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Indicator polychlorinated biphenyl residues in muscle tissue of fish from Black Sea coast of Bulgaria

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Abstract. *Polychlorinated biphenyls (PCBs) are characterized by high lipophilicity and persistence in the environment and will therefore bioaccumulate and biomagnify in the food chain. PCBs were determined in muscle tissue of four fish species: goby (Neogobius melanostomus), horse mackerel (Trachurus Mediterraneus ponticus), shad (Alosa pontica pontica) and turbot (Psetta maxima maeotica). Samples were collected from Bulgarian Black Sea coast during 2007 – 2011. The PCBs were analyzed in order to examine the time trends of PCB concentrations in fish from Black Sea. The six individual PCBs congeners were determined by capillary gas chromatography system with mass spectrometry detection. PCBs were found in all fish species at concentrations ranging between 2.32 ng/g ww (wet weight) and 32.87 ng/g ww in goby and shad, respectively. PCB profiles have been found to be similar in all the fish species tested. The most abundant PCB congeners in fish species were hexa- and heptachlorinated PCBs 138, 153 and 180. PCB 153 was the dominant congener in all fish studied and were found in the range from 0.95 ng/g ww (horse mackerel 2011) to 11.67 ng/g ww (shad 2010). The sum of six indicator PCBs in all fish species did not exceed the European maximum limit of 75 ng/g ww. The levels of PCBs in fish from Bulgarian Black Sea coast were found lower than in fish species from other seas – the Aegean Sea and the Mediterranean Sea.*

Keywords: polychlorinated biphenyls, fish, Black Sea, Bulgaria

Introduction

Polychlorinated biphenyls (PCBs) are lipophilic and persistent compounds and were listed as persistent organic pollutants (POPs) in the Stockholm Convention (UNEP, 2001). They are transported by air, water and migratory species and accumulate in terrestrial and aquatic ecosystems. The properties of resistance to degradation and low volatility of PCBs lead to their ubiquitous presence as persistent environmental pollutants. Many investigations have previously proved that contamination of PCBs can pose a threat for living organisms by inducing a wide range of adverse effects (Fisk et al., 2001; Falandysz et al., 2004). Because of possible impacts on human health and the environment, the use and production of PCBs have been banned since the 1980s (Smith and Gangoli, 2002; Beyer and Biziuk, 2009). PCBs are usually divided into two groups according to their toxicological properties: dioxin-like PCBs (dl-PCBs) and non dioxin-like PCBs (ndl-PCBs). The sum of the six PCBs (IUPAC № 28, 52, 101, 138, 153 and 180) comprises about half of the amount of total non dioxin-like PCBs present in feed and food (EFSA, 2010). That sum is considered as an appropriate marker for occurrence and human exposure of ndl-PCB and therefore European Commission has set a maximum level in fish 75 ng/g fresh weight (EU, 2011). World Health Organization (WHO) defined the set of six PCBs (IUPAC No 28, 52, 101, 138, 153, 180) as important for evaluating the risk to human health and they are called indicator PCBs (I-PCBs).

During recent years, the bioaccumulation process of PCBs and other persistent organochlorine pollutants (POPs) in marine biota has been intensively studied in relation to biological variables and spatial plus time variations ((Voorspoels et al., 2004; Covaci et al., 2006; Szlinder-Richert et al., 2009). The fish species are an appropriate indicator for the environmental pollution monitoring because they accumulate pollutants directly from water and also through their diet (Storelli and Marcotrigiano, 2006; Serrano et al., 2008; Tanabe et al., 1997; Weijs et al., 2010).

This article presents the results from the investigations about the levels of six PCBs (IUPAC № 28, 52,101, 138,153 and 180) in four fish species collected from the Black Sea during 2007 – 2011. The fish species were selected according to their characteristic feeding behavior: goby *(Neogobius melanostomus),* horse mackerel *(Trachurus Mediterraneus ponticus)*, shad *(Alosa pontica pontica)* and turbot *(Psetta maxima maeotica)*. The goby was chosen because it is very common in Black Sea, and therefore may be a good tool for monitoring. It is nonmigratory species and feed mainly with benthic organisms. Horse mackerel is pelagic, summer spawning fish with Mediterranean origin, it feeds on different small fish, crustaceans and worms. Shad is pelagic fish, it migrates to middle reaches of large rivers for spawning. It feeds on a wide variety of zooplankton and small fish. The turbot is a predator species that lives on sandy, rocky or mixed bottoms. Adults feed mainly on other bottom-living fish (sand eels, gobies, etc.) and to a lesser extent, on crustaceans and bivalves. The goby and the turbot could be considered the key species for monitoring of the Black Sea environment.

The aim of the present study was to examine the time trends of PCB levels in fish species from Black Sea during the period from 2007 to 2011.

Materials and methods

Sampling and sample preparation

Samples were collected from Black Sea, in area of cape

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Kaliakra, Bulgaria from 2007 to 2011. The sampling campaigns took place during September – November in every year. Four different edible fish species (goby, horse mackerel, shad and turbot) were caught by local professional fishermen by net. The samples were transferred immediately to the laboratory in foam boxes filled with ice and were stored in a freezer (-20°C). Each sample of selected fish species is composed of edible tissues of several individuals. The samples of goby and horse mackerel were comprised 15 – 20 individuals and samples of shad and turbot contained between 5 and 8 individuals. The length and weight of each specimen were measured and they were rinsed with distilled water to remove sand and impurities. Fish samples were filleted, the head and internal organs were removed.

Analytical method

Twenty grams of homogenized fish tissue were mixed with 100 g of anhydrous sodium sulfate and was extracted with hexane / dichloromethane (3/1, v/v) in Soxhlet extractor for 16 h. Each sample was spiked with internal standards PCB 30 and PCB 204. The solvent was carefully evaporated and the lipid content was determined gravimetrically of an aliquot of the extract (1/5th). The extract was cleaned-up on a glass column (10x250 mm) packed with 2 g neutral silica, 4 g acid silica and 2 g neutral silica (Merck KGaA, Darmstadt, Germany). PCBs were eluted with 50 ml n-hexane (Sigma-Aldrich Chemie, Taufkirchen, Germany). The eluates were concentrated to near dryness and reconstituted in 0.5 ml in hexane. One micro liter of purified extract was injected into GC/MS.

Gas chromatographic analysis of PCBs were carried out by GC FOCUS (Thermo Electron Corporation, Austin, Texas, USA) using POLARIS Q Ion Trap mass spectrometer and equipped with an AI 3000 autosampler. Experimental MS parameters are the following: Ion source and transfer line temperatures were 220°C and 250°C, respectively. The splitless Injector temperature was 250°C. The PCBs experimental temperature program was 90°C for 1 min, then programmed 30°C/min to 180°C, 2°C/min to 270°C, 30°C/min to 290°C with a final hold for 3.0 min. Splitless injections of 1 µl were performed using a TR-5MS capillary column (Bellefonte, PA, USA) with a length of 30 m, 0.25 mm ID and a film thickness of 0.25 µm. Helium was applied as carrier gas at a flow of 1 ml/min.

Pure reference standard solution PCB Mix 20 (Dr. Ehrenstorfer Laboratory, Augsburg, Germany) was used for instrument calibration, recovery determination and quantification of compounds. The PCB mix includes the set of six indicator PCBs IUPAC No 28, 52, 101, 138, 153, 180 (I-PCBs). The limits of quantification (LOQ) varied for individual PCBs from 0.02 to 0.1 ng/g ww. Each sample was analyzed three times and was taken an average of the results obtained.

Quality control

The quality control was performed by regular analyses of procedural blanks and certified reference material BB350 (PCBs in Fish oil) – Institute for Reference Materials and Measurements, European Commission. PCBs recovery from certified reference material varied in the range 85 – 106% for individual congeners. Procedural blanks and a spiked sample with standards were analyzed between each 5 samples to monitor possible laboratory contamination. Blanks did not contain traces of contaminants.

Statistical analysis

The statistical analysis of the data was based on the comparison of average values by a t-test and a significance level of $p<0.05$ was used. All statistical tests were performed using SPSS 16 software.

Results and discussion

The six PCB congeners (IUPAC № 28, 52, 101, 138, 153 and 180) have been selected by the International Council for the Exploration of the Sea (ICES) for their abundance in environmental samples and have also been recommended by the European Union as indicators of PCB contamination (Giandomenico et al., 2013). Our previous studies showed that the most abundant PCB congeners in fish species were the indicator PCBs constituting more than 80% of the total amount of PCBs (Georgieva et al., 2012). PCB 153 was the dominant congener in all investigated species at concentrations ranging between 0.95 ng/g ww (wet weight) (horse mackerel 2011) and 11.67 ng/g ww (shad 2010) (Figure 1).

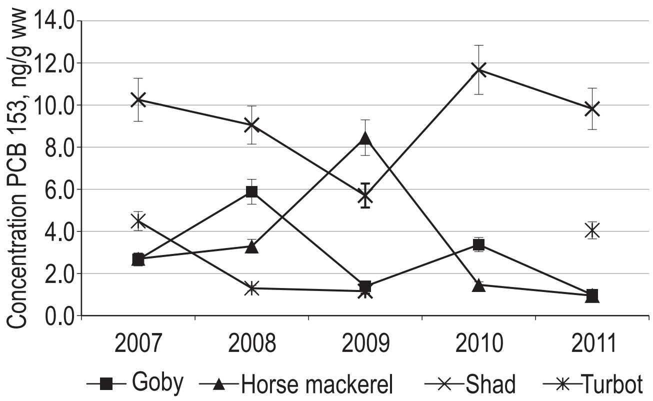

Figure 1. Concentrations of PCB 153 (annual mean, ng/g ww) in fish samples from Black Sea

In horse mackerel samples, the concentrations of PCB 153 showed high variability but during the period under study, they declined to about 0.95 ng/g ww. In goby samples, the concentrations of PCBs 153 varied between 0.99 (2011) and 2.66 ng/g ww in 2007. In goby, turbot and horse mackerel samples, a decrease in the concentrations of PCBs 153 in 2010 and 2011 was observed. In shad samples was observed increase of PCB 153 concentration.

The relative proportions of Indicator PCB congeners were determined as the concentration ratios between individual congeners and total PCB content (Table 1). PCB pattern found in fish showed a predominance of the hexa- and heptachlorinated PCBs 138, 153 and180. The amount of PCB 138, 153 and 180 represents more than 76% (in goby samples) of the total content of the Indicator PCBs and can be used to evaluate the temporal trends throughout the study period. In goby samples, hexachlorobiphenyl PCB 153 represent over 32% of the total PCBs until in horse mackerel it represents only from 26.0% to 38.6%. The heptachlorobiphenyl PCB 180 represent from 9.1% in shad to 33.2% in horse mackerel.

Habitat, physiologic factors and geographical origin are important aspects that explain accumulations and pollutants elimination of PCBs in marine organisms (Perugini et al., 2004; Bodiguel et al., 2009). Turbot samples showed similar percentage distribution of PCB 138, 153 and 180 (average 29.2%, 34.8% and 19.3%, respectively) as opposed to other species investigated. In agreement with our data, these congeners 153, 138 and 180 are the prevalent ones usually reported in marine samples (Bayarri et al., 2001; Falandysz et al., 2004; Storelli et al., 2006).

Table 1. Indicator PCBs (% of total I-PCBs) and lipid content (mean values) determined in fish species collected from the Black Sea

Species/Year	n	Lipids, %	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180
Goby								
2007	4	3.1	10.9	0.0	10.0	29.2	34.0	16.0
2008	5	2.7	7.2	6.0	7.7	27.1	41.7	10.2
2009	4	1.2	14.8	0.0	9.6	24.7	31.7	19.2
2010	3	1.1	20.1	0.0	0.0	14.3	45.5	20.1
2011	5	0.7	0.0	0.0	0.0	28.0	42.7	29.3
Horse mackerel								
2007	4	10.8	13.5	13.1	10.7	23.2	26.4	13.1
2008	5	7.4	12.4	12.6	12.7	23.4	26.0	12.9
2009	3	18.0	6.5	7.0	9.9	29.4	32.7	14.5
2010	4	11.5	37.3	0.0	0.0	24.1	38.6	0.0
2011	5	11.7	35.5	0.0	0.0	0.0	31.3	33.2
Shad								
2007	5	14.4	5.7	7.9	10.8	29.8	34.3	11.4
2008	5	23.6	8.0	9.5	14.0	28.6	30.9	9.1
2009	4	23.5	13.7	11.6	11.1	18.9	30.0	14.7
2010	4	18.1	9.5	7.6	9.5	23.1	35.5	14.9
2011	5	25.3	11.7	0.0	17.3	27.1	30.6	13.3
Turbot*								
2007	3	2.2	0.0	7.5	9.3	34.9	32.2	16.1
2008	3	0.9	0.0	0.0	12.4	31.1	34.2	22.4
2009	3	1.7	17.9	0.0	10.0	24.6	27.7	19.8
2011	3	1.5	0.0	0.0	9.5	26.4	45.1	19.0

n – number of samples, *Turbot – not sampled in 2010

The sum of six Indicator PCBs of the annual mean concentrations in fish species are shown on Figure 2. The lowest level of the I-PCBs was found in goby samples 2011 (2.32 ng/g ww) and the highest level – in shad 2010 (32.87 ng/g ww).

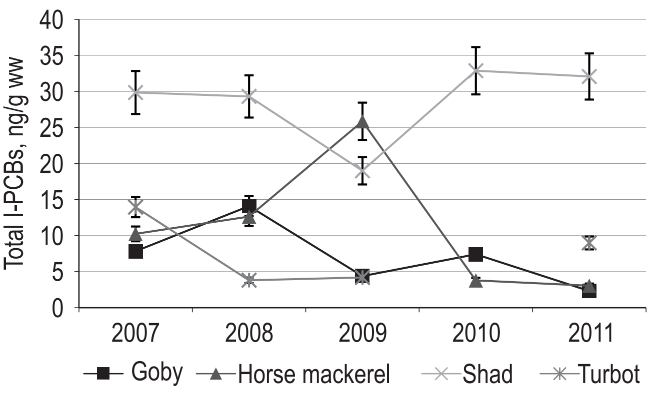

Figure 2. Sum of Indicator PCBs (ng/g ww) determined in fish collected from the Black Sea

The residual levels of PCBs varied significantly among the fish species studied. The differences in concentrations of PCBs may be attributable to various factors such as the nature of the habitat, feeding preferences and lipid contents. The highest level of I-PCBs (for all period of study) was found in order: shad > horse mackerel > goby = turbot. The higher levels of PCBs in shad compared to other fish species may be due to its higher lipid content. The high contamination levels detected in this species may also be ascribed to their feeding habit - shad is a top predator, and hence the dietary uptake is an important route of entry for organochlorines in this species (Loizeau et al., 2001; Naso et al., 2005). The statistical test indicated that the levels of PCBs found in shad were significantly higher than those detected in goby and turbot ($p<0.05$, $t=0.001$). The experimental results showed no significant differences between mean annual sum of I-PCBs in goby and turbot. The European Union has recommended a maximum level of 75 ng/g wet weight, calculated as the sum of the six I-PCBs in muscle meat of fish (European Commission, 2011). Our results for I-PCBs in all fish species did not exceed this limit.

The comparison of PCB concentrations between the present study and other surveys is very complicated due to the large variation of measured congeners (specific congener and number of congeners). The experimental results indicated that PCB contamination of fish from the Bulgarian Black Sea coast was found lower than the results from the Italian Adriatic Sea, where average PCB concentrations were found in the range from 50.08 to 88.17 ng/g ww (Stefanelli et al., 2004). The levels of indicator PCBs found in present study were lower than the results of eels from Thames River – 44 ng/g fresh weight, reported by Jürgens et al. (2015) and lower than concentration in golden grey mullet from Tunis Bay – over 52 ng/g ww (Masmoudi et al., 2007). Our results were found comparable to PCB concentrations in sardine and seabream (from 14.64 to 23.79 ng/g ww) collected from Spanish Atlantic Coast

(Bordajandi et al., 2006). The low levels of PCBs observed in fish tissues correspond with the fact that no industrial production of PCBs took place in Bulgaria.

Conclusion

This study provides data on levels of PCBs contamination in fish species from the Bulgarian Black Sea coast. PCB pattern found in fish showed a predominance of the hexa- and heptachlorinated PCBs 138, 153 and180. PCB 153 was the dominant congener in all investigated species. The highest total I-PCBs level was found in shad (mean 28.62 ng/g ww) and the lowest in goby (mean 7.21 ng/g ww). Levels of PCBs in goby, horse mackerel, shad and turbot were found lower than levels measured in the similar fish species from other investigations. Our results for sum of I-PCBs in all fish species did not exceed the European maximum limit of 75 ng/g ww.

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The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

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Mauff G, Pulverer G, Operkuch W, Hummel K and Hidden C, 1995. C3-variants and diverse phenotypes of unconverted and converted C3. In: Provides of the Biological Fluids (ed. H. Peters), vol. 22, 143-165, Pergamon Press. Oxford, UK.

Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows,IXth International Conference on Production Diseases in Farm Animals, September 11–14, Berlin, Germany.

Thesis:

Hristova D, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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