Bulgarian Chemical Communications, Volume 51, Special Issue D (pp. 227 – 232) 2019

Residual levels and tissue distribution of polychlorinated biphenyls (PCBs) in fish from the Danube River, Bulgaria

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Received October 30, 2018; Revised January 4, 2019

Polychlorinated biphenyls (PCBs) are widespread synthetic chemicals, which tend to accumulate in aquatic organisms, due to their lipophilic properties. Fish species accumulate persistent chemicals particularly in the fatty tissues and therefore they are widely used to assess pollution of the aquatic ecosystems. The PCBs occurrence was evaluated in tissues of six fish species: common carp *(Cyprinus carpio)*, catfish *(Silurus glanis)*, common nase *(Chondrostoma nasus)*, beluga *(Vimba vimba)*, bream *(Abramis brama)* and pike-perch *(Sander lucioperca)*. Sampling was carried out in spring and autumn 2015 in the Danube River, near Silistra, Bulgaria.

The aim of the study was to examine PCB concentrations in muscle and liver of fish from the Danube River in order to evaluate toxicological levels of PCB in different tissues. Six indicator and six dioxin-like PCBs were determined by capillary gas chromatography system with mass spectrometry detection.

In muscle tissues analyzed the sum of Indicator PCBs ranged from 2.51 to 10.67 ng/g wet weight in pike-perch and catfish, respectively, and did not exceed the European maximum level. The dominant congeners in all samples analyzed were PCB138 and PCB153. When concentration was calculated on a wet weight basis, tissue distribution of PCBs showed higher levels in muscle than in liver of carp and catfish. On the contrary, in common nase higher concentration of PCBs in liver than in muscle tissue were measured. On a lipid normalized basis, sum PCBs in the liver of all fish studied was higher compared to levels in muscle tissue.

The levels of dioxin-like PCBs measured varied from 0.52 to 3.76 ng/g ww. The results were used for calculation of Toxic equivalent quotient (TEQ) in order to assess the human health risk via consumption of fish. TEQs of dl-PCBs were calculated in the range 0.016 - 0.186 pg TEQ/g ww and did not exceed the EC limit of 3 pg TEQ/g ww.

***Keywords**: PCBs; fish; Danube River; Bulgaria*

INTRODUCTION

The Danube River was listed as one of the world's top 10 rivers at risk in report of World Wildlife Fund (WWF), 2007 [1]. One of the most significant factors affecting the water quality of the Danube River basin is the hazardous substances pollution [2]. Historically, the Danube has been home to 103 fish species, including seven fish species found nowhere else in the world, 88 freshwater mollusks and over 18 amphibian species [3].

Polychlorinated biphenyls (PCBs) are toxic contaminants of concern because they tend to disrupt the endocrine system, cause neurobehavioral deficits and possibly induce cancer [4]. Fish and other seafood can be used as indicator species for the evaluation of the environment pollution, because they accumulate organochlorinated compounds from water, including PCBs.

The persistent organochlorine substances have high solubility in fatty tissues, which may lead to higher concentrations in some fatty foods (dairy products, fish, meat). Mostly human exposure is through ingestion of contaminated food. Organochlorine compounds, including PCBs, accumulate in fatty tissue with typical half-lives of about 7 years by humans [5]. Several studies have investigated organochlorine pollution of fish species and the dietary exposure of general population to toxic organic chemicals, such as polychlorinated biphenyls, through fish consumption [6, 7, 8].

The most commonly PCBs found in food of animal origin are PCB 28, 52, 101, 138, 153 and 180 (2,4,4'-Trichlorobiphenyl, 2,2',5,5'-Tetrachlorobiphenyl, 2,2',4,5,5'-Pentachlorobiphenyl, 2,2',3,4,4',5'-Hexachlorobiphenyl, 2,2',4,4',5,5'-Hexachlorobiphenyl, 2,2',3,4,4',5,5'-Heptachlorobiphenyl, respectively) which account for approximately half of total non dioxin-like PCB congeners. These six congeners are called indicator PCBs (I-PCBs) [9]. The mono-ortho and non-ortho PCBs have toxicological properties similar to those of the highly toxic compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and are defined as ‘‘dioxin-like’’ (dl-PCBs). Each congener of dl-PCBs exhibits a different level of toxicity. The concept of ‘‘toxic equivalency factors’’ (TEFs) has been developed to facilitate evaluating of human health risk and

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regulatory control [10]. TEF represents the individual toxic potency of each dl-PCB compound compared to 2,3,7,8-TCDD, which is considered as the reference congener. The total WHO toxic-equivalency quotient (WHO-TEQ) concentration of a food product (including fish) can be calculated as a sum of the concentration of each dl-PCB congener multiplied by its TEF [10]. Commission Regulation (EU) No 1259/2011 has set maximum levels for I-PCBs and dl-PCBs in various food groups, including fish and fish liver [11].

The aim of the present study was to examine PCB concentrations in muscle and liver of freshwater fish from the Danube River in order to evaluate toxicological levels of PCB in different tissues.

EXPERIMENTAL

Sampling

Silistra is a hurbour in northeastern Bulgaria. The city lies on the southern coast of the lower Danube river and is also the part of the Romanian border. Fish species were sampled in the area of Silistra, Bulgaria from the Danube River: common carp *(Cyprinus carpio)*, catfish *(Silurus glanis)*, pike-perch *(Sander lucioperca)*, common nase *(Chondrostoma nasus)*, beluga *(Vimba vimba)* and bream *(Abramis brama).* Samples were caught by local professional fishermen in Spring 2015 and in Autumn 2015. All fish samples were transported into the laboratory in foam boxes filled with ice, where they were measured (cm), and weighted (g), before separating the muscles and livers. In the laboratory from each fish species a pooled sample of muscle tissue and livers of individuals was compiled by filleting and dissecting. The fish tissue from several individuals was homogenized by a blender (about 300 grams muscle tissue and about 70 grams liver tissue).

Analytical method

The extraction and clean-up of the samples, and quantitative determinations of PCBs in fish were conducted according to the method previously described by Stancheva *et al.* [12]. Fish muscles and livers (ten grams) were extracted with a mixture of dichloromethane: hexane in Soxhlet Extractor. Total lipid content was determined gravimetrically on an aliquot of each lipid extract of fish analyzed for PCBs. The extracts were concentrated by a rotary evaporator, cleaned up on a multilayer column - neutral silica and acidified silica (Merck KGaA, Darmstadt, Germany) and were eluted with hexane. The eluates were concentrated to near dryness and reconstituted in 0.5 ml in hexane. One microliter of purified extract was injected into GC/MS.

Gas chromatographic analyses of PCBs were carried out by GC FOCUS (Thermo Electron Corporation, Austin, Texas, USA) using POLARIS Q Ion Trap mass spectrometer. The gas chromatography oven was programmed as follows: 90 ºC for 1 min, then programmed 30 ºC/min to 180 ºC, 2º C/min to 270 ºC, 30 ºC/min to 290 ºC with a final hold for 3.0 min. The PCB chromatographic separation was achieved by splitless injections of 1 µl on 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 - a flow rate of 1 ml/min.

For instrument calibration, recovery determination and quantification of compounds were used pure reference standard solutions (PCB Mix 20 - Dr. Ehrenstorfer Laboratory, Augsburg, Germany). Measured compounds: six Indicator PCBs (I-PCBs IUPAC No. 28, 52, 101, 138, 153 and 180) and six dioxin-like PCBs (non-ortho PCBs 77, 126, 169 and mono-ortho PCBs 105, 118, 156). Each sample was analyzed three times and was taken an average of the results obtained.

Quality control

Quality Control procedures were implemented for each 5 samples, including procedural blanks, analysis of replicate samples, use of recovery surrogates, analysis of certified reference material BB350 (PCBs in Fish oil – Institute for Reference Materials and Measurements, European Commission). Recovery of PCBs from certified reference material varied in the range 85 -109% for individual congeners. Blanks did not contain traces of contaminants. The detection limits for individual PCBs ranged between 0.2 and 0.5 ng/g ww.

Fish weight and lengths were measured individually throughout the experiment. Fulton's condition factor (CF), used as an index of fish overall fitness, was calculated for each fish using [13]:

$$CF = (W/L^3) \times 100$$

whereby W is fish weight in grams and L is individual fish length in cm.

Statistical analysis

The statistical analysis of the data was based on the comparison of average values by a t-test. A significance was set at level of $p<0.05$. When the p value was lower than 0.05, it was considered statistically significant. Concentrations below LODs were considered as zero for all statistical analyses. All statistical tests were performed using SPSS 16 software.

RESULTS AND DISCUSSION

Biometry data, condition factor (CF) and total lipid content of fish species are presented in Table 1. Muscle tissue presented higher lipid content (0.6-14.1%) than liver - lipid content in the range 1.8–6.9% (Table 1). The value of CF is influenced by age of fish, sex, season, feeding preferences and amount of fat reserve. The condition factor provides information on the variation of fish physiological status and can be influenced by environmental contaminants [13].

Indicator PCB concentrations

Residue concentrations of individual PCBs congeners in each fish species are provided in Table 2 and Table 3.

Table 1 Biometry data, Condition factor (CF) and lipid content (%), in muscle and liver according to fish species

Fish	n	Weight, g	Length, cm	CF	Lipids (%)	
					muscle	liver
Cyprinus carpio	3	1717±65	49±12	1.5	9.8±0.4	1.8±0.2
Silurus glanis	3	1378±72	48±11	1.3	6.2±0.4	2.2±0.2
Chondrostoma nasus	3	1788±84	52±13	1.3	9.4±0.3	6.9±0.4
Vimba vimba	6	660±35	38±6	1.2	14.1±0.5	-
Abramis brama	5	660±26	37±7	1.3	6.3±0.3	-
Sander lucioperca	5	663±43	40±11	1.0	0.6±0.1	-

Table 2. Concentration levels of Indicator PCBs (ng/g wet weight) determined in fish species from Danube River

I-PCB	**common carp**	**catfish**	**common nase**	**beluga**	**bream**	**pike-perch**
PCB 28+31	0.72± 0.06	1.08± 0.08	0.73± 0.06	1.38± 0.11	1.62± 0.13	0.46± 0.03
PCB 52	1.07± 0.09	1.35± 0.11	1.09± 0.07	1.30± 0.10	1.36± 0.10	0.39± 0.03
PCB 101	0.79± 0.08	0.91± 0.08	0.72± 0.06	nd	nd	nd
PCB 153	2.40± 0.19	3.32± 0.22	3.18± 0.24	2.12± 0.18	1.97± 0.16	0.96± 0.08
PCB 138	2.08± 0.18	2.94± 0.21	2.16± 0.19	1.73± 0.15	1.42± 0.12	0.70± 0.06
PCB 180	1.03± 0.08	1.07± 0.08	0.80± 0.08	nd	nd	nd
Sum I-PCBs, ng/g ww	8.09	10.67	8.68	6.53	6.37	2.51

nd – not detected

The highest concentration on wet weight bases were observed in catfish (10.67 ng/g ww like Sum of Indicator PCBs) and the lowest in pike-perch (2.51 ng/g ww). The high levels of PCBs in catfish may be due to its feeding preference and nature of the habitat. European catfish (*Silurus glanis)* is the largest freshwater fish of Europe and is historically known to take a wide range of food items. European catfish is bottom feeder and is known to feed on many anadromous species, worms, insects, crustaceans, fish, frogs, mice, rats and some aquatic birds [14]. The European Legislation has recommended a maximum level of 75 ng/g wet weight (as the sum of the six I-PCBs) in muscle meat of fish [11]. Our results for I-PCBs in all species analyzed did not exceed the permissible limit.

The levels of I-PCBs in common carp and common nase were found 8.06 and 8.69 ng/g ww, respectively. These species are bottom feeders, do not migrate extensively, have long life spans and reproduce rapidly [15]. Bottom-feeding fish ingest and accumulate PCBs from sediment. For these reasons, carp and nase are a good species to assess bioaccumulation of organic pollutants.

Jankoviç S. *et al.* [16] reported results for PCBs level in ten freshwater fish species from Danube River, Serbia: I-PCBs content in fish in 2006 was in the range 14.4–107.2 ng/g ww, with median value of 42.9 ng/g. Our results for sum of indicator PCBs in catfish were about four times lower than reported by Jankoviç, S. *et al.* [16].

Our previous study found low levels of I-PCBs in freshwater fish gibel carp *(Carassius gibelio)*, roach *(Rutilus rutilus)* and perch *(Perca fluviatilis)* from Varna Lake, Burgas Lake and Mandra Lake, Bulgaria (1.60, 1.06 and 1.06 ng/g ww for Sum of I-PCBs) [17]. These findings showed the concentrations of PCBs in wild fish from the Danube River were higher than the levels in fish species from some lakes in Bulgaria.

Hexachlorinated PCB 153 accounted for the greatest proportion of I-PCBs (mean 32.6%), followed in order by PCB 138 (25.7%) and pentachlorinated PCB105 (15.3%). The percent distribution of I-PCB was in accordance with reports for cod from Baltic Sea [18], shark from Mediterranean [19] and in tissue of wild carp from eastern Lake Erie [15].

The congener profile in fish tissue depends on metabolizatione degree. The half-life of congeners varies from months to several years. The high chlorinated congeners (xexa- and hepta-chlorbiphenyls) metabolically degrade slowly and can accumulate higher levels in fish tissue [5, 20]. Thus, the profile of congeners detected may differ from the profile of the initially released PCBs in the environment [5].

Dioxin-like PCB concentrations

All the samples analyzed, with the exception of bream, were positive to the presence of dioxine-like PCBs, as shown in Table 3. The levels of dl-PCBs measured varied from 0.52 (pike-perch) to 3.76 ng/g ww (catfish).

The statistical test indicated that the levels of PCBs found in catfish were significantly higher than those detected in pike-perch ($p<0.05$, $t=0.001$). Concentrations of the most toxic non-ortho congeners (PCBs 77, 126, 156 and 169) were found below LOD for most of the samples.

The results were used for calculation of Toxic Equivalence (TEQ) in order to assess the human health risk via consumption of fish – Table 4. TEQ values were calculated by multiplying the congener concentrations measured in each sample with its TEF, expressed in pg WHO-TEQ/g ww [10].

Fish and seafood has been shown to be the main contributors to total toxic equivalent (TEQ) intake from PCBs for humans [6]. The TEQ values of dioxin-like PCBs in the fish studied are presented in Table 4. The means of total TEQ ranged from 0.016 to 0.186 pgTEQ-WHO/g wet weight (for pike-perch and catfish, respectively). The highest TEQ value observed in catfish may be due to its high capacity to accumulate dioxin-like PCB congeners. This results were comparable to TEQ values for fish from the Black Sea in our recent study, Stancheva *et al*. [21]. The European Commission has set a limit of 3.0 pg TEQ/g wet weight in muscle of fish for the sum of dioxin-like PCBs [11]. Our results for TEQ values of the six dl-PCBs for all fish studied did not exceed this limit.

Table 3. Concentration levels of dl- PCBs (ng/g wet weight) determined in fish species from Danube River

dl-PCB	**common carp**	**catfish**	**common nase**	**beluga**	**bream**	**pike-perch**
PCB 77	nd	1.04	1.04	nd	nd	nd
PCB 105	0.91± 0.07	1.29± 0.10	0.97± 0.08	1.27± 0.09	nd	0.52± 0.05
PCB 118	1.47± 0.11	1.43± 0.12	1.19± 0.10	nd	nd	nd
PCB 126	nd	nd	nd	nd	nd	nd
PCB 156	nd	nd	nd	nd	nd	nd
PCB 169	nd	nd	nd	nd	nd	nd
Sum dl-PCBs, ng/g ww	2.38	3.76	3.20	1.27	nd	0.52

nd – not detected

Table 4 Concentrations of dl-PCBs expressed in pg WHO-TEQ/g ww and total TEQ values estimated for fish studied

dl-PCBs UPAC №	**TEF, WHO 2005**	**common carp**	**catfish**	**common nase**	**beluga**	**bream**	**pike-perch**
PCB 77	0.0001	nd	0.104	0.104	nd	nd	nd
PCB 105	0.00003	0.027	0.039	0.029	0.038	nd	0.016
PCB 118	0.00003	0.044	0.043	0.036	nd	nd	nd
PCB 126	0.1	nd	nd	nd	nd	nd	nd
PCB 156	0.00003	nd	nd	nd	nd	nd	nd
PCB 169	0.03	nd	nd	nd	nd	nd	nd
Total TEQ, pg WHO-TEQ/g ww		0.071	0.186	0.169	0.038	nd	0.016

nd – not detected

Tissue distribution of PCBs

The distribution of Indicator PCBs was investigated in the muscles and liver of the three fish species (common carp, catfish and common nase) collected from the Danube River. Distribution of I-PCBs and dl-PCBs in different fish tissues was summarized in Fig. 1. The PCB concentrations reported for muscle and liver are expressed on the basis of lipid content because of the lipophilic properties of PCBs. Muscle tissues contain higher amount of lipids (6.9 - 9.8% lipid) than liver samples (1.8 – 6.9% lipid).

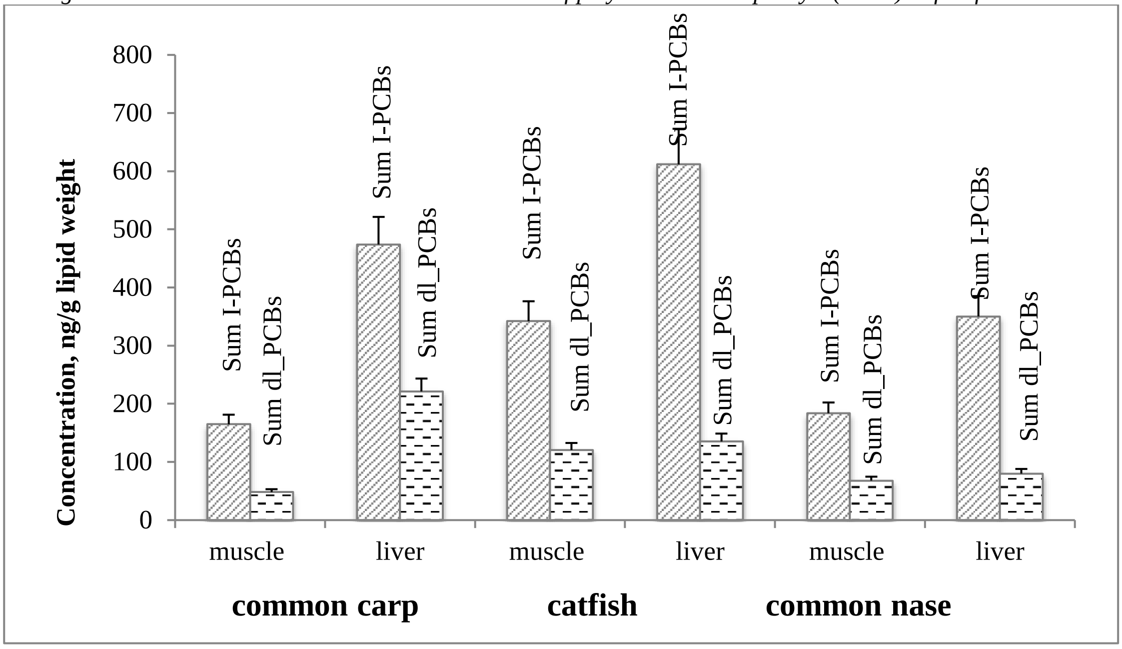

Figure 1 Sum of I-PCBs and Sum of dl-PCBs, ng/g lipids in muscle and liver of fish

The highest levels of I-PCBs were found in catfish: 342 ng/g lw in the muscle and 612 ng/g lw in the liver (Figure 1). Hexachlorine substituents (PCB 153 and 138) made the greatest contribution to the accumulation of PCBs in both tissues. The PCBs levels in all fish species studied revealed that liver tissue contains higher concentrations than muscles samples. Our results were in accordance with several previous studies investigated the presence of PCBs in various fish tissues. Solé *et al*. [22] reported higher concentrations of PCBs in tissues with greater lipid content (liver, gills and muscles) in fish from the Mediterranean. Higher concentrations of PCBs in liver than in muscle of fish from Huston River were observed by Monosson *et al.* [23]. Sapozhnikova *et al.* [24] report higher concentrations of PCBs in liver than in muscles in fish from the Salton Sea.

Stefanelli *et al.* [25] investigated the presence of PCBs in tissues of swordfish (*Xiphias gladius*) from Mediterranean Sea and Azores islands. They reported sum of 34 chlorobiphenyl congeners from 80.7 ng/g w.w. (in muscle) to 246.2 ng/g w.w. (in liver). In present study the concentration of PCBs in muscle tissue and liver of fish from the Danube River were observed to be much lower.

CONCLUSIONS

WHO recommended consumption of fish due to the high protein, unsaturated fatty acids, vitamins and minerals content. On the other hand, a frequent contaminated fish consumption may pose a potential risk for humans. The current investigation on toxicological levels of Indicator PCBs and dioxine-like PCBs in fish from the Danube River showed that the concentrations remained well below the existing European limits. We can conclude that the consumption of fish do not pose any significant risk to human health. However, it would be necessary the investigation of the PCBs in freshwater fish to continue, as they represent one of the major contributors to the pollution of Danube River waters.

Acknowledgment: *This study was supported by the EEA Grants and Ministry of Environment and water, Bulgaria, project contract № Д-33-49/30.06.2015.*

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