

PERSISTENT ORGANOCHLORINE COMPOUNDS (PCBs, DDTs, HCB & HBDE) IN WILD FISH FROM THE LAKE BURGAS AND THE LAKE MANDRA, BULGARIA

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Abstract

*This study assessed the presence and concentrations of polychlorinated biphenyls (PCBs), DDT and its metabolites in tissues of freshwater fish collected from the Lake Burgas and the Lake Mandra in autumn 2014. Concentrations of organochlorine compounds were determined in three fish species: gibel carp (*Carassius gibelio*), roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*). The fish tissues were analyzed also for hexachlorobenzene (HCB) and hexachlorobutadiene (HCBT), named as priority substances in current European legislation regarding environmental quality standards (EQS) in biota. The aims of the study were to investigate the presence of pollutants in wild fish species from the lakes near Burgas City, Bulgaria and to compare the results to the levels in other aquatic ecosystems.*

The fifteen congeners of PCBs, HCB, HCBT, DDT and its two main metabolites DDE and DDD were determined by capillary gas chromatography system with mass spectrometry detection. The levels of organochlorine pollutants in the wild fish were found in the order DDTs > PCBs. Among the DDTs, essentially only the metabolites p, p'-DDE and p, p'-DDD were found. The sum of DDT and its metabolites was determined from 1.81 to 3.99 ng/g wet weight (in perch and gibel carp, respectively) and PCBs concentrations were found below 1.60 ng/g ww. The other contaminants HCB and HCBT were found below the analytical detection limit.

The levels of persistent organochlorine pollutants investigated were found lower compared to those in fish species from other aquatic ecosystems.

Key words: fish, polychlorinated biphenyls, organochlorine pesticides, hexachlorobenzene, hexachlorobutadiene, Bulgaria

1. INTRODUCTION

Organochlorine pollutants such as polychlorinated biphenyls (PCBs), 1,1,1-trichloro - 2, 2 - bis (4-chlorophenyl) ethane (DDTs) and its metabolites, hexachlorobenzene (HCB) and hexachlorobutadiene (HBDE) are considered as harmful for the environment and especially for aquatic ecosystems. Due to their stable structure and lipophilic character, they tend to concentrate and magnify in the food chain particularly associated with fat (Bordajandi et al., 2006).

The European Union Water Framework Directive (WFD) lays down a strategy against pollution all european waters (rivers, lakes, ground and coastal waters) and requires Member States to implement specific measures for pollution control and prevention (EU, 2000). In this respect one of the EU Directives lays down the Environmental Quality Standards (EQS) for 33 priority substances and other 8 pollutants with the aim of achieving a good chemical status of surface water (EU, 2008, Majoros et al., 2013). The EQS is set for prey tissue (wet weight) and member states being able to choose "the most appropriate indicator from among fish, molluscs, crustaceans and other biota;" (EU Directive, 2008). Fish is a suitable indicator for the environmental pollution monitoring because they concentrate pollutants in their tissues directly from water, but also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web (Fisk et al., 2001). Data on the presence and distribution of organohalogenated contaminants in fish and especially edible fish species are therefore important not only from ecological, but also human health perspective (Macgregor et al., 2010).

Various studies have proved that organochlorine compounds exhibit several types of toxicity in humans and marine organisms, especially the neurotoxicity (Tanabe et al., 1997; Langer et al., 2003). For these reasons, most countries have restricted or banned the use of PCBs and organochlorine

pesticides since 1970s. Polychlorinated biphenyls have been widely used by a large variety of industries over the past 50 years. DDT is a chlorinated pesticide widely used in the past to control the spread of insects and other agricultural pests.

Hexachlorobenzene (HCB) is a hydrophobic and highly persistent chemical (Verweij et al., 2004). In 2002 it remained a consistent and widespread atmospheric contaminant across Europe (Jaward et al., 2004). HCB was mainly used as a solvent in the production of rubber and other polymers. Other uses were in agriculture as a seed dressing, in hydraulic fluids and a number of other industrial processes (Jürgens et al., 2013). HCB and HCBd are also named as priority substances under the EU Water Framework Directive (EU, 2013).

Lake Burgas (Lake Vaya) located near the Black Sea west of the city of Burgas, is the largest natural lake in Bulgaria, with an area of 27.60 km². It is up to 103 feet deep. An important fish-producing reservoir in the past, Lake Burgas lost much of its economic importance after the construction of the petrochemical plant near the city, but has witnessed an increasing number of species and decreasing pollution in recent years. Lake Mandra is the southernmost of the Burgas Lakes, located in the immediate proximity of the Black Sea and close to Burgas. It was a brackish natural lake until 1963, when it was turned into a reservoir with the construction of a dam to secure fresh water for the large Neftochim Burgas oil refinery. The rivers Izvorska, Fakiyska, Sredetska and Rusokastrenska flow into the lake. Parts of Lake Mandrensko are designated protected areas inhabited by a number of locally and globally endangered species of fish and birds.

To our knowledge, no data on levels of organohalogenated pollutants in fish in this aquatic ecosystem are present in the literature. The aim of the present study was to investigate the presence of organohalogenated contaminants PCBs, DDTs, HCB and HCBd in several fish species from Lake Burgas and Lake Mandra, lakes with great economic importance for the region.

The gibel carp (*Carassius gibelio*) is a member of the family Cyprinidae, which includes many other fish, such as the common carp. It is a medium-sized cyprinid, and does not exceed a weight of 6.6 pounds (3 kg) and a size of 45 cm. They are omnivorous and feed on plankton, invertebrates, plant material and detritus. They inhabit lakes, ponds, and slow-moving rivers throughout Europe and Asia. The roach (*Rutilus rutilus*), also known as the common roach, is a fresh and brackish water fish of the Cyprinidae family, native to most of Europe and western Asia. In the cold season, they migrate to deep waters, where they form large and dense shoals (small inland harbours are a favourite). The common roach eats plant material, bottom-dwelling (benthic) invertebrates, and plankton. Young fish feed mainly on plankton, while the mature fish feed mainly on benthos. The perch (*Perca fluviatilis*) is a carnivorous fish most commonly found in small ponds, lakes, streams, or rivers. These fish feed on smaller fish, shellfish or insect larvae.

2. EXPERIMENTAL

2.1. Sampling and sample preparation

Three wild fish species: gibel carp (*Carassius gibelio*), roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) were caught by local professional fishermen by net along the Lake Burgas and the Lake Mandra between September and November 2014 (Fig. 1).



Fig. 1. The map of sampling areas – Lake Burgas and Lake Mandra.

Samples were immediately transferred to the laboratory in foam boxes filled with ice and were stored in a freezer (-18°C) until analysis. The edible tissue of each fish was homogenized using a blender; pools of about 300 g were made with fillets taken from several individual fish. The fish species was selected according to their characteristic feeding behaviour (Table 1).

Table 1. Biometric data of fish, habitat and feeding habit

Fish species	n	Length, cm	Weight, g	Habitat	Feeding habit
gibel carp (<i>Carassius gibelio</i>)	9	17.9 ± 2.5	117 ± 58	benthic species: gravely and muddy bottoms	omnivorous and feed on plankton, invertebrates, plant material and detritus
roach (<i>Rutilus rutilus</i>)	11	11.7 ± 1.1	21.4 ± 3.7	fresh and brackish water	plant material, bottom-dwelling (benthic) invertebrates, and plankton
perch (<i>Perca fluviatilis</i>)	12	22.8 ± 1.5	228 ± 17	small ponds, lakes, streams	small fish, shellfish and insect larvae

n – number of samples

2.2. Chemical analysis

Samples were prepared according to a previously described method (Stancheva et al., 2013). Briefly, the edible tissues of fish were homogenized and twenty grams were taken for extraction. Each sample was spiked with internal standards PCB 30 and PCB 204. The compounds were extracted with hexane / dichloromethane (3/1, v/v) in Soxhlet Extractor. After lipid determination, the extract was cleaned-up

on a glass column packed with neutral and acid silica. PCBs and DDTs were eluted with 80 ml n-hexane followed by 50 ml n-hexane/ dichloromethane (80:20 v/v). The eluates were concentrated to near dryness and reconstituted in 0.5 cm³ in hexane.

Gas chromatographic analysis of the DDTs, PCBs, HCB and HCBd were carried out by GC FOCUS (Thermo Electron Corporation, USA) using POLARIS Q Ion Trap mass spectrometer and equipped with an AI 3000 autosampler. Experimental mass spectrometry parameters are the following: the Ion source and Transfer line temperatures were 220°C and 250°C, respectively. The splitless Injector temperature was 250°C. For DDTs, HCB and HCBd determination the oven was programmed as follows: 60°C (1 min), 30°C/min to 180°C, 5°C/min to 260°C, 30°C/min to 290°C with a final hold for 3.0 min. The PCBs experimental temperature program - 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 coated with cross-linked 5% phenyl methyl siloxane 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 solutions (EPA 625/CLP Pesticides Mix 2000 µg/ml - Supelco and PCB Mix 20 - Dr. Ehrenstorfer Laboratory, see Fig.2 and Fig. 3) were used for instrument calibration, recovery determination and quantification of compounds. Measured compounds were p,p'-DDT, p,p'-DDD and p,p'-DDE, HCB, HCBd and PCB congeners: IUPAC № 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 170, 180). Each sample was analyzed three times and was taken an average of the results obtained. The limits of quantification (LOQ) varied for individual OCs from 0.02 to 0.05 ng/g ww.

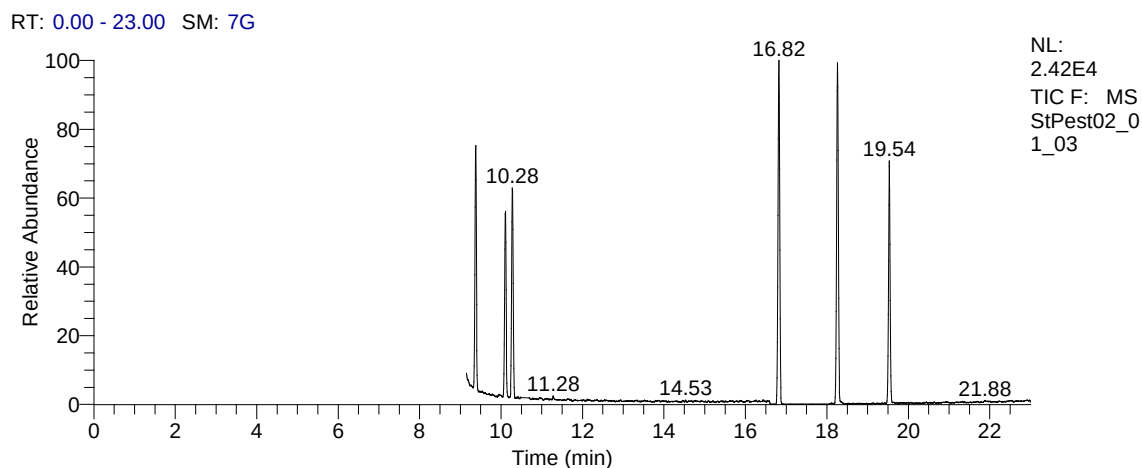


Fig. 2. GC-MS chromatogram of DDTs standard solution, 100 ng/ml

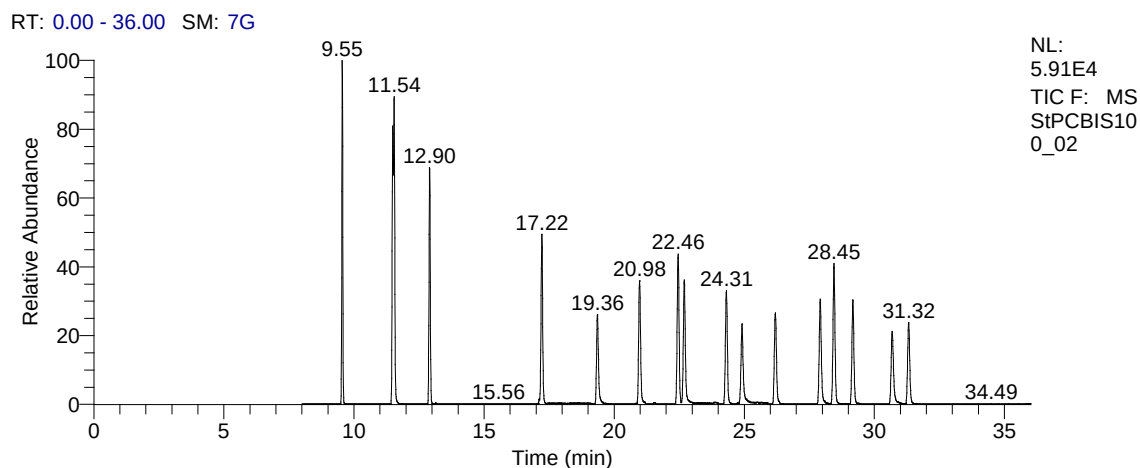


Fig. 3. GC-MS chromatogram of PCB standard solution, 100 ng/ml

2.3. Quality control

The quality control was performed by regular analyses of procedural blanks and certified reference materials: BCR - 598 (DDTs in Cod liver oil) and BB350 (PCBs in Fish oil) – Institute for Reference Materials and Measurements, European commission. 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.

3. RESULTS AND DISCUSSION

3.1. DDTs, HCB, HCBd levels

Because of their wide distribution in air, water, soil and food, p,p'-DDT and its metabolites remain a human health concern and have been quantified in the present study. Lipid contents and concentrations of p,p'-DDE, p,p'-DDD, p,p'-DDT, HCB, HCBd found in the samples, are present in Table 2. The lipid percentage ranged from 0.6% in perch to 2.2% in roach. The lipids in fish tissue are influenced by several factors, such as sex, age, species, nourishment and spawning status (Voorspoels et al., 2004). In the environment DDT metabolised slowly and the metabolite DDE is particularly persistent compound.

Table 2. Lipid content (%) and concentrations of DDTs, HCB and HCBd (ng/g ww)

Fish species	Lake Burgas		Lake Mandra	
	Gibel carp (<i>Carassius gibelio</i>)	Roach (<i>Rutilus rutilus</i>)	Roach (<i>Rutilus rutilus</i>)	Perch (<i>Perca fluviatilis</i>)
Lipids, %	1.3	2.2	1.3	0.6
HCB	< LOD	< LOD	< LOD	< LOD
HCBd	< LOD	< LOD	< LOD	< LOD
p,p' - DDE	2.53	1.97	2.23	1.46
p,p' - DDD	1.46	0.34	1.46	0.35
p,p' - DDT	< LOD	< LOD	< LOD	< LOD
Σ DDTs	3.99	2.31	2.59	1.81

The main metabolite p,p'-DDE was the abundant organochlorine contaminant in all samples. The concentrations of p,p'-DDE varied in the range from 1.46 to 2.53 ng/g wet weight (ww) in perch and gibel carp samples respectively.

The metabolite p,p'-DDE constituted more than 63% of the ΣDDTs for each species, followed by p,p'-DDD (14–37%) (Fig. 4). DDT did not found at detectable levels in investigate fish species.

Many authors assess the chronology of DDT inputs by using the DDE/ΣDDTs ratio [17]. In the present study, this value was calculated between 0.63 and 0.86 in gibel carp and roach, respectively. This suggests that these pesticides have not been recently used in agriculture after their ban. In all tested samples, the residues were found in the order of DDE > DDD > DDT and this is in agreement with the results of other studies (Covaci et al., 2006; Georgieva et al., 2012).

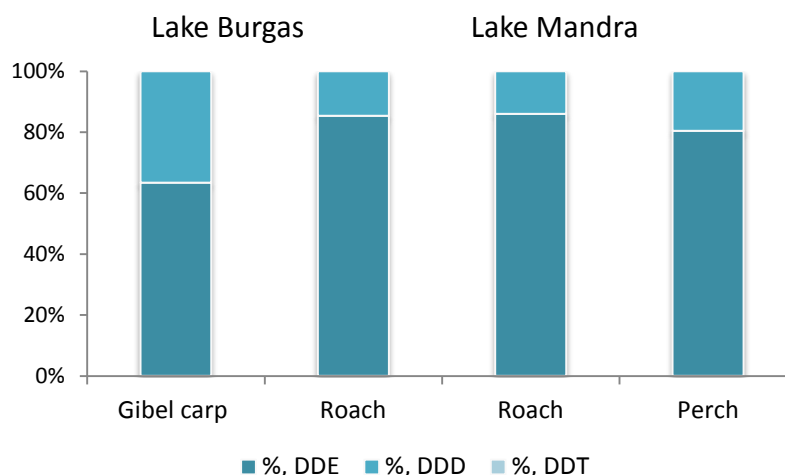


Fig. 4 Percentage distribution of DDE, DDD and DDT in fish species

The concentrations of DDTs in investigated samples gibel carp, roach and perch were found lower than those reported by Erdogrul, Ö., 2005 for carp from the Kahramanmaras, Turkey (median 77.4 ng/g ww) (Erdogrul et al., 2005), and those reported by Covaci, A. et al., 2006 from Danub Delta (2 847 ng/g lipid weight). Perch sampled from coastal waters of Latvia (Olsson et al., 1999) was found to contain 180-1100 ng/g lw DDTs and pikeperch from Danub Delta had concentration of DDTs 4 829 ng/g lw (Covaci, A. et al., 2006).

Regarding other organochlorine compounds determined concentrations of HCB and HCBd were all below detectable levels and did not exceed the European EQS of 10 µg/kg and 55 µg/kg, respectively. In a recent study of wild fish from four English rivers HCB was a maximum of 6 µg/kg in some eels (Jürgens et al., 2013). In a recent survey of eels in Scotland (Macgregor et al., 2010) HCBd was only detected in one of 150 samples at detection limits of either 1 or 3 µg/kg and the authors of a French study also failed to detect any HCBd in fish (Miege et al. 2012).

3.2. PCBs levels

The concentration of individual PCB congeners determined in fish species are shown in Table 1. 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 are called indicator PCB (noted with * in Table 3).

The PCB pattern found in fish showed a predominance of PCB 153 followed by PCB 138 for indicator PCBs. The predominance of hexachlorinated PCBs in fish species, especially PCB 153 and PCB 138, has been reported by several authors for different coastal areas in the Mediterranean (Naso et al., 2005) and in the Adriatic Sea (Perugini et al., 2004).

The lowest concentration of the PCBs was found in roach samples from Lake Burgas (0.98 ng/g ww) and the highest concentration - in gibel carp (1.60 ng/g ww). 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 experimental results showed no significant differences between mean concentrations of $\sum$ PCBs in investigated fish species. The higher levels of PCBs in gibel carp compared to other fish species may be due to its feeding habit. Gibel carp probably receive large quantities of organochlorine pollutants present in the water and in the sediments through a process of bioconcentration.

Table 3. PCBs (ng/g ww) concentration levels determined in fish species collected from the Black Sea

PCB congeners	Lake Burgas		Lake Mandra	
	Gibel (<i>Carassius gibelio</i>)	carp Roach (<i>Rutilus rutilus</i>)	Roach (<i>Rutilus rutilus</i>)	Perch (<i>Perca fluviatilis</i>)
28*+31	0.27	0.14	0.13	0.12
52*	0.25	0.17	0.15	0.20
101*	nd	nd	nd	nd
77	nd	nd	nd	nd
118	nd	nd	nd	nd
153*	0.45	0.26	0.33	0.36
105	nd	nd	nd	nd
138*	0.35	0.21	0.28	0.22
126	nd	nd	nd	nd
128	nd	nd	nd	nd
156	nd	nd	nd	nd
180*	0.28	0.19	0.23	0.16
169	nd	nd	nd	nd
170	nd	nd	nd	nd
ΣPCBs, ng/g ww	1.60	0.98	1.13	1.06

*Indicator PCB, nd – not detection

The contamination degree with PCBs of fish samples from the Lake Varna was found lower than PCB levels found in other studies. PCB concentrations found by Erdogru et al., 2005 in carp and wels muscle from the Kahramanmaras, Turkey ranged between nd - 4.8 and 0.39 - 42.3 ng/g ww, respectively. Barbel and chub from rivers of the North of Luxembourg contained Σ PCBs ranging from 29.6 to 158.2 ng/g ww and from 21.7 to 195.3 ng/g ww, respectively - reported by Boscher, A. et al., 2010.

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 (EC, 2011). Our results for I-PCBs in all fish species (max values 8.05 ng/g ww in golden grey mullet) did not exceed this limit. The toxic dl-PCBs in all samples showed concentrations below LOD.

4. CONCLUSION

DDTs were the predominant organohalogenated contaminants in all species, with the p,p- DDE contributing to more than 63% to the total DDTs. In all samples DDT was present only in the form of its metabolites p,p'- DDE and p,p'- DDD, suggesting previous contamination. The analysis of fish tissues of gibel carp, roach and perch showed a mean total load of DDT pollutants 2.68 ng/g ww, respectively. The concentrations of HCB and HCBd were found below detectable levels and did not exceed the European EQS of 10 µg/kg and 55 µg/kg, respectively.

The mean residue concentrations of PCBs in fish tissue quantified in our study were found 1.10 ng/g ww. The residues of PCBs varied in the range between 0.98 ng/g ww in roach (Lake Burgas) and 1.60

ng/g ww in gibel carp. The very low levels of PCBs observed in fish tissues correspond with the fact that no industrial PCBs production in Bulgaria.

In general, concentrations of DDTs, HCB, HCBd and PCBs in fish species: gibel carp, roach and perch from the Lake Burgas and Lake Mandra were found lower or similar to levels measured in the fish species from European rivers or other lakes.

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