# POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN TURBOT *(PSETTA MAXIMA MAEOTICA)* FROM BULGARIAN BLACK SEA

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## ABSTRACT

**Persistent organochlorine pollutants (POPs) like polychlorinated biphenyls (PCBs) and DDT residues (DDTs) were determined in muscle tissue of turbot *(Psetta maxima maeotica)*. The samples were collected from Black Sea, near cape Kaliakra, during 2007 – 2011. The fifteen congeners of PCBs, p,p'-DDT and its metabolites p,p'-DDE and p,p'-DDD were determined by capillary gas chromatography system with mass spectrometry detection. The Indicator PCBs UPAC № 180, 153, 138, 118 were found most frequently and abundantly. The sum of the Indicator PCBs ranged from 3.80 to 13.94 ng/g wet weight and did not exceed the European maximum limit. The values of toxic equivalency (TEQs) of the 6 "dioxin–like" PCBs were calculated in the range from 0.02 to 0.09 pg TEQ/g ww and did not exceed the European limit of 6.5 pg TEQ/g ww. Among the DDTs, only p,p'-DDE and p,p'-DDD were found at levels 14.45 and 5.05 ng/g wet weight, respectively. The levels of PCBs and DDTs in turbot were determined comparable to those found in fish species from other aquatic ecosystems.**

**Key words:** *PCBs, DDTs, TEQs, turbot, Black Sea, Bulgaria*

## INTRODUCTION

Persistent organic pollutants (POPs) are lipophilic and bioaccumulate in fatty tissue. Polychlorinated biphenyls (PCBs) and DDT (1,1,1-trichloro - 2,2 - bis (4-chlorophenyl) ethane) were listed as POPs in the Stockholm Convention (30). Because of their persistence they biomagnify through the food chain, and human exposure to POPs is mainly via the diet, especially from food of animal origin which have shown to contain the highest levels (29)

The marine environment is particularly vulnerable to POPs because it acts as the final sink and consequently contains the major portion of these compounds (27). The production and intensive agricultural or industrial use of organochlorine pesticides (such as DDT and its metabolites DDE and DDD) or PCBs have led to widespread contamination of the environment. The contamination of POPs is a significant health problem because POPs can cause several adverse effects to human health and wildlife survival (9, 11). In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems (15,21). For these reasons, most countries have restricted or banned the use of PCBs and DDTs since 1970s. PCBs are a group of chemicals primarily used in transformers, capacitors, paints and printing inks, and also in many other industrial applications. The set of 6 Indicator PCBs IUPAC No 28, 52, 101, 138, 153, 180 (I-PCBs) was recommended by the European Union for assessing the pollution by PCBs (5). Twelve non-ortho and mono-ortho PCBs are classified as dioxin-like PCBs (dl-PCB), they show a similar toxicity as polychlorinated dibenzodioxins and polychlorinated furans. The dl-PCBs are used in or-

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der to estimate the toxicity potential of PCB exposure as toxic equivalency (TEQ) (31). DDT is a chlorinated pesticide widely used in the past to control the spread of insects and other agricultural pests. In the environment DDT metabolize slowly and the metabolite DDE the 1-1 dichloro-2,2-bis (*p*-chlorophenyl) ethylene is particularly persistent compound (10). DDT is still used for vector control against malaria (34).

Fish species are a suitable indicator for the environmental pollution monitoring because they concentrate pollutants in their tissues directly from water, but also through their diet (20,25,26). To our knowledge, there is very little information available on residue concentrations of POPs in fish and mammals from the Black Sea (14,28,33) and particularly from the Bulgarian coast of the Black Sea (22,24).

The purpose of this study was to determine the levels of PCB congeners and DDT (including its metabolites p,p'-DDE and p,p'-DDD) in muscle tissue of turbot *(Psetta maxima maeotica)* from the Bulgarian Black Sea coast and to monitor the presence of these pollutants during the 2007 – 2011.

## MATERIALS AND METHODS

### *Sampling and sample preparation*

The turbot was selected according to its characteristic feeding behavior and importance to human consumption in Bulgaria. The turbot is a predator species that lives on sandy, rocky or mixed bottoms; it is common in brackish waters. When juvenile, its diet is based on crustaceans. Adults feed mainly on other bottom-living fish (sand-eels, gobies, etc.) and to a lesser extent, on crustaceans and bivalves. Besides, its importance for fisheries the turbot could be considered the key species for monitoring of the Black Sea environment.

Samples were collected from Black Sea, near cape Kaliakra, Bulgaria during 2007 – 2011. The map of the study area is presented on Figure 1. The samples were transferred immediately to the laboratory in foam boxes filled with ice and were stored in a freezer (-20°C) until analysis. The length and weight of each specimen were measured and they were rinsed with distilled water to remove sand and impurities.


*Fig. 1. Black sea map and sampling area*

### *Analytical methods*

20 g of homogenized fish tissue was mixed with 100 g of anhydrous sodium sulfate and was extracted with hexane/dichloromethane (3/1, v/v) in Soxhlet apparatus. Each sample was spiked with internal standards PCB 30 and PCB 204. The organochlorine compounds were extracted with hexane/dichloromethane (3/1, v/v) in Soxhlet apparatus. The lipid content was determined gravimetrically of an aliquot of the extract (1/5th). 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-hexan/dichloromethane (80:20). The eluates were concentrated to near dryness and reconstituted in 0.5 ml in hexane. One micro liter of purified extract was injected into GC/MS.

Gas chromatographic analysis of the DDTs and PCBs were carried out by GC FOCUS (Thermo Electron Corporation, Austin, Texas, 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 was 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 (Bellefonte, PA, USA) 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

(Ion Trap masspectrometry) 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 Bellefonte, PA, USA and PCB Mix 20 - Dr. Ehrenstorfer Laboratory, Augsburg, Germany), were used for instrument calibration, recovery determination and quantification of compounds. Measured compounds were: p,p'-DDT and its metabolites: 1,1,- dichloro-2,2-bis (4-chlorophenyl) ethane (p,p'-DDD) and 1,1,-dichloro- 2,2-bis (4-chlorophenyl) ethylene (p,p'-DDE) and 15 PCB congeners: IUPAC № 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 170, 180). The sum of all 15 measured congener concentrations is referred below as ΣPCBs. The sum of p,p-DDE, p,p-DDD and p,p-DDT is referred below as ΣDDTs. The concentrations of the individual dl-PCB in a sample are multiplied by their respective toxic equivalency factors (TEF) and subsequently summed to give total concentration of dioxin-like compounds expressed in TEQs (31). TEFs were developed by the World Health Organization (WHO-TEF) (31). Each sample was analyzed three times and was taken an average of the results obtained. The limits of quantification (LOQ) varied for individual PCBs and DDTs from 0.2 to 0.5 ng/g ww.

### *Quality control and statistical analysis*

The quality control was performed by regular analyses of procedural blanks and certified reference materials: BB350 (PCBs in Fish oil) and BCR - 598 (DDTs in Cod liver oil) – Institute for Reference Materials and Measurements, European Commission. PCBs recovery varied in the range 85 -108% for individual congeners. Recovery of DDTs from certified reference material BCR – 598 were: DDE – 97.5%, DDD – 103.4% and DDT – 85.4%. Procedural blanks and a spiked sample with standards were analyzed between each 5 samples to monitor possible laboratory contamination.

The statistical analysis of the data was based on the comparison of average values by a t-test and a significance level of $p<0.05$ was used. PCBs and DDTs concentrations were log10-transformed to approximate a normal distribution of the data. All statistical tests were performed using SPSS 16 software.

## RESULTS AND DISCUSSION

### *PCBs levels*

The concentrations of individual PCBs congeners measured in turbot are shown in Table 1. ΣPCBs were found at concentrations ranging between 4.41 ng/g ww (2008) and 18.34 ng/g ww (2007).

*Table 1. PCBs (ng/g ww) concentration levels determined in turbot collected from the Black Sea*

| | Turbot *(Psetta maxima maeotica)* | | | |
|---|---|---|---|---|
| PCB | 2007 | 2008 | 2009 | 2011 |
| 28*+31 | < LOD | < LOD | 0.75 | < LOD |
| 52* | 1.05 | < LOD | < LOD | < LOD |
| 101* | 1.29 | 0.47 | 0.42 | 0.85 |
| 77 | < LOD | < LOD | < LOD | < LOD |
| 118 | 1.80 | 0.61 | 0.63 | 1.42 |
| 153* | 4.49 | 1.30 | 1.16 | 4.05 |
| 105 | 1.28 | < LOD | < LOD | < LOD |
| 138* | 4.86 | 1.18 | 1.03 | 2.37 |
| 126 | < LOD | < LOD | < LOD | < LOD |
| 128 | < LOD | < LOD | < LOD | < LOD |
| 156 | < LOD | < LOD | < LOD | < LOD |
| 180* | 2.25 | 0.85 | 0.83 | 1.71 |
| 169 | < LOD | < LOD | < LOD | < LOD |
| 170 | 1.32 | < LOD | < LOD | < LOD |
| ΣPCBs, ng/g ww | 18.34 | 4.41 | 12.70 | 5.91 |

** Indicator PCBs, LOD – limit of detection*

PCB pattern found in turbot showed a predominance of the hepta-, hexa-, and pentachlorinated PCBs 180, 153, 138, and 118. PCB 153 was the dominant congener in all investigated samples. The 153, 138, 180 and 118 congeners turned out to be the most abundant also due to their high lipophilicity, stability and persistence in the aquatic ecosystem. The predominance of hexachlorinated PCBs in marine fish species, especially PCB 153 and PCB 138, has been reported by several authors for different coastal areas in the Mediterranean Sea (17,23), in the Adriatic Sea (1,19) and in Marmara Sea (4).



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The sum of the six Indicator PCBs (∑Indicator PCBs) constituted from 76% to 87% of the total PCBs. The lowest concentration of the Indicator PCBs were found in turbot samples 2008 (3.80 ng/g ww) and the highest concentration - in 2007 (13.94 ng/g ww). The European Union has recommended a maximum level of 75 ng/g wet weight, calculated as the sum of the six Indicator PCBs in muscle meat of fish (8). Our results for Indicator PCBs in all samples did not exceed this limit.

The toxic dl-PCBs showed the lowest levels, specially the non-ortho congeners (PCBs 77, 126, and 169) with concentrations below LOD for most of the samples. Comparison of levels of Indicator PCBs and dioxin-like PCB is presented in Fig. 2. The results showed that the levels of ∑Indicator PCBs are much higher than the ∑dl-PCB throughout the study period. The experimental results showed significant differences between concentrations of ΣIndicator PCBs and ∑dl-PCBs ($p<0.05$). In our study mean values of toxic equivalency (TEQs) of the 6 dl-PCBs were calculated 0.04 pg TEQ/g ww. The comparison of our TEQ values in fish with those in the literature showed lower levels than the TEQs in sardine from Spanish Atlantic southwest coast (0.75 pg TEQ /g ww) (3) and lower than those in salmon from the Baltic Sea (12.6 pg TEQ /g ww) (13). The European Union has set a limit of 6.5 pg TEQ/g wet weight in muscle meat of fish for the sum of dioxins and dioxin-like PCBs (8). In our study TEQs for all investigated samples did not exceed this limit.

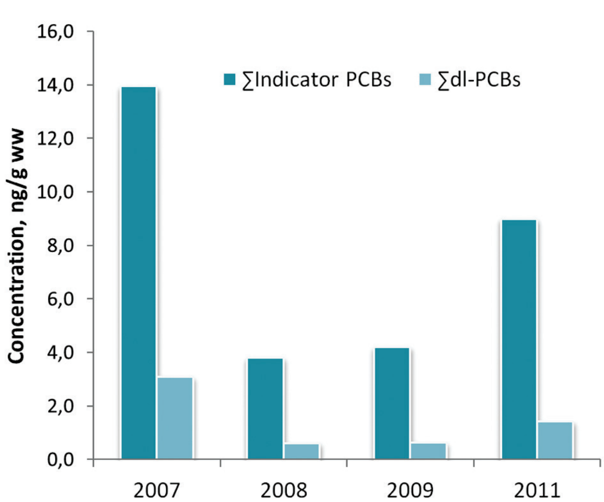


*Fig. 2. Comparison of Indicator PCBs and dl-PCBs in turbot from Black Sea*

The sum of I-PCB congeners are expressed also on a lipid weight basis (mean 472.5 ng/g lw) in order to compare our results with data cited in the literature. The experimental results indicate that PCB contamination in turbot from the Bulgarian Black Sea coast is lower than PCB concentrations in Atlantic mackerel (1096 ng/g lw) collected in the Central Adriatic Sea (19). The levels of indicator PCBs found in present study are lower than the results of fish species from Gulf of Naples, the Mediterranean Sea (1921.9 – 39406.5 ng/g lw), reported by Naso et al. 2005 (17) and from Tunis Bay (16). The low levels of PCBs observed in fish tissues correspond with the fact that no industrial production of PCBs took place in Bulgaria.

### *DDT and its metabolites*

The lipid content and concentrations of p,p'-DDT and its metabolites in investigated fish from the Black Sea coast of Bulgaria are presented in Table 2. The lipid percentage ranged from 0.89% in 2008 to 2.21% in 2007. The lipids in fish tissue are influenced by several factors, such as sex, age, species, nourishment and spawning status (2,32).

*Table 2. Lipid content (%) and concentrations of p,p'-DDT and its metabolites (ng/g ww)*

| Turbot (*Psetta maxima maeotica*) | | | | | |
|---|---|---|---|---|---|
| Year | Lipids, % | p,p-DDE, ng/g ww | p,p-DDD, ng/g ww | p,p-DDT, ng/g ww | ΣDDT, ng/g ww |
| 2007 | 2.21 | 33.32 | 13.03 | 3.17 | 49.51 |
| 2008 | 0.89 | 6.40 | 2.16 | < LOD | 8.56 |
| 2009 | 1.72 | 10.52 | 3.28 | 2.04 | 15.84 |
| 2011 | 1.53 | 7.57 | 1.76 | < LOD | 9.33 |

*LOD – limit of detection*

The main metabolite p,p'-DDE was the most frequently detected compound and was present in much higher concentrations than the other DDTs (ranging from 6.40 to 33.32 ng/g ww). p,p'-DDD was found in all samples at levels ranging from 1.76 ng/g ww (2010) to 13.03 ng/g ww (2007). The highest concentration of ∑DDTs was measured in turbot samples in 2007 (49.51 ng/g ww). DDT was presented mainly in the form of its metabolites p,p'-DDE and p,p'-DDD. The concentrations of individual DDTs were referred to ΣDDTs and percentage distribution of DDT and its metabolites in turbot is present in Fig. 3.

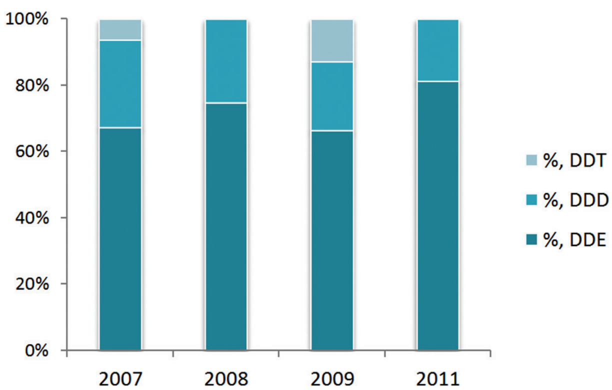


*Fig. 3. Percentage distribution of DDE, DDD and DDT in turbot. Each compound refers to ∑DDTs.*

In the environment decrease in DDT levels has been associated with a parallel increase in the relative abundance of its metabolized forms. The residues of DDT were found in the order of DDE > DDD > DDT and this is in agreement with the results of other studies (6,12,24). This suggests that these pesticides have not been recently used in agriculture after their ban.

Maximum residue limits for DDT and their metabolites in some foods of animal origin (meat, milk and eggs) were established by the European Union, but not yet for these organic compounds in fish. The established limit in those animal products is 1000 mg/kg on a lipid basis (7). Our results were found below this level.

It can be seen that the mean levels of DDTs (1103 ng/g lipid weight) detected in turbot from Black Sea coastal areas are comparable to those reported for fish species from other areas (4,10,17). In an earlier study by Tanabe et al., 1997 (28), total DDT concentrations in horse mackerel samples from the Black Sea were found higher - 3800 ng/g fat. Our results for DDTs in turbot were found lower than ∑DDTs in horse mackerel from Konya markets (Turkey) - 60.6 ng/g ww (14).

### *Estimated daily intake (EDI) of POPs*

More than 90% of the human exposure to persistent organic pollutants is estimated to come from food, especially fish and fish products (29). Dietary intake estimations are therefore important tools when estimating the exposure of POPs in a population. The results for contaminants in turbot were used to evaluate daily intake of DDTs by human through fish consumption. The exposure level for a particular contaminant is calculated by use of estimated daily intake EDI (ng/kg body weight/day).

The equation is as follows:

$$EDI = C \times FC / BW$$

EDI is the estimated daily intake ; C is the mean concentration of chemical contaminant (ng/g ww); BW is the consumer body weight (70 kg); FC is the per capita fish consumption (g/day).

The rates of the consumption of fish for the Bulgarian population were estimated to be 13.2 g/day per capita (18). The consumer body weight was 70 kg. The estimated daily intake of DDTs was calculated 3.9 ng/kg body weight/day and was far below the ADI (Acceptable daily intake) recommended by FAO/WHO – 20 000 ng/kg body weight/day (35). Estimated daily intake of DDTs in this study indicating that consumption of turbot does not represent human health risk.

## CONCLUSIONS

This study provides data on levels of organochlorine contamination in turbot from the Bulgarian Black Sea coast. The highest PCB concentrations were found in turbot in 2007 (13.94 ng/g ww) and lowest in 2008 (3.80 ng/g ww). The mean concentration of DDTs was found 20.20 ng/g ww. Levels of PCBs and DDTs in turbot were found comparable to levels measured in the marine fish species from other seas - Marmara Sea, Adriatic Sea and Mediterranean Sea. Our results for sum of Indicator PCBs in all samples did not exceed the EU maximum level of 75 ng/g ww. TEQs of the 6 “dioxin–like” PCBs were found 0.04 pg TEQ/g ww for turbot and did not exceed the EU limit of 6.5 pg TEQ/g ww. Estimated daily intake of DDTs in this study indicating that consumption of turbot does not represent a human health risk.

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