibenzofuran (E6f), since the same pattern appears also among the herring from the middle Gulf of Finland (the samples K1–K3). However, the measurement error is not known and the data should not be over-interpreted. The congener profiles of sprat from this area (samples K4 and K5) differ so much that one would contemplate an error in the analysis of the sample K5 and ask for a duplicate analysis, possibly also for sample B5. Sample K5 contains many more male fish than sample K4, but fish in both samples are of approximately the same age, and there is no obvious reason for the very high concentration of 1,2,3,4,6,7,8,9-octachlorodibenzofuran in sample K5.

The CDD and CDF profiles of sprat from the western Gulf of Finland (samples T4–T7), with the exception of the sample T5, are in a cluster above the samples of B cluster. The cluster T is situated above the cluster B primarily because of the relatively lower concentration of 2,3,7,8-tetrachlordibenzo-*p*-dioxin (66d) and higher concentration of 2,3,4,6,7,8-hexachloro-dibenzofuran (E7f) in the former.

The CDD and CDF congener profiles in herring from the Gulf of Riga (R1–R3) follow the pattern of the other herring samples (see Figs. 2, 3). Their cluster is below that of the B herring (B1–B3), mainly because of the higher concentrations of

Table 3: Concentration of CDD and CDF congeners (%) in edible tissues of Baltic herring and sprat. For the origin of the samples, see Table 1

Herring												
Congener	B1	B2	B3	K1	K2	K3	T1	T2	T3	R1	R2	R3
2378D	1.83	2.17	2.74	0.97	3.75	1.69	2.88	0.86	2.14	2.50	3.01	2.06
12378D	5.42	4.92	4.89	6.85	3.93	3.81	6.34	3.03	2.92	3.62	3.01	2.54
123478D	0.99	0.60	0.08	0.18	0.19	0.11	1.08	0.17	0.21	1.02	0.56	0.11
123678D	3.21	2.95	3.10	4.96	3.58	2.40	4.15	4.39	3.90	1.53	2.21	1.27
123789D	0.27	0.43	0.50	0.18	0.17	0.11	0.12	0.14	0.21	0.11	0.13	0.10
1234678D	1.60	1.18	2.03	0.20	0.84	1.69	1.84	3.48	3.31	1.27	2.21	1.95
12346789D	7.71	6.01	7.51	2.60	4.65	7.19	9.80	17.12	15.40	3.34	6.42	4.03
2378F	24.59	33.19	36.02	28.69	38.98	32.43	25.24	29.08	31.20	31.43	38.30	39.44
12378F	5.42	6.30	5.84	3.42	3.22	2.68	3.11	2.73	2.14	5.28	4.41	4.03
23478F	34.44	31.12	30.42	44.98	32.72	26.79	33.89	26.36	20.67	42.97	30.48	35.62
123478F	1.60	2.07	1.31	1.07	0.88	0.78	1.04	1.26	0.68	1.04	1.68	1.23
123678F	2.52	2.56	1.91	1.89	1.00	0.63	1.84	1.06	1.60	1.32	1.08	1.57
123789F	0.01	0.03	0.02	0.05	0.07	0.06	0.04	0.05	0.05	0.03	0.05	0.04
234678F	2.21	2.07	2.15	2.13	2.15	1.69	2.07	2.57	2.14	1.53	1.86	1.93
1234678F	1.60	1.28	0.79	0.97	1.97	3.67	1.50	2.73	3.12	0.74	1.10	1.04
1234789F	0.05	0.05	0.13	0.17	0.39	0.18	0.09	0.12	0.14	0.06	0.09	0.08
12346789F	6.49	3.05	0.55	0.71	1.52	14.10	4.96	4.85	10.14	2.22	3.41	2.97

Sprat									
Congener	Code in figures	B4	B5	K4	K5	T4	T5	T6	T7
2378D	66d	2.52	0.77	1.44	0.55	0.82	2.40	1.21	1.65
12378D	76d	4.25	2.04	3.06	1.22	4.62	4.16	4.78	4.09
123478D	F6d	0.60	0.32	0.73	0.85	0.65	0.09	0.72	0.67
123678D	77d	4.09	1.83	3.77	1.99	3.81	4.00	4.36	4.83
123789D	7Ed	0.60	0.05	0.13	0.88	0.31	0.08	0.32	0.24
1234678D	F7d	2.68	2.32	2.07	2.80	2.86	2.08	4.36	3.90
12346789D	FFd	8.03	14.61	6.13	22.12	8.84	4.16	14.77	6.14
2378F	66f	31.64	14.89	38.41	7.52	29.38	34.84	26.44	35.14
12378F	76f	7.40	3.09	3.77	1.03	5.30	7.35	5.20	5.58
23478F	E6f	27.08	13.63	30.87	5.01	30.88	29.40	24.90	26.40
123478F	F6f	2.05	1.83	0.82	0.88	1.29	1.60	1.69	1.54
123678F	77f	3.46	1.55	1.96	0.88	2.45	1.60	2.95	2.23
123789F	7Ef	0.03	0.04	0.07	0.66	0.02	0.04	0.04	0.05
234678F	E7f	3.31	3.37	2.22	1.03	3.40	3.04	2.67	3.53
1234678F	F7f	1.57	7.16	3.53	3.54	1.90	1.60	1.97	2.05
1234789F	FEf	0.11	0.21	0.26	1.81	0.06	0.07	0.09	0.09
12346789F	FFf	0.58	32.31	0.75	47.20	3.40	3.52	3.52	1.86

2,3,7,8-tetrachlorodibenzofuran (66f) and 2,3,4,7,8-pentachlorodibenzofuran (E6f) relative to the herring B.

On the whole, it appears that the CDD and CDF congener profiles are more varied in sprat than in herring. Additional analyses are needed to confirm the observed patterns. The cost of the analyses is high, but it would be desirable to have data from duplicate analyses of duplicate extracts. However, the variability of the profiles seems to correspond to the current state-of-the-art of the measurement of CDD and CDF in fish samples (**Fig. 4**).

Huestis et al. (1997) reported the concentrations of CDD and CDF in 4-year-old lake trout from Lake Ontario, Canada from 1977 to 1993, and of 3–9-year-old lake trout in 1988. The 'scatter' of the profiles (see Fig. 4) is approximately the same as observed in this work.

2.4 The effect of the age of the fish

The median age of the herring samples (Table 1) allowed a tentative assessment of the relationships between the concentration of the CDD and CDF congeners and the age of the fish. As can be seen from **Table 4**, the concentration of one penta- and two hexachlorodibenzo-*p*-dioxins, and the concentration of two penta-, two hexa- and one heptachlorodibenzofuran increase with the age of the fish. Considering the small number of samples and the ranges of ages within and between the samples, one cannot place too much weight on these estimates. However, most of the same congeners increased with age in the Lake Ontario trout in 1988. The concentrations of the CDD and CDF congeners in 4-year-old trout from Lake Ontario have not changed much over the period of 16 years. This may mean that the CDD and CDF concentration in fish from the Baltic may also remain fairly constant for a number of years.

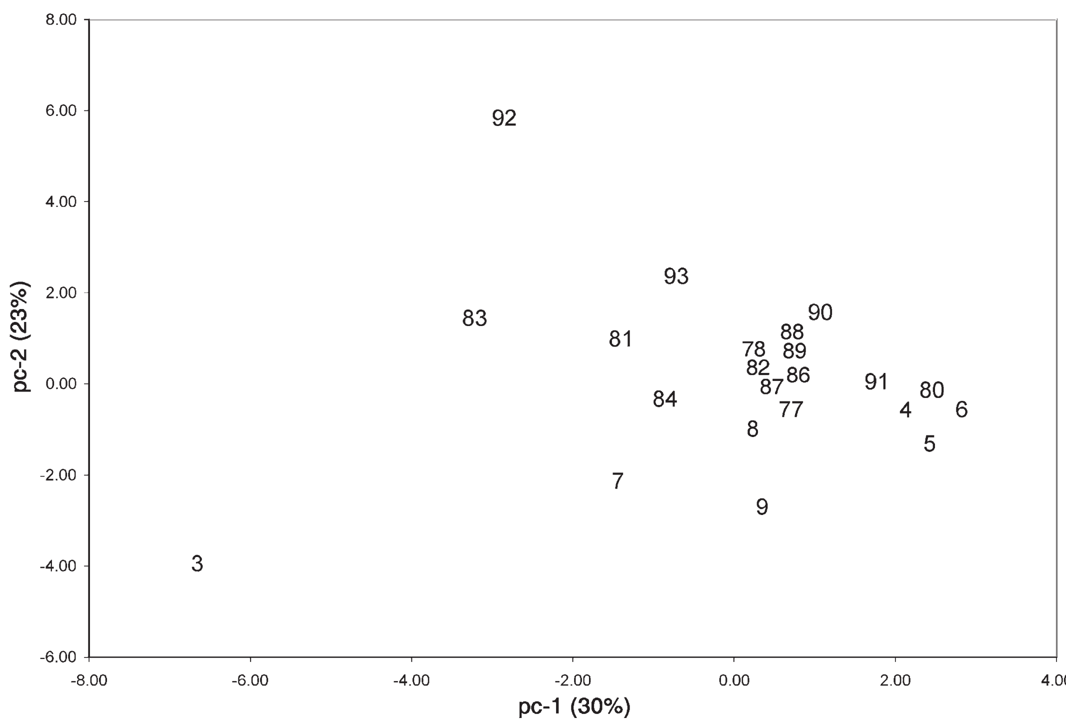

Fig. 4: The score plot of CDD and CDF congener profiles of lake trout from Lake Ontario, Canada (Huestis et al. 1997) is presented to compare with the variability of the profiles in this study (Fig. 2). Four-year-old trout were analyzed from 1977 to 1993 (two-digit codes), and 3–9 years old trout in 1988 (one-digit codes). The projection captures a similar fraction (53%) of the original variability as the projection of the profiles discussed in this paper (59%, Fig. 2)

Table 4: Time trends of CDD and CDF congeners. Slopes of linear least squares-fitted trends, expressed as percent of the starting value. B, K, and T are the slopes of concentration vs age relationships for Baltic herring from the central Baltic, middle Gulf of Finland, and western Gulf of Finland, respectively. Trends of concentrations in Lake trout from Lake Ontario (Huestis et al. 1997) are included for comparison. Four-year-old trout were sampled from 1977 to 1993 and 3–9-year-old trout in 1988

Congener	B	K	T	1977–1993 (4years)	1988 (3–9years)
2378D	1.50	–28.29	97.79	–3.13	24.50
12378D	29.54	74.16	185.71	–1.49	15.07
123478D	720.51	51.43	538.96	4.24	3.35
123678D	24.70	90.35	50.71	–1.49	13.95
123789D	–5.35	59.79	–4.55	2.83	–7.32
1234678D	7.83	–47.07	–9.24	–0.86	6.18
12346789D	23.37	–30.20	–0.72	–4.05	11.96
2378F	3.11	4.13	23.48	–1.06	9.03
12378F	18.91	33.63	94.81	–0.70	7.61
23478F	31.07	63.52	117.52	–2.17	12.30
123478F	39.62	42.32	92.65	–2.77	90.70
123678F	44.29	157.70	70.03	1.48	85.71
123789F	–7.45	0.00	22.86	13.58	32.14
234678F	24.59	31.39	37.66	–0.63	–3.84
1234678F	89.52	–36.51	–15.18	–0.26	11.08
1234789F	–20.23	–2.93	3.06		
12346789F	707.82	–48.76	–6.87	1.74	50.93

3 Concluding Remarks

The CDD and CDF congener profiles of Baltic herring from the central Baltic differ from those from the Gulf of Riga. In both groups, the profiles do not change much with the age of the fish. In comparison, the profiles of Baltic herring from the Gulf of Finland are more varied and affected by the age of the fish. The CDD and CDF profiles of herring from the western Gulf of Finland are similar to those of herring from the central Baltic. The profiles of CDD and CDF of herring from the middle Gulf of Finland are similar to those from the Gulf of Riga.

The variability of the CDD and CDF profiles in sprat is much higher than the variability of the profiles in herring. In particular, the profiles in two samples of sprat are so unusual that errors in the measurement of CDD and CDF cannot be excluded.

The information is not sufficient for the identification of the CDD and CDF sources.

The data on the relationship between CDD and CDF concentrations and the age of the fish are limited, but it appears that the concentrations of several pentachloro- and hexachloro-dibenzo-*p*-dioxins and dibenzofurans increase with the age of Baltic herring.

Monitoring of the CDD and CDF concentrations should continue, preferably in intervals of several years, but on age- and gender-stratified replicate samples, with at least duplicate extracts and duplicate measurements.

Acknowledgments. The project was financially supported both by the Ministry of Agriculture and the Ministry of Environment

References

Billo EJ (2001): Excel for Chemists. A Comprehensive Guide. Wiley-VCH, New-York, 463p

Hallikainen A, Kiviranta H (2002): EU-kalat dioksiinitutkimushanke <http://www.elintarvikevirasto.fi>

Henkelmann B, Schramm KW, Klimm C, Kettrup A (1996): Quality criteria for the Isotope dilution method with HRGC/MS. Fresenius J Anal Chem 354, 818–822

Huestis SY, Servos MR, Whittlle DM, van den Heuvel M, Dixon DG (1997): Evaluation of temporal and age-related trends of chemically and biologically generated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in Lake Ontario Lake trout, 1977 to 1993. Env Toxicol Chem 16, 154–164

Klinkhard M (2001): How contaminated are fish and fish products? Eurofish 4, 102–104

Naito W, Jin J, Kang Y-S,Yamamura M, Masunaga S, Nakanishi J (2003): Dynamics of PCDDs/DFs and coplanar-PCBs in an aquatic food chain of Tokyo Bay. Chemosphere 53, 347–362

Olsson M, Bignert A, Aune M, Haarich, M, Harms U., Korhonen M, Poutanen E, Roots O, Sapota G (2002): Organic Contaminants. In: Environment of the Baltic Sea area 1994–1998. Baltic Sea Env Proc No 82B, HELCOM, 133–140

Otsa E, Roots O, Simm M (2003): Dioxin content in the Baltic Herring and Sprat in autumn 2002. Report-Estonian Environmental Research Centre (Contract No. 397 with Ministry of Agriculture), Tallinn <http://www.agri.ee/eng>

Roots O, Zitko V (2002): Polychlorinated biphenyls patterns in grey seals (*Halichoerus grypus*). Ecological Chemistry, Thesa 11, 68–71

Roots O, Lahne R, Otsa E, Simm M, Schramm K-W (2003): Dioxins in the Baltic herring and sprat in Estonian coastal waters. Organohalogen Compounds, 62, 201–203

The Baltic Marine Environment 1999–2002 (2003). Baltic Sea Environmental Proceedings, No 87, 47 pp

Vartiainen T, Parmanne R, Hallikainen A (1997): Ympäristömyrkkyjen kertyminen silakkaan. YMPÄRISTÖ JA TERVEYS-LEHTI 7–8, 18–22

Vuorinen P, Parmanne R, Vartiainen T, Keinänen M, Kiviranta H, Kotovuori O, Halling F (2002): ICES Journal of Marine Science 59, 480–496

Wise BM, Gallagher NB (1998): PLS-Tool 2.0, Eigenvector Inc., Manson WA 98831, USA

Zitko V (2004): Chlorinated dibenzo-*p*-dioxins and dibenzofurans in an aquatic food chain. An evaluation by chemometrics <http://preprint.chemweb.com/envchem/0403001>

Zitko V (1994): Principal Component Analysis in the evaluation of environmental data. Mar Pollut Bull 28, 718–722

Zitko V (1985): 'Shorthand' numbering of chlorinated dibenzodioxins (CDD) and dibenzofurans (CDF). Chemosphere 14:165–170

Received: January 6th, 2004
Accepted: April 23rd, 2004
OnlineFirst: April 24th, 2004

Otsa E, Roots O, Simm M (2003): Level of dioxins and poly-chlorinated biphenyls (PCB), similar to dioxins, in fish in the coastal sea of Estonia in 2003 Estonian Environmental Research Center, Contract No: 133 (the work began on May 28th, 2003 and was closed on October 24th, 2003) <http://www.agri.ee/eng>

Introduction. The EC Council Regulation No 2375/2001 of November 2001, which came into force on 20.07.2002 in the Member States, has laid down the limits for dioxin level in fish which amounts to 4 pgTEQ/g in wet weight, therefore practically banning the use of the Baltic herring and salmon for human consumption. Estonia has unofficially informed the European Union of its intent to apply for a transition period for the approximation and implementation of the Council regulation No 466/2001/EC, laying down the limits for dioxin level, which has been supplemented with the EC Council Regulation No 2375/2001/EC, as laid down with Article 5.2, Part 5, Annex 1 to the Regulation No 466/2001/EC, applicable until 31.06.2006. According to the Decree of the Government of the Republic of 17.09.2002, the Ministry of Agriculture has initiated a survey to determine the dioxin level in fish caught from the Baltic Sea.

The concentration of dioxin in samples of herring and sprat, analyzed in 2002, did not exceed the limits, established by the European Union, as a rule. The only exception were the herring older than six years, caught from the open part of the Baltic Sea, where the dioxin level was 4,5 pg TEQ/g wet weight. Therefore, in 2003, more attention was paid to the dioxin level in older herring. As there is limited information concerning the dioxin level in sprats, the samples of sprat of different size and age were analyzed. There is no information available in Estonia concerning the other species of fish. Both the sea bass and pikeperch have been announced to be fish not posing any threat of dioxin in Finland; nevertheless, in 2003 the aforementioned fish was caught for analyzing and distinction of local differences purposes from the Gulf of Pärnu.

Some of the PCB compounds (in total, 12) have toxic effect, similar to dioxins. The Euro-regulation have stipulated that the respective limits for these compounds shall be established in 2004. According to published materials, the level of PCB compounds, similar to dioxins, is rather high in fish and comparable to the dioxin levels. Therefore, the concentration of these compounds was determined simultaneously with the dioxin level in the samples taken in 2003.

The terms of reference concerning the collection of fish samples and biological and chemical analysis have been met. The results of the analysis allow to assess the size/age dynamics of the level of dioxins (PCDD), furans (PCDF), and PCBs similar to dioxins (non-ortho PCB and mono-ortho PCB), in the Baltic herring and sprat, sea bass and pike perch in the coastal water of Estonia.