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PCDD/F, dl-PCB and Indicator PCBs in Whiting, Horse Mackerel and Anchovy in Black Sea in Turkey

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Abstract

Samples of three mainly consumed three edible fish species, whiting, horse mackerel and anchovy, from the Black Sea were analyzed for PCDD/Fs, dioxin-like PCBs and indicator PCBs. The results showed that PCDD/F-TEQ, dl-PCBs-TEQ and indicator PCBs concentrations ranged between 0.28-0.91 pg/g fresh weight product, 0.56-1.89 pg/g fresh weight product and 3.05-10.94 ng/g fresh weight product, respectively. It is observed that with increasing fat content of the fishes most of congeners' concentrations increased. Measured values were below the maximum residue levels indicated in the EU Commission Regulation "Setting maximum levels for certain contaminants in foodstuffs".

Keywords: Dioxin, fish, GC-HRMS, PCB, Turkey, Black Sea.

Karadeniz'den (Türkiye) Yakalanan Mezgit, İstavrit ve Hamsi Balıklarında PCDD/F, dl-PCB ve İndikatör PCB'ler

Özet

Karadeniz'den yakalanan ve sıklıkla tüketilen mezgit, istavrit, ve hamsi örnekleri PCDD/F'ler, dioksin benzeri PCB'ler ve indikatör PCB'ler açısından analiz edilmiştir. Sonuçlar PCDD/F-TEQ, dl-PCB-TEQ ve indikatör PCB'ler için sırasıyla 0.28-0.91 pg/g yaş ağırlık, 0.56-1.89 pg/g yaş ağırlık ve 3.05-10.94 ng/g yaş ağırlık olarak bulunmuştur. Balıkların yağ oranları arttıkça, bileşenlerin çoğunun konsantrasyonlarının arttığı gözlenmiştir. Tespit edilen değerler AB "Gıdalarda bulaşanların maksimum seviyelerinin belirlenmesi" mevzuatında belirlenen maksimum kalıntı seviyelerinin altındadır.

Anahtar Kelimeler: Dioksin, balık, GC-HRMS, PCB, Türkiye, Karadeniz.

Introduction

Since Turkey is surrounded by the seas, toxic contaminant levels of these seas are very important for human health, fisheries and state economics. The Black Sea provides 70-90% of fish production of Turkey and plays a very important role in our fishery industry and economy. Fish and fishery products play a significant role in the Turkish diet especially for the people in the Black Sea region. The Black Sea is a semi-enclosed sea and because of being an inner sea, the Black Sea is exposed to more pollution. The high level of pollution burden of the Black Sea is constituted by the natural effects and discharge of big rivers receiving industrial countries' waste waters. Generally around the Black Sea, there is manure and copper industries in Samsun, iron-steel in Ereğli and Karabük, cellulose-paper in Çaycuma, cement factories in Trabzon and Ünye, mine plants in Murgul and Zonguldak (Anonymous, 1998).

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are groups of toxic and persistent polychlorinated chemicals ubiquitous in environment of all industrialized countries. PCDDs/PCDFs, contrary to PCBs have never been commercially produced, they are formed as by-products of different processes, in which combustion (the incineration of hospital, hazardous and municipal waste) has major importance (Chovancova *et al*., 2005). These contaminants are lipophilic and they accumulate and bioconcentrate especially in fat tissue (Yao *et al*., 2002). Contaminated commercial chemical products, incineration of wastes and fossil fuels, fires, chlorophenol usage in wooden materials, usage of PCB liquids in electrical equipments and also wastes of paper mill industry are the most important routes of PCDD/F contamination (WHO, 1989).

PCBs have been produced as manmade to be used in industry commercially. Although there is no more PCB production, because of the PCBs' chemical and physical properties that are convenient to use in industry, they have been used widely in the past all around the world. And PCBs are still being used in electrical equipments especially in condensators and transformers as cooling liquid (WHO, 1992).

In Table 1, the daily intake of PCDD/F and indicator PCBs by food and the contribution of fish as percentage to this intake in European countries are given by Baeyens *et al*. (2007). It can be seen that intake by fish is very high and if fish is contributed to daily nutrition at high level like in Finland then fish is contributed to dioxin and PCB intake more than the other food groups.

This study is very important since these results are the first dioxin results for fish from Turkey and also the results are comparable with the Commission Regulation and all the congeners that should be analysed according to EU Regulation have been done.

Materials and Methods

Fish Samples

Whiting (*Gadus merlangus* L. 1758), anchovy (*Engraulis encrasicolus* L. 1758) and horse mackerel (*Trachurus mediterraneus* S. 1868) samples were collected in the beginning and at the end of March 2008. These fish species were chosen since they can be caught from every part of the Black Sea which is important to compare. Also they have economic importance and their biology is well known. The fish were taken from Ankara Fish Market. For the analysis 10 whiting, 26 anchovy, and 19 horse mackerel fish were pooled, homogenized both in first and second round analysis. Before analysis total lengths were measured and until analysis they were kept at -20ºC.

Standards and Solvents

All the standards were bought from Cambridge Isotope Laboratories (EDF-7999, TCDD/F-OCDD/F; EDF-8999, ^{13}C-TCDD/F-OCDD/F; EC-4986, Non-ortho PCBs; EC-4987, Mono-ortho PCBs; EC-5179, Indicator PCBs; EC-4187, ^{13}C-NO-PCBs; EC-4188, ^{13}C-MO-PCBs; EC-4058, ^{13}C-Ind-PCBs; ED-911, ^{13}C-1,2,3,4-TCDD; ED-996, ^{13}C-1,2,3,7,8,9-HxCDD; ED-907, $^{37}Cl_4$-2,3,7,8-TCDD) and all the solvents used were gas chromatography grade.

7 point calibration curve was used for qualitative and quantitative measurement of dioxins. Concentration ranges were 0.02-0.20 pg/µl, 0.05-0.50 pg/µl, 0.10-1.0 pg/µl, 0.20-2.0 pg/µl, 0.50-5.0 pg/µl, 1.00-10.0 pg/µl, 2.0-20 pg/µl for standard 1, 2, 3, 4, 5, 6, 7 respectively. 8 point calibration curve was used for determination of non-ortho, mono-ortho and indicator PCBs and the concentration range was 0.10-50 pg/µl.

^{13}C labelled internal, recovery and clean up standards were added to all calibration standards. During sample extraction, a procedure blank and a quality sample were also extracted. All the standards were injected at the same time with the samples, blank and quality sample.

Our laboratory participated in Ninth Round of Interlaboratory Comparison on Dioxins in Food 2008 coordinated by Norwegian Health Institute. Good results were found in eel sample and good z scores obtained (lab code 27).

Extraction and Clean-up

Total lengths of the fish were measured and edible parts of the fish were homogenized. 10-15 g was taken and ^{13}C labelled standards were added to

Table 1. Daily intake of indicator PCB ve PCDD/Fs' by food (Baeyens *et al*., 2007)

Country	PCB ng-PCB/day	Fish contribution (%)
Swiss	615	57
Netherlands	420	26
England	1960	
Finland	1200	58
Belgium	1690–2210	15–19
Country	PCDD/F pg-TEQ/day	Fish contribution (%)
Swiss	96	32
Netherlands	90	16
England	81.8	
Finland	46	81
Spain-Tarragona	59.6	33.7
Spain-Catalonia	95.4	31
Japan	81.9	37
Belgium	65.3	40
Germany	50	29.9
Belgium	80.5–122	34–50

the portions. 2-propanol and cyclohexane were added and then homogenized via ultra turrax and distilled water was added. And then homogenized again via ultra turrax and centrifuged. Upper layer was pipetted to the funnel filled with anhydrous sodium sulphate fitted to flask tared before. This procedure was repeated 2 more times. After that the solvent was evaporated via the rotary evaporator and then the flask was left to stay in the oven 60ºC for overnight. After determining the fat amount and before clean up step with power prep the fat was pastor pipetted to measuring cylinder with a portion of hexane.

In the power prep clean up system all the fish samples were treated with the jumbo acidic silica, silica (acidic and basic layers), alumina and carbon columns. At this step first of all mono-ortho and indicator PCBs were collected in the a fraction and the non-ortho and dioxin congeners were collected in the B fraction.

Afterwards the solvents were evaporated in the Turbo Vap system. Hexane was added to the Turbo Vap tubes and then hexane was pipetted to little tubes. These tubes were concentrated to dryness under the gentle nitrogen stream in the heating mantle. Recovery standards were added to the tubes and after vortex they were pipetted into insert vials fitted in 2 ml vials. A fraction and B fraction vials were injected in HRMS system at 10000 resolution separately.

Quantification

PCDD/PCDF/dl-PCBs were determined on a Autospec Ultima HRMS system using EPA method 1613 and 1668A analytical procedures. Samples were analyzed on an Agilent 6890 GC equipped with DB5MS column (250 µmx0.25 µmx60 m). Carrier and make up gas was helium with 99.9999% purity.

The mass spectrometer was operated in positive electron impact ionization mode using multiple ion detection. The source temperature was 260ºC, electron energy was 35 eV, trap current was 625 uA, detector voltage was 350 and ion energy was 13. PFK was used as the mass reference. Inlet temperature of the GC method used in the determination of the dioxin and non-ortho PCB congeners was 280ºC and the injection system used was splitless mode. The temperature ramps of the oven programme were adjusted in between 110ºC to 300ºC with the incrementation of 20ºC up to 200ºC, 4ºC up to 280ºC after 20 minutes holding time, 5ºC up to 300ºC after 8 minutes holding time (EPA 1613, EPA 1668A).

Results and Discussion

Mean length (cm), and fat values (%) were in between 10.7 (anchovy) - 14.4 (whiting) cm, and 0.95% (whiting) - 7.44% (horse mackerel) respectively and were given in Table 2. Total dioxin and dl-PCBs, and indicator PCBs' concentrations were found between 0.84 (whiting) and 2.80 (horse mackerel) pg TEQ/g fresh weight, and 3050 (whiting) and 10900 (horse mackerel) pg/g fresh weight, respectively. The concentrations of the congeners are given in Table 3 and total contamination graphs of the dioxins and PCBs are illustrated in Figure 1 and 2. In all fish species, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD, PCB 126, PCB 153 were determined as dominant congeners.

The concentrations are in accordance with the fat amounts of the fishes. While the lowest dioxin and PCB concentrations were determined in whiting with the lowest amount of fat, the highest dioxin and PCB

Table 2. Mean length values (cm) and fat amounts (%) of fish species

	Whiting 1 (n=10)	Whiting 2 (n=10)	Anchovy 1 (n=26)	Anchovy 2 (n=26)	H. mackerel 1 (n=19)	H. mackerel 2 (n=19)
Length (cm)	14.4±0.3	14.3±0.3	11.7±0.2	10.7±0.2	13.1±0.2	13.0±0.2
Fat (%)	1.41	0.95	4.97	6.01	7.44	7.19

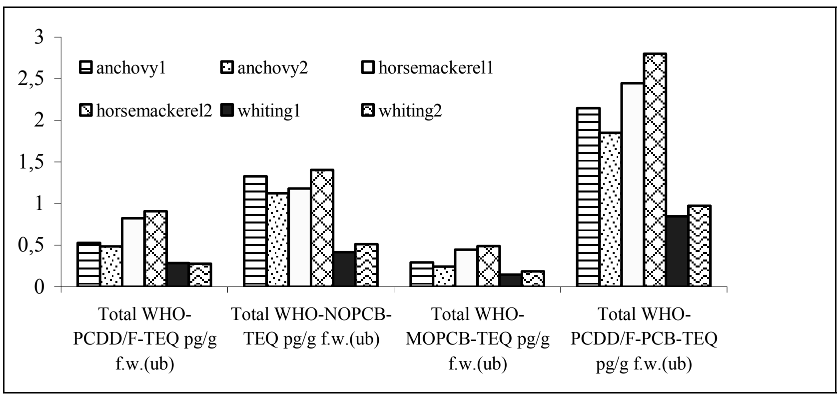

Figure 1. Total dioxin and dl-PCB concentrations (pg TEQ/g fresh weight) according to fish species.

Table 3. PCDD/F, dl-PCB ve indicator PCB concentrations in anchovy, horse mackerel, whiting (WHO-TEQ pg /g fresh weight)

CONGENER	TEQ Anchovy1	LOQ Anchovy1	TEQ Anchovy2	LOQ Anchovy2	TEQ Horsemackerel1	LOQ Horsemackerel1	TEQ Horsemackerel2	LOQ Horsemackerel2	TEQ Whiting1	LOQ Whiting1	TEQ Whiting2	LOQ Whiting2
2,3,7,8-TCDF	0.028	0.019	0.032	0.027	0.142	0.028	0.151	0.035	0.023	0.022	0.016	0.021
1,2,3,7,8-PeCDF	0.008	0.019	0.010	0.026	0.013	0.025	0.013	0.043	0.005	0.018	0.004	0.015
2,3,4,7,8-PeCDF	0.271	0.017	0.230	0.029	0.334	0.026	0.431	0.041	0.141	0.018	0.154	0.020
1,2,3,4,7,8-HxCDF	0.007	0.010	0.008	0.020	0.005	0.028	0.005	0.020	0.008	0.015	0.005	0.011
1,2,3,6,7,8-HxCDF	0.010	0.010	0.010	0.020	0.006	0.027	0.006	0.020	0.008	0.015	0.006	0.011
2,3,4,6,7,8-HxCDF	0.011	0.011	0.011	0.023	0.006	0.029	0.006	0.022	0.007	0.019	0.005	0.013
1,2,3,7,8,9-HxCDF	0.001	0.013	0.003	0.028	0.003	0.034	0.003	0.027	0.002	0.022	0.002	0.015
1,2,3,4,6,7,8-HpCDF	0.001	0.006	0.001	0.011	0.001	0.018	0.000	0.013	0.001	0.009	0.000	0.015
1,2,3,4,7,8,9-HpCDF	0.000	0.008	0.000	0.009	0.000	0.028	0.000	0.020	0.000	0.015	0.000	0.022
OCDF	0.000	0.010	0.000	0.020	0.000	0.010	0.000	0.019	0.000	0.009	0.000	0.003
2,3,7,8-TCDD	0.070	0.017	0.062	0.030	0.147	0.040	0.147	0.024	0.041	0.019	0.041	0.017
1,2,3,7,8-PeCDD	0.108	0.019	0.106	0.038	0.156	0.036	0.137	0.04	0.037	0.018	0.037	0.017
1,2,3,4,7,8-HxCDD	0.002	0.013	0.002	0.011	0.003	0.029	0.002	0.025	0.002	0.024	0.002	0.021
1,2,3,6,7,8-HxCDD	0.005	0.014	0.005	0.020	0.003	0.028	0.003	0.026	0.005	0.024	0.004	0.022
1,2,3,7,8,9-HxCDD	0.002	0.013	0.002	0.019	0.003	0.027	0.003	0.026	0.002	0.024	0.002	0.022
1,2,3,4,6,7,8-HpCDD	0.001	0.024	0.001	0.029	0.000	0.041	0.000	0.027	0.001	0.012	0.000	0.019
OCDD	0.000	0.023	0.000	0.016	0.000	0.030	0.000	0.010	0.000	0.007	0.000	0.001
PCB81	0.000	0.029	0.000	0.035	0.000	0.089	0.000	0.055	0.000	0.031	0.000	0.022
PCB77	0.002	0.020	0.003	0.027	0.002	0.065	0.002	0.043	0.001	0.023	0.001	0.020
PCB126	1.30	0.016	1.10	0.025	1.16	0.046	1.38	0.035	0.407	0.019	0.499	0.022
PCB169	0.025	0.007	0.019	0.017	0.016	0.022	0.020	0.023	0.009	0.012	0.011	0.012
PCB 123	0.000	0.688	0.000	0.587	0.000	1.17	0.000	1.58	0.000	0.416	0.000	0.551
PCB 118	0.136	0.733	0.114	0.649	0.214	1.35	0.232	1.80	0.068	0.440	0.086	0.584
PCB 114	0.013	0.533	0.012	0.483	0.022	0.923	0.028	1.20	0.008	0.398	0.010	0.525
PCB 105	0.055	0.793	0.046	0.664	0.080	1.44	0.090	1.87	0.027	0.436	0.033	0.582
PCB 167	0.001	1.11	0.001	1.26	0.001	1.13	0.001	3.42	0.000	0.858	0.000	0.692
PCB 156	0.069	0.899	0.054	1.09	0.103	0.89	0.109	2.95	0.034	0.798	0.044	0.609
PCB 157	0.018	1.22	0.014	1.44	0.024	1.24	0.026	3.96	0.008	0.885	0.011	0.728
PCB 189	0.001	0.603	0.001	0.648	0.002	1.40	0.002	1.15	0.001	0.311	0.001	0.427
PCB 028	321	0.314	355	0.454	566	0.216	585	0.146	128	0.271	98.8	0.334
PCB 052	509	0.305	478	0.367	955	0.426	961	0.774	218	0.387	197	0.361
PCB 101	1110	0.53	964	0.489	1810	0.831	1970	1.156	536	0.413	626	0.725
PCB 153	1850	0.103	1470	0.151	3740	0.308	3100	0.194	1020	0.196	1210	0.389
PCB 138	1510	0.488	1180	0.627	2730	0.388	2570	1.29	846	0.669	1030	0.564
PCB 180	600	0.186	434	0.217	1130	0.399	830	0.309	297	0.259	360	0.490
Σ WHO-PCDD/F-TEQ (ub)	0.53		0.48		0.82		0.91		0.28		0.28	
Σ WHO-NOPCB-TEQ (ub)	1.33		1.12		1.18		1.40		0.42		0.51	
Σ WHO-MOPCB-TEQ (ub)	0.29		0.24		0.45		0.49		0.15		0.18	
Σ WHO-IP (ub)	5890		4880		10900		10000		3050		3520	
Σ WHO-DL-PCB-TEQ (ub)	1.62		1.36		1.63		1.89		0.56		0.69	
Σ WHO-PCDD/F-PCB-TEQ (ub)	2.15		1.84		2.45		2.80		0.84		0.97	
Dioxin-Furan/dl-PCB ratio	0.33		0.35		0.51		0.48		0.50		0.40	

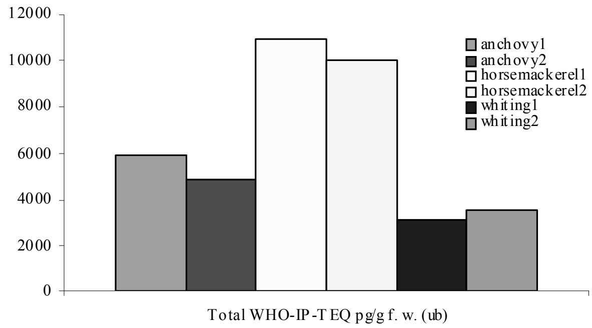

Figure 2. Total indicator PCB concentrations (pg/g fresh weight) according to fish species.

concentrations were determined in horse mackerel with the highest amount of fat. This might be explained with the fact that dioxins and PCB congeners accumulate in fatty tissue (WHO 1993).

Sediment layer has important impact on accumulation of dioxins and PCB congeners (WHO, 1993). In spite of the fact that whiting is a deep water low level of dioxin and PCB contamination might be explained in the following ways. Firstly, it can be thought that fat amount is more important factor in accumulation of these congeners. Secondly, the Black Sea sediment layer is not that much contaminated by these congeners as Fillmann *et al.* (2002) reported. In 1995, PCBs' concentrations were determined in sediment samples collected from Ukraine, Russian Federation, Turkey (Bosphorus) and Romania. PCB concentrations of the Black Sea sediments were determined lower than the samples collected from the other regions of the world (Fillmann *et al.*, 2002). Moreover, March is in spawning period of whiting fish. Some whiting fish detected with eggs. Since spawning plays an important role on excretion of dioxin and PCB congeners, low concentrations of congeners in whiting may be attributed to this situation (WHO, 1992).

Higher dioxin and PCB concentrations determined in horse mackerel and anchovy fish might be explained by the following facts; dioxins accumulate in tissues via water, sediment and food according to species, habitat and behaviours. Horse mackerel and anchovy are surface fish. Although lower concentrations of dioxins and PCBs are found in water, if there is an oil film layer on water surface, then higher concentrations can be detected. Moreover, since dioxin and PCB congeners also accumulate in phytoplanktons, zooplanktons and all other organisms found in water, contaminated fish and organisms that were being feed of horse mackerel and anchovy might also be the contamination sources (WHO, 1992). Furthermore, the spawning periods of anchovy and horse mackerel are May-September and May-July, respectively. So, they were not in spawning period during the analysis just on the contrary of whiting.

Anchovy consumes planktons especially copepods and the other little crustaceans and horse mackerel mainly consumes little fish like anchovy, sardines and little crustaceans. And also, horse mackerel fish are generally coastal fish and they never go to high sea (Atay and Bekcan, 2000). The high levels of horse mackerel's compared to anchovy can be explained by this situation.

According to Commission Regulation EC No 1881/2006 of 19 December 2006 "Setting maximum levels for certain contaminants in foodstuffs" the maximum residue limit is 4.0 pg/g fresh weight for the dioxins (WHO-PCDD/F-TEQ) and 8.0 pg/g fresh weight for the sum of dioxin and dioxin-like PCBs (WHO-PCDD/F-dl-PCBs-TEQ) in fish and other aquaculture products (except eel). Maximum residue limit is 2.0 pg/g fat for the dioxins (WHO-PCDD/F-TEQ) and 10 pg/g fat for the sum of dioxin and dioxin-like PCBs (WHO-PCDD/F-dl-PCBs-TEQ) in marine oils (fish body oil, fish liver oil and oils of other marine organisms intended for human consumption) (EC Commission 2006a). In the 2006/13/EC commission directive the maximum residue limit for sum of the dioxins (WHO-PCDD/F-TEQ) is 6 ng/kg fat for fish oil, 1.25 ng/kg whole weight for fish meal and for sum of dioxin and dioxin-like PCBs (WHO-PCDD/F-TEQ) it is indicated as 24 ng/kg fat, 4.5 ng/kg whole weight, for fish oil, and fish meal respectively (EC Commission 2006b).

Dioxin and dioxin like PCBs' concentrations of anchovy, horse mackerel and whiting were below the maximum residue levels determined for fish in "Commission regulation EC No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs" (EC Commission, 2006a). If these fish are wanted to be used to prepare fish meal the contamination values would be within the EU Regulation (EC Commission, 2006b). However, they can not be used in fish oil both for human consumption and as an ingredient for fish feed (EC Commission, 2006a-b).

Contamination levels of toxic coplanar PCBs and indicator PCBs found in this study are much lower than the concentrations determined in European anchovy, whiting and horse mackerel from the Black Sea by Tanabe *et al.* (1997a,b). Levels of indicator PCBs determined are lower than the levels found in the same fish species in the Marmara Sea by Coelhan *et al*. (2006). Contamination levels in anchovy and horse mackerel show values, in terms of total PCDD/Fs (TEQ) concentration, similar to those found by Bayarri *et al*. (2001) at the Adriatic Sea. In terms of indicator PCBs, the levels found in this study are much lower than the levels found by Bayarri *et al*. (2001). Dioxin and dl-PCBs (TEQ) contamination levels of whiting found in this study are higher, while the levels of anchovy are similar compared to the results found by Karl and Ruoff (2008). It is seen that the dioxin and PCB levels found in this study are higher than the results found in Greece by Papadopoulos *et al*. (2004) and dioxin levels are similar to the results found in Italy by Taioli *et al*. (2005).

Conclusion

In this study three fish species were selected based on human consumption patterns, and were collected from popular fishing area of the Black Sea. Fish are indicator organisms of persistent organic contaminants and are directly consumed. This study is very important to get the first results of the dioxin contaminations of Turkish coastal zone of the Black Sea. It is aimed to research different regions, water sources and fish species at different seasons in the future. And sediment pollution of the region also should be investigated to point out the source of the contamination. Although these fish are consumed too much, dietary exposure analysis could not be done due to lack of scientific data for consuming levels.

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