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ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS IN FRESHWATER FISH

St. Georgieva, M. Stancheva, L. Makedonski

Department of Chemistry, Medical University – Varna,
Marin Drinov 55, 9002 Varna, Bulgaria,
e-mail: stanislavavn@mail.bg

ABSTRACT

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were determined in muscle tissue of three freshwater fish species: common carp *(Cyprinus carpio)*, grass carp *(Ctenopharyngodon idella)* and bighead carp *(Hypophthalmichthys nobilis)*. Fish samples were collected in 2010 from the Pyasachnik Dam Lake. The OCPs and PCBs were analysed in order to evaluate the status and potential sources of pollution in area of the lake. The species were selected because of their importance to local human fish consumption.

The fifteen congeners of PCBs, p,p'-DDT and its two main metabolites p,p'-DDE and p,p'-DDD were determined by capillary gas chromatography system with MS detection. DDTs were the predominant organohalogenated contaminants in all species, with the p,p' – DDE contributing to more than 67% to the total DDTs. All samples of muscle tissue examined contained detectable levels of p,p'-DDE and p,p' – DDD. The residues of p,p' – DDT were not detected in all samples. The sum of the individual PCB congeners

was determined lower than those found in similar fish species from other aquatic ecosystems.

Key words: *organochlorine pesticides; polychlorinated biphenyls; fish; Bulgaria*

INTRODUCTION

Organochlorine pesticides (such as DDT and its metabolites DDE and DDD) and polychlorinated biphenyls (PCBs) are widely distributed environmental pollutants. They characterised by a high bioaccumulation potential in food chains and therefore may pose a serious threat to upper trophic levels of aquatic communities [1]. Chlorinated pesticides such as DDT are effective pest control chemicals, wich were used in agriculture worldwide in the past and are still in use in many developing countries (malaria eradication, etc.). Polychlorinated biphenyls are group of chemicals primarily used in transformers, capacitors, paints and printing inks, and also in many other industrial applications. DDTs and PCBs are readily accumulated by aquatic organisms [2], and although banned decades ago, remain a concern in many aquatic systems [3].

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. In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems [4]. Once released into the aquatic environment, these chemicals biomagnify in the food web, and consequently high trophic level organisms may accumulate high concentration in their fatty tissues [5, 6].

The Pyasachnik Dam Lake is located 50 km from Plovdiv, Bulgaria. It is the largest dam lake in this region. Its water are used for water-supply and irrigation. Potential sources of pollution are agricultural activity along the dam and long-range atmospheric transport. To our knowledge, no studies carried out on levels of organohalogenated pollutants in fish in this aquatic ecosystem.

The aim of the present study was to investigate the presence of organochlorine pesticides (DDT and its metabolites DDE and DDD) and poly-

chlorinated biphenyls (PCBs) in several fish species from Pyasachnik Dam Lake.

MATERIALS AND METHODS

Sampling

Three freshwater fish species: common carp *(Cyprinus carpio)*, grass carp *(Ctenopharyngodon idella)* and bighead carp *(Hypophthalmichthys nobilis)* were obtained from fishermen along the Pyasachnik Dam Lake between May and June 2010. The samples were transferred immediately to the laboratory in foam boxes filled with ice and were stored in a freezer (-18°C) until analysis.

The species were selected for their importance to local human fish consumption. Common carp is omnivorous – can eat water plants, but prefer to scavenge the bottom for insects, crustaceans, crayfish and benthic worms. The grass carp is herbivorous – adults of the species feed primarily on aquatic plants. Bighead carp is primarily filter feeder and preferentially consume zooplankton but also phytoplankton and detritus.

DDTs and PCBs analysis

The analytical method for determination of residues of OCP and PCB was based on BDS EN 1528:2001. Briefly, the edible tissues of fish were homogenized and sub-samples of 20 g were taken for extraction. Each sample was spiked with internal standards PCB 30 and PCB 204. These standards were used to quantify the overall recovery of the procedures. The OCs were extracted with hexane / dichloromethane (3/1, v/v) in Soxhlet apparatus. After lipid determination, the extract was cleaned-up on a glass column packed with neutral and acid silica. PCBs and OCPs were eluted with 80 ml n-hexane followed by 50 ml n-hexan/dichloromethane (80:20). The eluates were concentrated to near dryness and reconstituted in 0.5 ml in hexane.

Gas chromatographic analysis of the DDTs and PCBs 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 MS 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 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. The selectivity of the IT–MS/MS method was based on the appropriate selection of parent ions for the detection of each analyte by mass spectrometry extracted ion mode.

Pure reference standard solutions (EPA 625/CLP Pesticides Mix 2000 μg/ml – Supelco and PCB Mix 20 – Dr. Ehrenstorfer Laboratory), were used for instrument calibration, recovery determination and quantification of compounds. Measured compounds were p,p'-DDT, p,p'-DDD and p,p'-DDE, PCB congeners: IUPAC № 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 170, 180).

The detection limit of the method (LOD) varied from 2 to 5 ng/g lipid weight for PCBs and from 1 to 5 ng/g for the DDT and its metabolites. The recoveries were within 73–108 %. Recoveries were determined by adding known amounts of PCBs and DDTs standards (at three levels of concentrations) to empty samples before extraction. The RSD values with five times repeatedly determined was less than 16%.

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.

RESULTS AND DISCUSIONS

DDT and its metabolites

Lipid contents and concentrations of p,p'-DDE, p,p'-DDD and p,p'-DDT found in muscle of selected fish species, average of duplicate measurements, are present in Table 1. In the environment DDT metabolised slowly and the metabolite DDE is particularly persistent compound [7]. The metabolite p,p'-DDE constituted more than 67% of the ΣDDTs for each species, followed by p,p'-DDD (12 – 32%). The concentrations of p,p'-DDE in common carp, grass carp and bighead carp muscle samples were 18.81, 7.45, 20.42 ng/g ww (wet weight), respectively. DDE concentration found in common carp of the present study is lower then concentration levels of carp from the Kahramanmaras, Turkey (4–156 ng/g ww) [8].

Table 1. *Lipids (%) and concentrations of DDTs (ng/g wet weight) in freshwater fish*

fish / compound	common carp *(Cyprinus carpio)*	grass carp *(Ctenopharyngodon idella)*	bighead carp *(Hypophthalmichthys nobilis)*
Lipids, %	8.39	3.24	0.66
p,p' – DDE	18.81	7.45	20.42
p,p' – DDD	9.15	3.27	2.95
p,p' – DDT	0.00*	0.00	0.00
Σ DDTs (ng/g ww)	**27.06**	**10.72**	**23.37**

**Values below LOD were set to zero.*

In our study the p,p'-DDE / p,p'-DDT ratio in the muscle of the investigated species correlated with exposure to DDT in the past (high p,p'-DDE concentrations and low p,p'-DDT content). Another metabolite of DDT, p,p'-DDD, was also found, but in lower amounts than p,p'-DDE. The p,p-DDT in all analyzed fish species were found under limit of detection of the method.

ΣDDTs (sum of p,p-DDE, p,p-DDD and p,p-DDT) in common carp, grass carp and bighead-carp muscle samples were measured 27.06, 10.72, and 23.37 ng/g ww, respectively (Table 1). The concentrations of DDTs in common carp samples from Pyasachnik Dam Lake were found lower than those reported by Erdogrul, Ö., 2005 for carp and nose carp from the Kahramanmaras, Turkey (4.5 – 170 and 8.4 – 246 ng/g ww, respectively) [8], and those reported by Covaci, A. et al., 2006 from Danub Delta (2 847 ng/g lipid weight) [9]. Grass carp and bighead carp sampled from Pearl River Delta, China (Kong et al., 2005) [10] were found to contain 7.4 – 20.57 ng/g ww and 1.5 – 25.33 ng/g ww DDTs, respectively.

PCBs contamination

The concentrations of individual PCBs congeners found in different fish species are summarized in Table 2. The most persistent PCB congeners (PCB 138, 153 and 180) were found only in fish tissues of common carp and bighead carp, but with a lower contribution to PCBs than reported for similar species from other rivers and lakes in Europe [8, 11]. They are defined by WHO as important for evaluating the risk to human health and are called indicator PCBs, such as PCBs 28, 52, 101, 118 (noted with * in Table 2).

ΣPCBs (sum of 15 congeners) were found in detectable levels in muscle tissues of common carp (7.52 ng/g ww), grass carp (0.98 ng/g ww) and of bighead carp (1.65 ng/g ww). The contamination degree with PCBs of the freshwater fish samples from the Pyasachnik Dam Lake were lower than PCB levels found in other countries. The average PCB levels in common carp from Lake Zemplinska Sirava (Slovak Republic) were identified 33.41 mg/kg fresh weight [11]. ΣPCB concentrations found by Erdogrul et al., 2005 [8] in carp and wels muscle from the Kahramanmaras, Turkey ranged between nd – 4.8 and 0.39 – 42.3 ng/g ww, respectively.

Table 2. *Concentrations of individual PCBs congeners (ng/g wet weight) in freshwater fish*

fish / **compound**	**common carp** ***(Cyprinus carpio)***	**grass carp** ***(Ctenopharyngodon idella)***	**bighead carp** ***(Hypophthalmichthys nobilis)***
PCB 28*+31	1.29	0.98	0.69
PCB 52*	nd	nd	0.08
PCB 77	nd	nd	nd
PCB 101*	nd	nd	nd
PCB 105	nd	nd	nd
PCB 118*	nd	nd	nd
PCB 126	nd	nd	nd
PCB 128	nd	nd	nd
PCB 138*	1.12	nd	0.02
PCB 153*	0.94	nd	0.19
PCB 156	nd	nd	nd
PCB 169	nd	nd	nd
PCB 170	1.96	nd	nd
PCB 180*	2.21	nd	0.67
Σ PCBs (ng/g ww)	**7.52**	**0.98**	**1.65**

*nd – not detection, * Indicator PCBs*

Other freshwater fish species – 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 [12].

CONCLUSIONS

In present study the analysis of fish tissues of carp, grass carp and bighead carp showed a mean total load of DDT pollutants 27.06, 10.72, and 23.37 ng/g ww, respectively. DDTs were the predominant organohalogenated contaminants in all species, with the p,p – DDE contributing to more than 67% 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 highest residue concentrations of PCBs quantified in our study were found in muscle tissue of common carp 7.52 ng/g ww. The residues of PCBs in bighead carp and grass carp were found lower. The very low levels of PCBs observed in fish tissues correspond with the fact that no industrial activities that lead to the spread of persistent organic pollutants in the area of Pyasachnik Dam Lake.

In general, concentrations of DDTs and PCBs in fish species: carp, grass carp and bighead carp from the Dam Lake Pyasachnik were found lower than levels measured in the same fish species from rivers and lakes in Europe and Asia.

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