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# ORGANOCHLORINE POLLUTANTS IN BLUEFISH *(POMATOMUS SALTATRIX)* FROM BULGARIAN BLACK SEA COAST

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**ABSTRACT**

Concentrations of 14 polychlorinated biphenyls (PCBs) and organochlorine pesticide dichlorodiphenyl- trichloroethane (p,p’- DDT) including its metabolites (p,p’- DDE and p,p’- DDD) were measured in muscle tissue samples of bluefish *(Pomatomus saltatrix)*. Samples were collected from Black Sea (region of Varna, Bulgaria) in the period of 2003–2006. DDTs and PCBs were determined by gas chromatograph equipped with electron-capture detector and mass spectrometry allowing better identification of compounds.

Total PCB concentration (sum of 14 congeners) in muscle tissue of bluefish varied in the range of 1.2 to 384.9 ng/g lipid weight. Concentrations in bluefish ranged from 367.1 to 879.5 ng/g lipid weight for total DDTs (sum of p,p’-DDT, p,p’-DDD and p,p’-DDE).

The levels of DDTs and PCBs in bluefish from region of Varna were found comparable or slightly higher than those found in fish from other parts of the Black Sea coast and from neighbor seas Marmara Sea and Aegean Sea.

*Keywords: polychlorinated biphenyls; organochlorine pesticides; fish; Black Sea; Bulgaria*

## INTRODUCTION

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are long-lived organic chemicals that are generally resistant to chemical and biological degradation processes. Organochlorine compounds (OCs) are among the most dangerous pollutants because of their high liposolubility and tendency to bioaccumulate along the food chain. As a consequence, they are widespread in the biotic compartment of the environment [1].

Pesticides and polychlorinated biphenyls (PCBs) are found in various parts of the environment in quite small concentrations, but they accumulate and thus become a threat to human health and life [2, 3]. PCBs and OCPs were measured in sediments collected in 2000 from the mouth of the Danube Delta and it was found that the Danube river is a potential source of contamination to the Black Sea [4, 5].

Fish are an excellent indicator for pollution in aquatic ecosystems, where trace contaminants are difficult to analyze directly. Fish consumption is the main source of human exposure to different environmental contaminants like PCBs and DDTs. There are only few data available on residue concentrations of OCPs in fish from the Bulgarian coast of the Black Sea [6].

The bluefish *(Pomatomus saltatrix)* enters the Black Sea in spring for feeding and spawning and moves back to the Sea of Marmara in winter. It feeds primarily on fish (anchovy, horse mackerel and young mackerel) and partially on crustaceans (shrims).

The purpose of this study was to determine the levels of persistent organochlorine contaminants in bluefish from the Bulgarian Black Sea coast and to monitor the accumulation of these pollutants during the period 2003–2006.

## MATERIALS AND METHODS

Fish samples were collected from Black Sea – region of Varna, Bulgaria in the period of 2003 – 2006. The fish were immediately frozen (-18$^{o}$C) after sampling.

Conventional methods for the determination of organochlorine compounds in fatty samples usually involve clean-up steps, including multiple digestion of extracts, acid digestion followed by liquid chromatography or gel permeation chromatography (GPC) combined with adsorption liquid chromatography [7, 8]. Most of these methods use GC/ECD or GC/MS for analytical determination [9, 10]. GC with IT-MS/MS detection provides high confidence in identification of target analytes, based on a selected parent ion and a whole mass spectrum of its daughter ions, high sensitivity and selectivity.

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 subsamples of 20 g were taken from it 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.

The PCBs were analyzed by a Perkin Elmer Autosystem XL gas chromatograph equipped with an electron capture detector. A Restek Rtx-5 capillary column (60m

length, 0.25mm ID, 0.25 μm film thickness) was used for separation of organochlorines. The experimental conditions were as follows: split/splitless injector temperature – 250°C, detector temperature – 310°C, oven temperature – 120°C for 1 min, then programmed at a rate of 2°C/min to 320°C and hold 15 min. Helium was used as the carrier gas. Pure reference standard solutions were used for instrument calibration, recovery determination, and quantification (PCB Mix 20, – Dr. Ehrenstorfer Laboratory, IUPAC № 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 180).

Gas chromatographic analysis of the DDTs were carried out by GC FOCUS (Thermo Electron Corporation, USA) equipped with an AI 3000 autosampler and using POLARIS Q Ion Trap mass spectrometer. Experimental mass spectrometer 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. The oven was programmed as follows: 60°C (1 min), 30°C/min to 180°C, 2°C/min to 260°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) were used for instrument calibration, recovery determination and quantification of p,p'-DDT, p,p'-DDD and p,p'-DDE.

The detection limit of the method varied from 0.2 to 0.5 ng/g lipid weight for PCBs and from 0.2 to 0.8 ng/g for the DDT and its metabolites.

Recoveries were determined by adding known amounts of PCBs and DDTs standards (at three levels of concentrations) to empty samples before extraction. The recoveries were within 73–108%.

The RSD values with five times repeatedly determined was less than 16%. A procedural blank and a spiked sample with standards were run to check for the interference and cross-contamination.

## RESULTS AND DISCUSIONS

### 1. PCBs levels.

Concentrations of individual PCBs congeners found in the fish studied, average of duplicate measurements, are listed in Table 1. Total PCB concentration (sum of 14 congeners) in muscle tissue of bluefish varied in the range of 1.2 to 384.9 ng/g lipid weight. Results were reported as „not detectable" (nd) when the concentrations were lower than the detection limits.

For monitoring of PCB burden, food samples are analyzed usually for seven indicator PCBs (IUPAC № 28, 52, 101,118, 138, 153, 180), noted with *. They are defined by WHO as important for evaluating the risk to human health.

**Table 1.** *Lipid content (%) and concentrations of individual PCBs congeners (ng/g lipid weight) in bluefish*

| **Bluefish** | **2003** | **2004** | **2006** |
|---|---|---|---|
| Lipids (%) | 10.6 | 14.7 | 12.9 |
| PCB 28* | nd | 0.4 | nd |
| PCB 31 | 2.1 | nd | nd |
| PCB 52* | 2.1 | nd | 43.6 |
| PCB 77 | 7.0 | nd | nd |
| PCB 101* | 3.2 | nd | 38.5 |
| PCB 105 | 6.0 | 0.2 | 47.5 |
| PCB 118* | 4.4 | nd | 56.7 |
| PCB 126 | nd | nd | nd |
| PCB 128 | 3.7 | 0.6 | nd |
| PCB 138* | nd | nd | 78.9 |
| PCB 153* | 7.9 | nd | 70.1 |
| PCB 156 | nd | nd | nd |
| PCB 169 | nd | nd | nd |
| PCB 180* | nd | nd | 49.6 |
| Sum PCBs (ng/g lw) | **36.4** | **1.2** | **384.9** |
| Sum PCBs* (ng/g lw) | 17.6 | 0.4 | 337.4 |

In general, our results indicate that PCB contamination of bluefish from the Black Sea in 2006 is slightly higher compared to the results from bluefish of the Marmara Sea, where concentrations as sum of 7 congeners were found 319.0 ng/g fat [11]. In the period 2003 -2004 the levels of PCBs in muscle tissue of bluefish from Bulgarian coast of Black Sea were found lower than results in 2006.

**2. DDT and its metabolites.**

Figure 1 showed the distribution pattern of p,p'-DDE, p,p'-DDD and p,p'-DDT in bluefish from Bulgarian part of the Black Sea. Concentrations of individual metabolites found in the fish were reported like average of duplicate measurements.

In all tested fish samples, the residues were found in the order of DDE > DDD > DDT and this is in agreement with the results of other authors [3, 4]. In all samples DDT was present mainly in the form of its metabolites p,p'-DDE and p,p'-DDD, suggesting previous contamination.

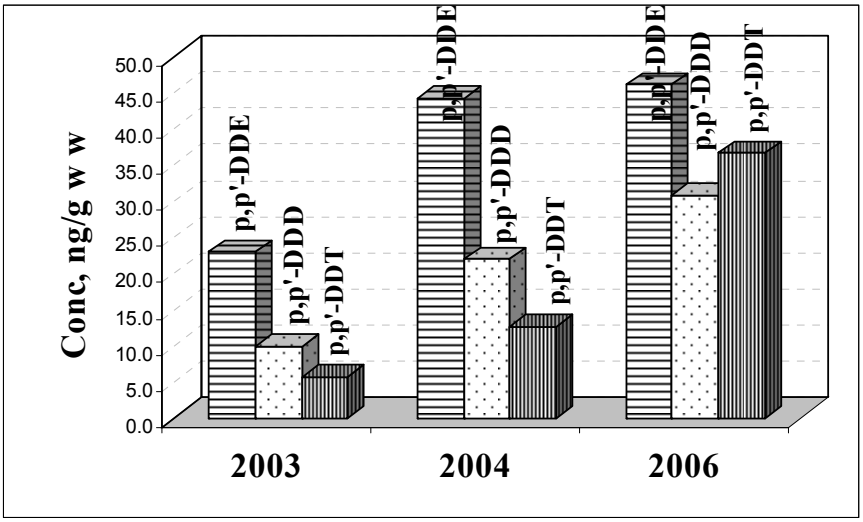


**Figure 1.** *Distribution pattern of p,p'-DDE, p,p'-DDD, p,p'-DDT in bluefish*

Concentrations in muscle tissue of bluefish ranged from 367.1 to 879.5 ng/g lipid weight for total DDTs (sum of p,p'-DDT, p,p'-DDD and p,p'-DDE). The results are presented in Figure 2. The analysis of bluefish during the study period 2003 – 2006 showed a mean total load of DDT pollutants 595.0 ng/g lipid weight.

Comparison between levels of the PCBs and DDTs contamination in bluefish are presented in Figure 2.

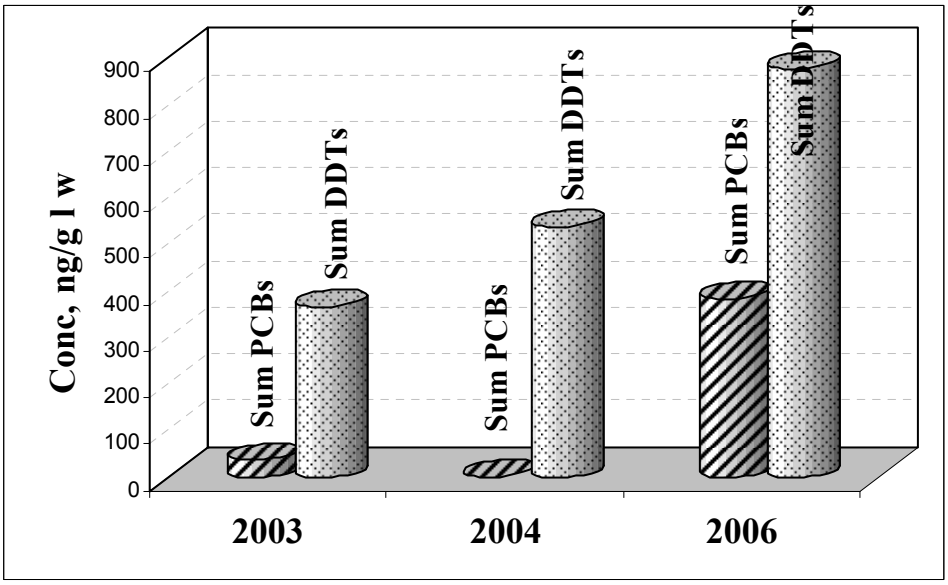


**Figure 2.** *Levels of DDTs and PCBs in bluefish during the period of study 2003 – 2006*

DDT group components are the main organochlorine contaminant in muscle tissue of bluefish during the all period of study. The levels of DDTs increased during the period 2003 – 2006.

## CONCLUSIONS

The mean residue concentrations of PCBs in muscle tissue of bluefish quantified in our study are between 1.2 and 384.9 ng/g lipid weight. The analysis in muscle tissue of bluefish during the study period showed a mean total load of DDT pollutants 595.0 ng/g lipid weight.

Concentrations of organochlorine contaminants in bluefish increased during the period of study 2003–2006.

The levels of DDTs and PCBs in bluefish from region of Varna were comparable or slightly higher than those found in fish from other parts of the Black Sea coast and from neighbor seas Marmara Sea and Aegean Sea.

The experimental data present initial investigations from a profound study of PCBs and DDTs in fish and seafood from Black Sea.

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