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# ORGANOCHLORINE PESTICIDES IN FRESHWATER FISH FROM THE ZAGREB AREA

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The aim of this study was to determine the level of organochlorine pesticides in freshwater fish from the Zagreb area, Croatia. The study included 215 freshwater fish samples from three sites: the Sava River, Lake Jarun, and five fishponds from the Zagreb surroundings. Organochlorine pesticides DDT and derivates, HCH, HCB, lindane, aldrin, dieldrin, endrin, heptachlor with epoxide, and methoxychlor were determined using the GC-ECD method. The determined amounts of organochlorine pesticides were within allowed concentration limits in all analysed fish samples. Median values ranged from below the detection limit of 0.01  $\mu$ g kg<sup>-1</sup> for dieldrin and metoxychlor to 2.00  $\mu$ g kg<sup>-1</sup> for DDT in the *Cyprinidae* fish family from the Sava River, Zagreb sampling site (group 1).

This study has confirmed pesticide persistence in the overall ecosystem in our country despite the ban of some thirty years ago, like in many other parts of the world.

**KEY WORDS:** aldrin, DDT, dieldrin, endrin, environment, epoxide, HCB, HCH, heptachlor, lindane, methoxychlor, pollution indicators

Worldwide pesticide production doubled between 1970 and 1985 and the annual sales soared from US\$ 2,700 million in 1970 to US\$ 40,000 million by the end of the century. Approximately 2,800 million kilograms were sold during this period. The percentage of these substances used in less industrialized countries has increased over the last three decades from 20 % to close to 40 %, with the USA as the major pesticide manufacturer and consumer worldwide, i.e. accounting for more than 1,100 million kilograms of various pesticides (1, 2). Due to their wide and large-scale use, pesticides are ubiquitous in the environment irrespective of the setting. It is known that only 0.1 % of the total amount of a pesticide used will reach the target site, the rest being dispersed around (3). Therefore, pesticides are found in foodstuffs, drinking water, and in all segments of the ecosystem (4). Considering all these facts, international scientific community has shown a

growing interest in determining the level and studying the effects of pesticides on human health.

All analyzed organochlorine pesticides are characterised by high lipophilic properties and slow biodegradability, which results in their bioaccumulation in the adipose tissue of various animal species (5). This is how these compounds contaminate food of animal origin and enter the food chain of humans (6).

Living organisms can accumulate chlorinated hydrocarbons from the surrounding media as well as from food. Intake *via* water predominates in aqueous organisms, whereas in terrestrial animals the main source is contaminated food (7). Pesticides can enter water system directly through area treatments aimed at destroying the vegetation, algae and insects (8), or indirectly through the inflow of sewage and industry wastewater, through rainfall washing and leaching the surrounding contaminated soil, as well as through accidental or deliberate contamination. Aqueous flora accumulates pesticides in an attempt to eliminate them from the water, thus initiating the first step of bioaccumulation (9). The grade of bioaccumulation can be expressed as a factor of bioaccumulation representing the ratio of a substance concentration in a living organism and its concentration in the medium or food. In some fish, molluscs and insect species this ratio may reach a figure of several dozen or even hundred thousands (10).

People generally show an increasing interest in healthy diet, in which fish has a prominent place for its energy and bionutritive values. Fish-based diets are associated with climate and tradition; however, the overall consumption of fish is on a steady increase all over the world, including Croatia, for a number of reasons. First of all, it should be noted that fish contains dominantly unsaturated fatty acids that are more readily degraded in the gastrointestinal system (11). In addition, fish is a significant source of oligoelements, their dietary role being lately ever more emphasised.

The aim of this study was to determine the level of organochlorine pesticides in freshwater fish from the Zagreb area as the largest urban, industry and traffic centre in Croatia. The aim was also to assess possible differences in the levels of organochlorine pesticides between fish from the Sava River as the major recipient of industry wastewater, sewage and agricultural wastewater in Croatia and fish originating from other sites.

# MATERIAL AND METHODS

### Sampling

The study included a total of 215 freshwater fish samples collected in 2000 from five sites in Zagreb and its surroundings in collaboration with the Zagreb fishing association: 103 fish samples from the Sava River (group 1), 82 fish samples from Lake Jarun communicating *via* underground waters with the Sava River (group 2), and 31 fish samples from five fishponds around Zagreb with no communication with the Sava River (group 3).

The fish from all three sampling sites belonged to either of the two families: *Ictaluridae* (n=31) and *Cyprinidae* (n=184). 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. 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 species (*Ictalurus nebulosus Lesneur*) (12,13).

# Analytical method

Each sample was examined individually and 215 individual analyses were performed. Each analysis required about 50 grams of the edible part of the fish, which was then homogenised. Extraction was performed using redestilled *n*-hexane and the sample was cleaned until it became a clear and colourless fluid, concentrated to the final volume between 0.25 mL and 1 mL and ready for gas chromatography (14-17). Sampling and sample preparation have been described in detail elsewhere (18).

The levels of organochlorine pesticides in fish samples were determined using the external standard method on a Unicam Pro GC capillary column gas chromatograph (Cambridge, United Kingdom) (19) with electron capture detection (GC-ECD). The detection limit was 0.01  $\mu$ g kg<sup>-1</sup>(20). The GC-ECD had a capillary column SP-2331 (Supelco, Bellefonte, PA) of 60 m x 0.32 mm ID x 0.20  $\mu$ m film thickness; the temperature program of the column was 150 °C (5 min)/10 °C min<sup>-1</sup>/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 a flow rate of 1 mL min<sup>-1</sup>. The detection limits and recoveries were determined using the standard addition method with known amounts of certified reference standard mixture of organochlorine pesticides EPA Pesticide Mix Supelco Cat. No 4-8858 established by standard testing (20). Recoveries ranged between 92 % and 98 %. To verify the results and 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. The detection limit was 0.01 µg kg<sup>-1</sup> (20).

# **RESULTS AND DISCUSSION**

Analyzed organochlorine pesticides were DDT, all HCH isomers, HCB, aldrin, dieldrin, endrin, heptachlor with epoxide and methoxychlor. DDT results are presented as a sum of the selection of 4,4'-DDT, 2,4'-DDT, 4,4'-DDE, 2,4'-DDE, or 4,4'-DDD, 2,4'-DDD. HCH results are presented as a sum of the selection of  $\alpha$ -HCH,  $\beta$ -HCH and  $\delta$ -HCH. All results are expressed in fresh weight of the edible part, in lower and medium determination limits.

Study results are presented in Tables 1-3. Table 1 shows the frequency of organochlorine pesticides detected in fish collected from the listed sampling sites. Table 2 shows the levels of organochlorine pesticides in freshwater fish from the indicated sites. Table 3 shows the levels of nine organochlorine pesticides in freshwater fish by sampling sites and by fish families. Table 3 also shows the number of samples with pesticide levels below the detection limit.

According to the sampling sites, fish samples from the Sava River showed the highest median levels of DDT as well as HCH isomers. In Lake Jarun fish samples, the highest median levels were detected for DDT, HCH isomers and aldrin. In fishponds, the highest median levels were detected for HCH, DDT and aldrin (Table 2).

The highest median levels in fish samples were 2.00  $\mu$ g kg<sup>-1</sup> for DDT (*Cyprinidae*, the Sava River, group 1) and 1.60  $\mu$ g kg<sup>-1</sup> for methoxychlor (*Ictaluridae*, Lake Jarun, group 2), but the lowest median levels were below the detection limit of 0,01  $\mu$ g kg<sup>-1</sup> for dieldrin, endrin, heptachlor+epoxide and metoxychlor (*Ictaluridae*, fishponds, group 3) (Table 3).

Median levels did not reach the maximal allowed concentration in any of the 215 fish samples, but they were higher in samples from the *lctaluridae* fish family collected at the Sava – Zagreb sampling site than at the other sampling sites.

Maximal allowed concentration in Croatia are 1000  $\mu$ g kg<sup>-1</sup> for DDT, 100  $\mu$ g kg<sup>-1</sup> for the sum of  $\alpha$ -,  $\beta$ - and  $\delta$ -HCH isomers, 200  $\mu$ g kg<sup>-1</sup> for HCB, 2000  $\mu$ g kg<sup>-1</sup> for lindane, 200  $\mu$ g kg<sup>-1</sup> for aldrin, 100  $\mu$ g kg<sup>-1</sup> for dieldrin, 50  $\mu$ g kg<sup>-1</sup> for endrin, 200  $\mu$ g kg<sup>-1</sup> for heptachlor with epoxide, and 100  $\mu$ g kg<sup>-1</sup> for methoxychlor (21).

One fourth of the population of Croatia, i.e. more than 1.2 million inhabitants, live in the City of Zagreb and its surroundings (Zagreb County), which clearly indicates the high potential of environmental pollution. Yet, there are a number of other factors that should be taken into consideration. Zagreb is the largest industrial area in Croatia with more than 90,000 industry workers, most of whom employed in food industry, chemical industry and technical plants. The water supply of the City of Zagreb relies on the Sava River (22).

The long lasting uncontrolled draining of sewage and wastewater through underground waters directly to the Sava River has ultimately led to the contamination of some of the sources drinking water on the one hand, and precluded the use of the river water for recreation, bathing and fishing on the other (22). Industries who use the Sava River upstream of Zagreb as a convenient wastewater recipient also contribute significantly to the alarming situation that has been persisting for decades now, resulting in serious contamination and influence on all river organisms. In addition, it should be noted that the very

Number of organochlorine	Number of samples							
pesticides detected	Sava River	Jarun Lake	Fishponds	Total				
0	21	0	1	22				
1	0	0	0	0				
2	2	2	5	9				
3	19	7	13	39				
4	30	31	9	70				
5	13	10	2	25				
6	11	21	1	33				
7	7	9	0	16				
8	0	1	0	1				
9	0	0	0	0				
Ν	103	81	31	215				

Table 1 Frequency of organochlorine pesticides detected in fish collected from three sampling sites

N = number of analysed samples

Mass freaction / $\mu$ g kg <sup>-1</sup>										
									Heptachlo	r
Sampling site		DDT	HCH	HCB	Lindane	Aldrin	Dieldrin	Endrin	+	Methoxychlor
									epoxide	
The Sava River (N=103)	Min	0	0	0	0	0	0	0	0	0
	Max	25.00	18.00	3.30	14.80	9.00	2.60	16.00	12.00	5.80
	Median	1.80	0.50	0	0.20	0	0	0	0	0
Lake Jarun (N=81)	Min	0	0	0	0	0	0	0	0	0
	Max	6.00	4.00	4.10	2.30	3.10	8.80	2.10	1.10	5.80
	Median	0.80	0.30	0	0.10	0.20	0	0	0	0
"Ecological"	Min	0	0	0	0	0	0	0	0	0
fishponds not	Max	3.40	4.00	0	3.60	3.10	5.10	4.00	1.40	0
communicating										
with the Sava River $(N=31)$	Median	0.10	0.20	0	0	0.10	0	0	0	0
Maximal allowed level (21) 100			100	200	2000	200	100	50	200	100

Table 2 Levels ( $\mu g \ kg^{-1}$ ) of organochlorine pesticides in freshwater fish from three sites

 $\overline{0}$  = values below the detection limit of 0.01 µg kg-1, N = number of analysed samples

			Mass fraction / $\mu$ g kg <sup>-1</sup>								
Sampling Site	Fish Family	1	DDT	HCH	HCB	Lindan	Aldrin	Dieldrin	Endrin	Heptachlor + epoxide	Metho- xychlor
			n=6	n=6	n=7	n=6	n=8	n=10	n=7	n=7	n=10
		Min	0	0	0	0	0	0	0	0	0
Group 1	Ictaluridae	Max	25.00	18.00	0.30	12.00	9.00	0	16.00	1.80	0
	N=10	Median	0	0	0	0	0	0	0	0	0
Sava			n=18	n=26	n=90	n=36	n=59	n=87	n=45	n=57	n=81
River		Min	0	0	0	0	0	0	0	0	0
	Cyprinidae	Max	14.80	6.20	3.30	14.80	3.00	2.60	7.00	12.00	5.80
	N=93	Median	2.00	0.50	0	0.30	0	0	0.20	0	0.01
				n=2	n=17	n=3	n=4	n=5	n=16	n=3	n=8
		Min	0.10		0	0	0	0	0	0	0
Group 2	Ictaluridae	Max	6.00	1.20	3.00	2.30	0.80	8.80	0.40	1.10	5.80
	N=18	Median	0.70	0.15	0	0.35	0.30	1.55	0	0.20	1.60
Lake Jarun		M	n=1	n=2	n=50	n=23	n=23	n=43	n=28	n=48	n=56
		Min	0	0	0	0	0	0	0	0	0
	Cyprinidae	Max	4.00	2.00	4.10	1.70	3.10	5.10	2.10	1.10	2.30
	N=63	Median	0.90	0.30	0	0.10	0.20	0	0.30	0	0
					n=3		n=2	n=3	n=3	n=3	n=3
		Min	0.50	0.10	0	0.10	0	0	0	0	0
Group 3	Ictaluridae	Max	0.80	0.30	0	0.10	0.10	0	0	0	0
	N=3	Median	0.60	0.20	0	0.10	0	0	0	0	0
Fishponds			n=15	n=5	n=31	n=16	n=8	n=25	n=14	n=22	n=31
		Min	0	0	0	0	0	0	0	0	0
	Cyprinidae	Max	3.40	4.00	0	3.60	3.30	5.10	4.00	1.40	0
	N=28	Median	0	0.25	0	0	0.10	0	0.05	0	0*

**Table 3** *Levels* (μg kg<sup>1</sup>) of nine organochlorine pesticides in freshwater fish by sampling site and by fish family

0= values below the detection limit of 0.01  $\mu g kg^{-1}$ , N = number of analysed samples; n = number of samples below the detection limit

long course of the Sava River through the Croatian territory also entails a considerable burden of various compounds, primarily of different pesticides draining into the river from the surrounding agricultural areas through underground waters.

Concentrations of organochlorine pesticides in the fish from the Sava River in our study were below the maximal allowed concentration of each study parameter, yet they are far from negligible (22). The Sava River flows through agricultural areas, and the burden accumulated on its course has resulted in contamination of the Sava River fish with DDT, HCH, lindane, endrin and heptachlor with epoxide. This is also expected in countries with highly developed agriculture, as demonstrated by the analyses performed not only in edible fish tissue but also in some specific fish organs (9, 23-25). These reports, although occasionally exceeding the values recorded in our study, clearly confirm the existence of organochlorine pesticide accumulation in fish tissue

As there is direct communication between the Sava River and Jarun Lake through underground waters, it is no surprise that the fish originating from this site also contained relatively high levels of organochlorine pesticides which, however, were lower than those measured in the fish from the Sava River. As expected, the lowest levels of these contaminants were found in fish samples from the so called "ecological" fishponds located in Zagreb surroundings, with no nearby agricultural areas and communication with the Sava River.

In an attempt to detect the organochlorine pesticide that would best reflect the level of the respective water system contamination, we noticed a higher presence of DDT than of other study pesticides; this finding suggests that DDT concentration may be used as an indicator of persistent pesticide contamination taking into account its chemical properties (26). The results obtained for lindane were lower than those for DDT, but they nevertheless demonstrate its persistence in the environment even many years after it has been banned.

Comparable results have been reported from India and the United States (27, 28). Singh and Sahai (India) showed the effect of three organochlorinated pesticides on the gill structure of Puntius ticto, freshwater teleost. Exposure to HCH was followed by an inflammatory reaction and complete dystrophy of the lamellar structure of the gills. Lindane-treated fish showed a disruption of the epithelial covering of the gills and excessive haemorrhage in the blood vessels. Due to exposure to endosulphane the gill lamellae shrink and become thiner (27).

Organochlorine pesticides and PCBs were detected in streambed sediment and fish from streams in the Central Columbia Plateau of central Washington and northern Idaho, one of the 60 study units in the U. S. Geological Survey's National Water Quality Assessment (NAWQA) Program. Concentrations of some compounds were at levels that may pose a threat to fish and wildlife of the region (28).

Low pesticide residue levels in our study confirmed their use in Croatia and their steady presence in the overall ecosystem despite the fact that most of them have been banned for thirty years.

In conclusion, great rivers such as the Sava River, serving as sewage and industry wastewater recipients throughout their course, have a major impact on the entire living world around them, indirectly including humans. These effects are depend on contaminant burden accumulating along the course of a river, particularly of contaminants with a carcinogenic potential, including organochlorine pesticides which bear a considerable risk for human health because of their generally chronic action. These bitter but real and indisputable facts obviously call for appropriate solutions to reduce the exposure of all living organisms, including humans, to these hazardous compounds.

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#### Sažetak

#### ORGANOKLORIRANI PESTICIDI U SLATKOVODNIM RIBAMA ZAGREBAČKOG PODRUČJA

Cilj istraživanja bio je utvrditi količinu organokloriranih pesticida u slatkovodnim ribama zagrebačkog područja. Obrađeno je ukupno 215 uzoraka slatkovodne ribe sa sljedećih triju lokacija: rijeka Sava, jezero Jarun i pet tzv. ekoloških jezera u okolici Zagreba. Organoklorirani pesticidi DDT i derivati, HCH izomeri ( $\alpha$ -HCH,  $\beta$ -HCH and  $\delta$ -HCH), HCB, lindan ( $\gamma$ -HCH), aldrin, dieldrin, endrin, heptaklor, heptaklorepoksid i metoksiklor) određivali su se metodom plinske kromatografije uz elektronapsorpcijski način detekcije i uz postignutu granicu detekcije od 0,01  $\mu$ g kg<sup>-1</sup>. Vrijednosti ispitivanih organokloriranih pesticida u svim su uzorcima riba bile mnogo niže od maksimalno dopuštenih koncentracija. Vrijednosti medijana kretale su se od najnižih 0,01  $\mu$ g kg<sup>-1</sup> za dieldrin i metoksiklor do najviših 2,00  $\mu$ g kg<sup>-1</sup> za DDT u uzorcima riba porodice Cyprinidae s lokacije Sava - Zagreb.

Utvrđeni ostaci preostalih ispitivanih pesticida potvrđuju kako njihovu uporabu na našim područjima tako i njihovu postojanost i prisutnost u cjelokupnom ekosustavu unatoč činjenici da je većina zabranjena u Hrvatskoj prije gotovo 30 godina, kao i u mnogim dijelovima svijeta.

KLJUČNE RIJEČI: aldrin, DDT, dieldrin, endrin, HCB, HCH izomeri, heptaklor, heptaklorepoksid, lindan, metoksiklor, okoliš, pokazatelji onečišćenja

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