# Short Communication Contamination of Fish and Sediments from the Vistula River by Organochlorine Pesticides and PCBs

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## Abstract

The aim of our study was to assess the occurrence and to determine the concentrations of organochlorine pesticides and indicator PCB congeners in muscle tissue of freshwater fish species and, additionally, in sediments collected from three sampling sites of the Vistula River in the vicinity of Cracow. The determinations of HCH isomers, HCB, DDTs (p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT), and six indicator PCB congeners (28, 52, 101, 138, 153, and 180) were carried out in 52 fish samples and 35 sediments samples using capillary gas chromatography.

The mean concentration of DDTs in fish was 281  $\mu$ g/kg wet weight (11.8-1921  $\mu$ g/kg) and PCBs 83  $\mu$ g/kg wet weight (10.5-790  $\mu$ g/kg). The highest levels of contaminants were detected in the muscles of bream. The high levels of DDT and its metabolites, exceeding the PEL values (probable effect levels) were also detected in sediments (mean 270  $\mu$ g/kg dry weight, range 12.9-927  $\mu$ g/kg). In fish and sediments from Cracow and downstream from Cracow the levels of DDTs were higher than upstream of the Vistula River. The levels of contaminants remain of concern due to the potential risk to fish consumers and the ecological risk they pose, and indicate a need for a continuous monitoring.

Keywords: organochlorine pesticides, PCBs, fish, sediments

## Introduction

Persistent organic pollutants (POPs), such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), are chemical substances that persist in the environment, bioaccumulate through the food chain, and pose a risk of causing adverse effects to human health and the environment. They are widespread in the air, water, soil, and organisms, including wildlife and humans. Environmental pollution caused by these compounds has been a serious global problem for over half a century. Exposure to certain OCPs and PCBs may result in serious environmental and health effects, including adverse effects on the nervous, immune, and endocrine systems, impairment of reproductive function, and contribution to the spread of certain types of cancer [1, 2]. Although the production and use of OCPs in agriculture and of PCBs in industry have been prohibited in many countries, their residues are still detected, mainly in foods of animal origin. In Poland, for over 30 years HCB-, lindane-, and DDT-containing pesticides were completely banned, and PCBs have never been produced on a technical scale. The most important human food intake sources for the POPs are fatty foods including fish, meat, eggs, and dairy products.

The levels of OCPs and PCBs in wildlife animals reflect environmental contamination by these compounds.

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Sampling site (Year, river, nearest city)	Number of samples	Fish species (number of samples)
2012 Vistula- Cracow/Laczany	15	roach (7), white beam (6), perch (2)
2012 Vistula-Cracow/Dabie	16	bream (9), roach (3), white beam (2), catfish, perch (1)
2011 Vistula-Cracow/Grabie	12	bream (6), roach (3), chub (3)
2012 Vistula-Cracow/Grabie	9	roach (4), white beam (1), chub (1), barbel (1), asp (1), catfish (1)

Table 1. Sampling sites and tested fish species.

Freshwater fish are good indicators of environmental contamination and possible toxicological risk to the fish consumer [3-6]. We have previously reported the levels of OCPs and PCBs in fish (bream and roach) collected from 10 river and lake sampling sites [7]. The highest levels of contaminants were detected in samples from the Vistula River in the vicinity of Cracow. The aim of this study was to assess the occurrence and to determine the concentrations of organochlorine pesticides and indicator PCB congeners in muscles of different freshwater fish species, as well as in sediments collected from three sampling sites of the Vistula River in the vicinity of Cracow, and to evaluate the possible risk to fish meat consumers and ecological risk.

## **Materials and Methods**

Sampling sites for fish and sediments were located in three different parts of the Vistula River in the vicinity of Cracow: Laczany – a village west of Cracow, 28 km upstream; Dabie – a district of Cracow; and Grabie – a village east of Cracow, 14 km downstream. A total of 52 fish samples and 35 sediment samples were collected during the fishing seasons of 2011 and 2012. Fish species of the cyprinids included bream (*Abramis brama*), roach (*Rutilus rutilus*), white bream (*Blicca bjoerkna*), chub (*Leuciscus cephalus*), asp (*Leuciscus aspius*), and barbel (*Barbus barbus*). Predatory species included perch (*Perca fluviatilis*) and catfish (*Silurus glanis*). The sampling sites and the numbers of fish examined are shown in Table 1.

Organochlorine pesticides (HCB,  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH, p,p'-DDE, p,p'-DDD, o,p'-DDT, p,p'-DDT) and six indicator PCB congeners (PCB IUPAC No. 28, 52, 101, 138, 153, 180) were determined by capillary gas chromatography with electron capture detection. The contaminants were extracted from fish muscles and dried sediment samples using a mixture of hexane and acetone (2+1). The extracts were cleaned with sulphuric acid (H<sub>2</sub>SO<sub>4</sub>+H<sub>2</sub>SO<sub>4</sub> 25% SO<sub>3</sub>), and the separation of PCB congeners from organochlorine pesticides was achieved with the use of alkaline hydrolysis (10% KOH in ethanol).

The analyses were performed on an Agilent Technologies chromatograph, model 6890 Plus equipped with 7683B series autosampler, split-splitless injector in pulsed splitless mode, and  $^{63}Ni_{\mu}EC$  detector. Chromatographic separation was achieved on HP-5MS capillary column (60 m×0.25 mm ID×0.25 µm film thickness, J&W Scientific, USA). The following oven tempera-

ture programme was used: initial temperature of 60°C for 2 min, increased to 180°C at 40°C/min, and held for 2 min, increased to 245°C at 2°C/min and held 2 min, increased to 265°C at 4°C/min and held for 15 min, and then increased to 290°C at 8°C/min and held for 20 min. For confirmatory second capillary column DB 1701 (60 m×0.25 mm ID×0.25 µm film thickness, J&W Scientific, USA) was used. The GC was controlled by ChemStation software.

The validation of the methods was performed in accordance with SANCO/12495/2011 (method validation and quality control procedures for pesticide residues analysis in food and feed). Validation parameters demonstrated that the analytical method fulfilled the method performance acceptability criteria. Recoveries from fortified samples ranged from 88% to 103%, coefficients of variation (CV) indicating the precision of the methods were <12% for repeatability and <15% for reproducibility, uncertainty 25%, limit of determination was 0.1 µg/kg for each analyte. The accredited laboratory regularly and successfully participated in international proficiency testing organized by Food Analysis Performance Assessment Schemes (FAPAS, UK) and the European Union Reference Laboratory (CVUA Freiburg). In addition, the compliance of the results for the following analysed certified reference materials (CRM 598-Organochlorine pesticides in cod liver oil, CRM 349-Chlorobiphenyls in cod liver oil) was confirmed.

## **Results and Discussion**

The presence of DDT and its metabolites and PCB congeners have been observed in the majority of fish muscle samples. Residues of PCBs, p, p'-DDE and o,p'-DDT occurred in all samples – p,p'-DDT in 98% and p,p'-DDD in 90% of the samples. The low concentrations of HCB were found in 98% of fish samples, in the range from traces to 32 µg/kg (mean 5.8 µg/kg). Low contents of the HCH isomers were also detected in more than 92% of the samples; the mean concentrations were 1.8 µg/kg ( $\gamma$ -HCH), 6.0 µg/kg ( $\alpha$ -HCH), and 10 µg/kg ( $\beta$ -HCH).

The DDT and PCB concentrations in fish muscles expressed in  $\mu$ g/kg wet weight are reported in Tables 2 and 3. The mean concentration of DDTs (sum of p,p'-DDE, p,p'-DDD, o,p'-DDT and p p'-DDT) was 281  $\mu$ g/kg (median 190  $\mu$ g/kg), in that p,p'-DDE 141  $\mu$ g/kg, p,p'-DDD 119  $\mu$ g/kg, o,p'-DDT 16.3  $\mu$ g/kg, and p,p'-DDT 5.2  $\mu$ g/kg. The results of individual samples ranged between 11.8 and 1921  $\mu$ g/kg. The percentage of p,p'-DDE in the total DDTs

Compounds	Sites in the vicinity of Cracow	Mean	MinMax.	Median	90 <sup>th</sup> percentile	95 <sup>th</sup> percentile
	Laczany	2.8	1.4-5.3	2.4	3.9	4.4
HCB	Dabie	11.9	0.8-31.7	8.6	25.3	27.4
	Grabie	3.4	<0.1-6.2	3.5	5.0	5.3
DDTs	Laczany	40	11.8-81.6	37.2	61.3	74.6
	Dabie	592	76.6-1921	363	1282	1556
	Grabie	216	24.2-424	215	343	378
	Laczany	44.3	14.3-122	42.6	56.6	76.3
PCBs	Dabie	68	10.5-238	45.3	120	155
	Grabie	122	13.6-790	58.9	168	356

Table 2. Concentrations of HCB, DDTs, and PCBs in fish species from different sampling sites (µg/kg wet weight).

Table 3. Concentrations of DDTs and PCBs in different fish species (µg/kg wet weight).

Fish species	DI	DTs	PCBs		
(Number of samples)	Mean±SD	MinMax.	Mean±SD	MinMax.	
Bream (15)	654±517	179-1921	75.9±55.9	23.8-238	
Chub (4)	289±105	167-424	126±30	99.5-168	
Asp (1)	267	-	160	-	
Barbel (1)	248	-	128	-	
Roach (17)	121±107	28.4-414	112±191	25.0-790	
White bream (9)	109±106	21.1-312	42.4±15.3	19.7-68.4	
Cat fish (2)	50	24.2-76.6	15	13.6-17.2	
Perch (3)	42.1±33.7	11.8-78.4	20±13.2	10.5-35.1	
Total (52)	281±371	11.8-1921	83±117	10.5-790	

was 50% for all tested fish. The mean concentrations of PCBs (sum of PCB No. 28, 52, 101, 138, 153 and 180) in fish muscle were 83  $\mu$ g/kg (median 48  $\mu$ g/kg), in that PCB 153-24.9  $\mu$ g/kg, PCB 138-19.7  $\mu$ g/kg, PCB 180-13.4  $\mu$ g/kg, PCB 101-15.3  $\mu$ g/kg, PCB 52-6.4  $\mu$ g/kg, and PCB 28-3.1  $\mu$ g/kg. PCB concentrations ranged from 10.5 to 790  $\mu$ g/kg. The percentage of the sum of PCB 153, 138, and 180 in total PCBs was about 70%.

The study indicated the occurrence of differences in the high levels of organochlorine pesticides and PCB congeners in fish from three locations. In fish from Cracow (Dabie) and downstream from Cracow (Grabie), the levels of DDTs were much higher than upstream of the Vistula River (Laczany). The highest levels of contaminants were detected in the muscles of bream. The mean concentrations of DDTs were 645  $\mu$ g/kg (median 378  $\mu$ g/kg, range 179-1921  $\mu$ g/kg) and PCBs 75.9  $\mu$ g/kg (median 56.4  $\mu$ g/kg, range 23.8-790  $\mu$ g/kg). These levels were up to 10 times higher than in fish from other sampling sites of the country [7]. The mean concentration of DDTs calculated for 74

samples of bream (without Cracow) was only 37  $\mu g/kg$  and PCBs 22  $\mu g/kg.$ 

Maximum levels (ML) for the sum of six non-dioxinlike PCB indicators in food are laid down in Commission Regulation (EU) No. 1259/2011 [8]. PCB concentrations above 125  $\mu$ g/kg wet weight (ML for muscle meat of wild caught freshwater fish) were found in seven fish samples (13.5%): in two samples from Cracow/Dabie and five samples from Grabie. There is no legislation regulating levels of organochlorine pesticides in fish, neither at the national level nor in the European Union.

According to the available literature, the presence of residues of some persistent organic pollutants in tissues of freshwater fish is still found in many countries [3, 6, 9-12]. The problem with DDT and PCB contamination of food and environment in the areas of their former manufacture still occurs. The impact of industrial activities on environmental pollution was investigated in many studies.

In eastern Slovakia, 25 years of the manufacture of PCBs (Delors, from 1959 to 1984) resulted in increased

Location (Number of samples)	p,p'-DDE		p,p'-DDD		p,p'-DDT		DDTs	
	Mean	MinMax.	Mean	MinMax.	Mean	MinMax.	Mean	MinMax.
Laczany (10)	98	9.4-200	2.2	<0.1-5.2	14.7	3.5-38.8	115	12.9-244
Dabie (10)	211	109-350	3.4	1.4-6.8	16.7	7.0-30.9	232	123-386
Grabie (15)	365	39.3-896	9.0	1.5-20.5	23.2	4.4-100	399	61.2-927
Total (35)	245	9.4-896	5.5	<0.1-20.5	18.9	3.5-100	270	12.9-927
TEL, µg/kg	1.42		3.54		1.19		-	
PEL, µg/kg	6.75		8.51		4.77		-	
Number of samples > PEL	35		7		33		-	

Table 4. Concentrations of DDT and its metabolites in sediments ( $\mu$ g/kg dry weight).

environmental pollution of the surrounding area [4]. Fish living in the contaminated Michalovce district contained about a hundred times higher PCB levels than those caught in the control area. While PCB levels in sediment samples from that district ranged between 1.7 and 6 mg/kg, sediment samples from the uncontaminated district did not exceed 0.05 mg/kg. Since 2005, commercial fishing and consumption of select species of fish were banned as a result of PCB contamination of the Rhone River and several tributaries in France. High PCB levels (up to hundreds of  $\mu$ g/kg) have been detected repeatedly in sediment and fish in the Rhone River, particularly downstream from Lyon to the Mediterranean Sea [13].

In Brescia, Italy, a PCB production plant operated from 1930 to 1984 polluted soil and forage of surrounding fields, and caused significant contamination of meat and milk of the local cattle forage [14]. The indicator PCB levels were about 100 times higher than the regional background. In the Czech Republic, the effect of three major chemical plants located on the River Elbe on fish (chub) was studied [10]. Fish from downstream sampling sites showed significantly higher levels of contaminants (PCBs, DDTs, HCHs, HCB) than fish from upstream locations. The highest levels of DDTs were found at Usti and Neratovice. In another study the occurrence of halogenated contaminants in several fish species collected from Czech rivers in highly industrialized areas, including Usti, was monitored. Levels of DDTs and PCBs in bream ranged from 6.4 to 791 and from 4.8 to  $210 \,\mu\text{g/kg}$  wet weight, respectively [9].

The main sources of DDT in the environment are historical application of technical DDT, atmospheric deposition, dicofol manufacturing and application, and wastes from DDT facilities. DDT pollution was detected in the sampling sites downstream from a factory manufacturing dicofol in the Cinca River (a tributary of the Ebro River in Spain). DDT was used as one of the intermediates in the production of dicofol. The highest DDT levels in fish (barbels and bleaks) exceeded value of 2000  $\mu$ g/kg wet weight [15]. Organochlorine pesticides still persist in fish from the Tombigbee River (Alabama, USA) near a former (from 1952 until 1963) DDT manufacturing facility at McIntosh [16]. In 2004, concentrations of DDTs in individual large-

mouth bass from that site ranged from 419 to 49,810  $\mu$ g/kg wet weight of whole fish. Levels of DDT isomers, PCB, HCB, and toxaphene represent a risk to fish and piscivorous wildlife. Contamination by DDT of industrial origin was detected in 1996 in Lake Maggiore (Northern Italy), causing concern for wildlife and human health [5]. The DDT levels exceeded the Italian limit for edible fish, so commercial fishing of several species was banned.

The bioaccumulation of contaminants such as DDTs (mainly as DDE), PCBs, dioxins, and heavy metals by fish represents a potentially significant risk to piscivorous wildlife [11, 12, 17]. Fish often comprise a substantial dietary portion of mammalian and avian species, and therefore represent a significant route of contaminant exposure. In addition, wildlife consume most or all of the fish, including internal organs, in which concentrations of many contaminants are generally greater than those in the muscle tissue (fillet) typically used to assess human health risks. For wildlife protection assessments, whole fish analyses are optimal. Because the susceptibility to contaminants varies among species, tissue residue guideline (toxicity threshold) for the protection of wildlife consumers of aquatic biota for DDTs was calculated as 0.15-3.0 µg/g and for PCBs 0.11-0.48 µg/g [17]. Although the concentrations of many persistent organochlorine chemical residues have declined over the past two decades, our and others authors' results indicate that contaminant residues still remain a risk to piscivorous wildlife at some sites [4, 12, 17, 18].

The results of OCP and PCB determinations in sediments indicate the contamination of the Vistula River in the vicinity of Cracow. The concentrations of DDT and its metabolites expressed in  $\mu$ g/kg dry weight are presented in Table 4. In sediments, p,p'-DDE were the most abundant in DDT derivatives, constituting up to 90% of the DDTs. Residues of p, p'-DDE, and p,p'-DDT occurred in all samples, with p,p'-DDD in 97% and o,p'-DDT in 37%. The HCB concentrations were found in 94% of the samples, ranging from traces to 358  $\mu$ g/kg (mean 34  $\mu$ g/kg). Additionally, low contents of the HCH isomers were detected with mean concentrations from 0.1  $\mu$ g/kg ( $\gamma$ -HCH) to 11  $\mu$ g/kg ( $\beta$ -HCH). The mean concentration of DDTs was 270  $\mu$ g/kg (median 210  $\mu$ g/kg, results from 12.9 to 927  $\mu$ g/kg)

and PCBs 2.2  $\mu$ g/kg (median 2  $\mu$ g/kg, results from traces to 6  $\mu$ g/kg, occurrence 91%). Mean levels of DDTs in sediment from these sampling sites were up to 50 times higher than in sediments from other regions of the country [7]. The mean concentration of DDTs calculated for 49 sediment samples (without Cracow) was 5.3  $\mu$ g/kg (results from traces to 24  $\mu$ g/kg) and PCBs 0.4  $\mu$ g/kg (results up to 3.8  $\mu$ g/kg).

DDTs and PCBs can accumulate in sediments and be taken up by fish and other aquatic organisms. Assessment of sediment as toxic (contaminated) or non-toxic (relatively uncontaminated) appears to be complicated. Numerical sediment quality guidelines (SQGs) have been developed using a variety of approaches and some SQGs have been adopted by various agencies in Europe and worldwide. Nowadays, sediment quality assessment methods mainly rely on a chemical approach, i.e. contaminant concentrations are measured and then compared to available reference, target, recommended, or quality values. Consensusbased SQGs were derived for 28 common chemicals (organochlorine pesticides, metals, PCBs, and PAHs) in freshwater sediment [19]. Some SQGs have certain advantages but also several limitations, and they are often not reliable for evaluation of sediment toxicity [19-21]. Pollutants in environmental sediments almost always are present in mixtures, so multi-contaminant toxicity should also be investigated. Among different SQG values, PEL (probable effect level) and TEL (threshold effect level) were developed [18, 19]. TEL represents the concentration of contaminants in sediment below which harmful effects are expected to occur only rarely. PEL represents the concentration above which adverse effects are expected to occur frequently.

In the current study, concentrations of p,p'-DDE exceeded PEL in all sediment samples and were on average 36 times higher than the PEL. The concentrations of p,p'-DDD above PEL contained 20% (seven sediment samples) of all samples. The PEL value for p, p'-DDT was exceeded in 33 samples. In 32 (91.4%) sediment samples low levels of PCB congeners were detected (mean 2.2 µg/kg). The levels of OCPs detected in sediments from the Vistula River/Cracow indicate a need for a continuous monitoring. In 2004, concentrations of chlorinated pesticides higher than PEL and TEL values were found in sediments collected from Polish rivers [22]. The high contents of pesticides were recorded mainly in sediments near urban-industrial centres (the Vistula/Oswiecim, the Brda/Bydgoszcz). However, in the sediments examined in our study and in sediments collected from the Odra River and from the Sulejowski Reservoir rather low levels of PCBs were detected [7, 23, 24].

## Conclusions

Due to environmental contamination marine and freshwater fish can contain higher levels of PCBs, organochlorine pesticides, and other chemical contaminants than farm animals. The results of the study indicate that freshwater fish species and sediments from the Vistula River/Cracow location are contaminated by the analysed chlorinated hydrocarbons. Further studies are needed to explain the presence of such high levels of DDTs in this ecosystem. In that case, competent authorities should inform the public, including fishermen and their families, about health risks of consuming contaminated fish. Fish advisories often recommend either limiting or avoiding consumption of certain fish from specific sites, especially for sensitive populations such as pregnant women, nursing mothers, and young children.

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