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POLYCHLORINATED BIPHENYLS IN FRESHWATER FISH FROM THE ZAGREB AREA

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The aim of this study was to determine the levels of six polychlorinated biphenyls (referred to as PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180, according to the IUPAC) in freshwater fish from the Zagreb area. A total of 216 samples was collected from five sites: from the Sava River upstream of Zagreb, in Zagreb, and downstream of Zagreb, from Lake Jarun, and from five fishponds from the Zagreb surroundings. Samples from all five sites contained polychlorinated biphenyls (PCBs). The maximal levels found in individual samples ranged between 20 μg kg^{-1} and 50 μg kg^{-1} depending on the congener. However, the total level of all examined congeners was far below the maximal allowed level of 2.0 mg kg^{-1} ww, set by Croatian authorities for total PCBs.

KEY WORDS: *Croatia, environment, PCB, pollution indicators*

Polychlorinated biphenyls (PCBs) are a group of industrial organochlorinated compounds that were first synthesised some hundred years ago, and have increasingly been used for about sixty years now. Since the end of the 1950s, over a million tonnes of PCBs have been produced (1).

PCBs are mostly absorbed orally, then by inhalation and then transdermally. The general population is mostly exposed to PCBs through fish and shellfish, which are characterised by high PCB accumulation. Their intestinal absorption is quite fast. PCBs are deposited from the blood into the adipose tissue or are metabolized in the liver to form metabolites that are excreted in the urine or bile. Beside adipose tissue, the highest amounts of PCBs were detected in the kidneys, liver and brain, and the lowest in muscles (2), and were found to cross the transplacental barrier (3). The compounds with a higher number of chlorine atoms are more stable. The rate of elimination decreases with the increasing number of chlorine atoms. According to the WHO report, the non-ortho substituted PCBs (PCB-77, PCB-81, PCB-126 and PCB-169) are the most toxic PCB congeners (4).

Data on PCB toxicity were based on the results obtained from experimental animals and accidental human exposure to high amounts of PCBs. Nowadays PCB toxicity is studied and evaluated separately for each congener (4, 5). Food associated with the highest human exposure to PCBs includes aquatic organisms (fish and shellfish), milk, and dairy products in which PCBs are deposited as their constituents in the form of residues. Through this food, PCBs are taken continuously in small amounts every day (6). It is not only the residues in the unchanged form that pose a health risk for humans, but also their degradation products, isomers or compounds formed by metabolism, which could be even more toxic than the original substance (7-9).

The main objective of this study was to obtain representative data on the levels of PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, and PCB-180 (according to the IUPAC numbering) (10) in freshwater fish from the Zagreb area in order to determine the baseline levels of these compounds in all fish samples and to compare them between fishing sites and between two major fish families. The objective was

also to assess the reliability of PCB monitoring in freshwater fish as one of the systems for monitoring environmental pollution.

MATERIAL AND METHODS

Sampling

This study included a total of 216 freshwater fish samples collected from five sites in Zagreb and its surroundings in 2000; 38 fish samples from the Sava River upstream of Zagreb (Group 1), 34 fish samples along the Sava in Zagreb (Group 2), 31 fish samples from the Sava downstream of Zagreb near the sewage outlet into the river (Group 3), 82 fish samples from Lake Jarun connected by underground waters with the Sava (Group 4), and 31 fish samples from five fishponds around Zagreb which are not connected with the Sava (Group 5).

The fish from all the five sampling sites belonged to either of the two families: *Ictaluridae* (31 sample) and *Cyprinidae* (185 samples). The family *Cyprinidae* comprises the carp genera, characterised by toothless jaw, but well developed pharyngeal teeth. Their body is mostly covered with scales. These fish live and search for food in the mud and sand at the bottom of still waters, and include the following species: chub (*Leuciscus leuciscus L.*), roach (*Rutilus rutilus L.*), bream (*Abramis brama L.*), barbel (*Barbus barbus L.*), goldfish (*Carassius auratus gibelio Bloch*) and other (11). The family *Ictaluridae* includes fish with no scales but naked body, spiked spines on the front and back fins, and eight barbs around the mouth. All our samples from this family were of the pygmy catfish (*Ictalurus nebulosis Lesneur*) (11, 12).

Each sample was examined individually and 216 individual analyses were performed. Each analysis required about 50 grams of fish sample, which was then homogenised. The prepared sample was an entirely clear and colourless fluid, concentrated to the final volume between 0.25 mL and 1 mL and ready for gas chromatography (13-16). More details about sampling and preparation of the samples for analysis are given in reference 17.

Analytical method

The levels of six different PCBs in fish samples were determined using the external standard method with a Unicam Pro GC capillary column gas chromatograph (Cambridge, United Kingdom) (18) with electron capture detection (GC-ECD). The detection limit for all PCB congeners was 0.01 μg kg^{-1} (19).

The GC-ECD had a capillary column SP-2331 (Supelco, Bellefonte, PA) of 60 m x 0.32 mm ID x 0.20 μm film thickness; the temperature programme of the column was 150 °C (5 min)/10 °C min^{-1}/280 °C (22 min). The temperature of the injector was 280 °C and of the detector 300 °C. The carrier gas was nitrogen (minimal purity 99.999 %) with the flow rate of 1 mL min^{-1}.

Recoveries ranged between 90 % and 102 %. To verify the results and the efficacy of the method, the Department of Health Ecology with related laboratories participated in interlaboratory exercises involving other laboratories in Croatia, and also in an international interlaboratory exercise conducted by the WHO on the six PCB congeners included in this study (19).

RESULTS AND DISCUSSION

The results of the analyses are summarized in Tables 1, 2, and 3. Tables 1 and 2 show the median levels and ranges of six PCB congeners by sampling site and fish family. They also show the number of samples with PCB levels below the detection limit. Of 216 samples, in only 14 were all analysed congeners below the detection limit. Table 3 shows the frequency of detected PCB congeners in 216 samples.

Croatian regulations (20) do not define the maximal allowed PCB concentration (MAC) for individual congeners, and the MAC of 2.0 mg kg^{-1} ww for total PCBs is based on the quantification against the standard Aroclor 1254. In none of the 216 analysed fish samples did the sum of all six congeners reach the MAC value, but was far below the 2.0 mg kg^{-1} ww limit. The maximum levels in individual samples were between 20 μg kg^{-1} and 50 μg kg^{-1}, depending on the congener.

Published data obtained by systematic monitoring and analysis of environmental pollution indicate that PCBs are frequently found in the environment (21, 22). They have been found in almost every component of the global ecosystem including the air, water, sediments, fish, and wildlife as well as in human adipose tissue, milk, and serum (23).

The major problems associated with these dangerous pollutants refer to their slow degradation and easy distribution across great distances. Thus,

Table 1 *Levels of six polychlorinated biphenyls (PCB) in freshwater fish collected from the indicated sites.*

Sampling site		Mass fraction / μg kg^{-1}					
		PCB-28	PCB-52	PCB-101	PCB-138	PCB-153	PCB-180
Group 1 Sava upstream of Zagreb N=38	Min	* n=13	* n=10	* n=6	* n=16	* n=14	* n=23
	Max	2.60	17.60	16.20	14.20	9.10	24.00
	Median	3.80	3.30	0.90	2.25	1.10	0.01
Group 2 Sava in Zagreb N=34	Min	* n=10	* n=5	* n=10	* n=12	* n=14	* n=19
	Max	17.00	9.00	3.10	6.20	5.20	2.30
	Median	1.80	4.60	5.80	3.80	1.60	1.60
Group 3 Sava downstream of Zagreb N=31	Min	* n=4	* n=4	* n=4	* n=4	* n=8	* n=7
	Max	17.00	42.00	26.40	15.90	27.00	12.00
	Median	1.80	4.60	5.80	3.80	1.60	1.60
Group 4 Lake Jarun N=82	Min	0.20	* n=2	* n=2	* n=2	* n=10	* n=21
	Max	6.50	54.00	18.00	9.50	10.50	29.00
	Median	1.80	4.70	4.10	2.20	2.20	1.10
Group 5 Fishponds N=31	Min	* n=5	* n=2	* n=22	* n=9	* n=27	* n=20
	Max	4.20	9.60	2.40	2.80	3.40	8.00
	Median	1.70	2.40	0	1.10	0	0

*values below the detection limit of 0.01 μg kg^{-1}, N = number of analysed samples, n = number of samples below the detection limit

PCBs are present in all countries all over the world, from highly to moderately or poorly industrialised (24-28).

The City of Zagreb, the capital of Croatia with a population of nearly one million, concentrates the majority of the industry in the country. Zagreb is supplied with drinking water from the Sava River (27). The long lasting uncontrolled draining of sewage and wastewater to the Zagreb underground waters and the Sava has led to the contamination of some of the water springs and to a considerable pollution load to the Sava waters, rendering the river unsuitable for swimming or fishing (29). Polluters upstream of Zagreb, from the neighbouring Slovenia, who had been using the Sava River as a suitable wastewater recipient for decades, also contributed to the devastation of its ecosystem and disappearance of almost all organisms living in or depending on the river.

The ecosystem of the Sava River has been accumulating PCBs from a number of adjacent industries along its course. In this study, we chose fish as one of the best bioindicators to evaluate environmental pollution with these pollutants. The Sava River was divided in three groups of sampling sites which showed distinct variation in PCB contamination, most probably due to the type of industry and variable composition of the wastewaters draining at particular sites along the river. The lowest median levels of all six congeners were found in fish samples from fishponds which are not connected to the Sava. As expected, the highest median PCB levels were determined in samples taken from the Sava downstream of Zagreb and in Zagreb. It is interesting to note that high median levels for PCB-52 and PCB-153 were found in the fish from Lake Jarun. These findings can be explained by the fact that Lake Jarun has a constant and direct connection with the Sava River through underground waters and that the water exchange in the lake is very slow because of its shape and bed soil. In addition, Lake Jarun is a sports and recreation centre favoured by the citizens of Zagreb, especially in the summer and on special occasions, offering a variety of aquatic amusements including boating which implies the risk of PCB-containing lubricating oil release into the environment. The obtained median PCB levels are comparable to values obtained in a similar study in Spain, where the highest mean level in fish and shellfish was 11864 ng kg^{-1} ww (30). The United Nations Environment Programme Mediterranean Regional Report, released in December

Table 2 *Levels of six polychlorinated biphenyls (PCB) in freshwater fish by sampling sites and by fish families.*

Sampling site	Fish family		Mass fraction / μg kg^{-1}					
			PCB-28	PCB-52	PCB-101	PCB-138	PCB-153	PCB-180
Group 1 Sava upstream of Zagreb	*Ictaluridae* N=2	Min	* n=2	* n=2	* n=2	* n=2	* n=2	* n=2
		Max	*	*	*	*	*	*
		Median	*	*	*	*	*	*
	Cyprinidae N=36	Min	* n=11	* n=8	* n=4	* n=14	* n=12	* n=21
		Max	2.60	17.60	16.20	14.20	9.10	24.00
		Median	1.0	3.65	1.65	0.4	1.0	0
Group 2 Sava in Zagreb	*Ictaluridae* N=4	Min	* n=4	* n=3	* n=4	* n=4	* n=4	* n=4
		Max	*	6.00	*	*	*	*
		Median	*	0	*	*	*	*
	Cyprinidae N=30	Min	* n=6	* n=2	* n=6	* n=8	* n=10	* n=15
		Max	17.00	9.00	3.10	6.20	5.20	2.30
		Median	5.6	3.3	0.9	2.85	1.35	0.1
Group 3 Sava downstream of Zagreb	*Ictaluridae* N=4	Min	* n=4	* n=4	* n=4	* n=4	* n=4	* n=4
		Max	*	*	*	*	*	*
		Median	*	*	*	*	*	*
	Cyprinidae N=27	Min	0.30	0.30	0.40	1.00	*	*
		Max	17.00	42.00	26.40	15.90	27.00	12.00
		Median	2.0	5.2	6.3	4.5	1.8	2.4
Group 4 Lake Jarun	*Ictaluridae* N=18	Min	0.20	2.80	* n=1	* n=2	* n=8	* n=9
		Max	2.50	15.00	7.20	4.50	5.40	3.10
		Median	1.4	6.5	2.4	2.0	0.2	0
	Cyprinidae N=64	Min	0.20	* n=2	1.10	0.40	* n=2	* n=12
		Max	6.50	54.00	18.00	9.50	10.50	29.00
		Median	1.9	3.6	4.3	2.3	2.5	1.1
Group 5 Fishponds	*Ictaluridae* N=3	Min	0.80	2.10	* n=2	* n=3	* n=2	3.40
		Max	1.70	9.60	2.40	*	1.80	8.00
		Median	1.6	2.5	0	0	0	4.6
	Cyprinidae N=28	Min	* n=5	* n=2	* n=20	* n=6	* n=25	* n=20
		Max	4.20	6.00	2.40	2.80	3.40	4.10
		Median	1.75	2.4	0	1.15	0	0

*values below the detection limit of 0.01 μg kg^{-1}, N = number of analysed samples, n = number of samples below the detection limit

2002, refers to PCBs in fish tissues from the Sava River in the range of 8 μg kg^{-1} to 177 μg kg^{-1} ww (31), which are higher than those obtained in this study. This can be explained by the fact that the 2002 report refers to measurements conducted immediately after the war in Croatia, when infrastructural damages near the Sava River caused several ecological disasters resulting in major ground water pollution.

According to the fish family, the median levels for almost all examined congeners were higher in the family *Cyprinidae* than in the family *Ictaluridae*. The highest median congeners levels were measured in the fish of the family *Cyprinidae* from the Sava river downstream of Zagreb. The highest median level for PCB-52 in the family *Ictaluridae* was recorded in the fish from Lake Jarun, followed by those from

Table 3 *Frequency of PCB congeners in fish collected from the listed sampling sites*

Number of detected PCB congeners	Frequency of detected PCB congeners					
	Group 1 Sava upstream of Zagreb	**Group 2** Sava in Zagreb	**Group 3** Sava downstream of Zagreb	**Group 4** Lake Jarun	**Group 5** Fishpounds	**Total**
0	4	3	4	0	3	14
1	0	0	0	0	2	2
2	2	0	0	0	0	2
3	6	3	0	0	12	21
4	9	8	1	7	9	34
5	12	8	5	19	4	48
6	5	12	21	56	1	95
N	38	34	31	82	31	216

N = number of analysed samples

fishponds (for PCB-180 and PCB-52) which have no underground connection with the Sava. As both of these are stagnant waters, this appears to be decisive for PCB accumulation in the fish of this family, as well as their living habits.

Systematic monitoring of environmental pollution at all levels has been introduced by almost all industrialised countries in the world, whereas it has been less common in the countries with a lower level of industrialisation, depending on their financial resources. In Croatia, which belongs to the latter group, the system of environmental pollution monitoring has not yet reached the level of highly industrialised countries (17). As it is well known that PCBs bioaccumulate in fish tissues due to their resistance to biodegradation and due to their lipophilic properties, (32) monitoring of environmental pollution with PCBs by monitoring the PCBs levels in freshwater fish seams quite acceptable in our country, allowing us to assess the environmental pollution of an ecosystem over a long period of time.

In conclusion, although the results obtained in this study show that PCB levels measured in freshwater fish from the Zagreb area are below the maximal allowed level (20), there is a need to monitor them systematically.

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Sažetak

POLIKLORIRANI BIFENILI U SLATKOVODNIM RIBAMA ZAGREBAČKOG PODRUČJA

Cilj istraživanja bio je utvrditi masene udjele šest kongenera polikloriranih bifenila (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 i PCB-180) u ribama te ocijeniti je li monitoringom navedenih kongenera u slatkovodnim ribama zagrebačkog područja moguće adekvatno pratiti onečišćenje ispitivanog ekosustava navedenim spojevima. Obrađeno je ukupno 216 uzoraka riba sa sljedećih pet lokacija: rijeka Sava uzvodno, kraj te nizvodno od Zagreba, jezero Jarun te pet "ekoloških" jezera u okolici Zagreba.

Uzorci sa svih pet lokacija bili su onečišćeni polikloriranim bifenilima iako ni u jednom ispitivanom uzorku utvrđena količina ispitivanih kongenera nije prelazila maksimalno dopuštenu količinu od 2,0 mg kg^{-1} u svježoj masi ribljeg uzorka, navedenu u Pravilniku o količinama pesticida, toksina, mikotoksina, metala, histamina i sličnih tvari koje se mogu nalaziti u namirnicama te o drugim uvjetima u pogledu zdravstvene ispravnosti namirnica i predmeta opće uporabe.

Iako je rijeka Sava najveći sabirnik svih vrsta otpadnih voda u Republici Hrvatskoj, razine polikloriranih bifenila u svim grupama uzoraka iz rijeke Save bile su unutar dopuštenih granica, a monitoring onečišćenja okoliša spomenutim spojevima putem praćenja njihovih količina u slatkovodnim ribama pokazao se vrlo prikladnim s obzirom na uvjete koji vladaju u ispitivanom ekosustavu te njegovu neposrednom okolišu.

KLJUČNE RIJEČI: *Hrvatska, indikatori onečišćenja, okoliš, PCB*

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