

RESIDUE LEVELS OF DDTs AND POLYCHLORINATED BIPHENYLS IN CULTURED FISH FROM FISH MARKETS IN BULGARIA

Stanislava Georgieva, Mona Stancheva, Lubomir Makedonski

Medical University - Varna, Marin Drinov 55, 9002 Varna, Bulgaria

Abstract

Organochlorine pesticides (DDT and its metabolites) and polychlorinated biphenyls (PCBs) were determined in four cultured fish species: gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*), brown trout (*Salmo trutta fario*) and rainbow trout (*Oncorhynchus mykiss*) from fish markets in Varna, Bulgaria in 2011. The species were selected regarding their importance to local human fish consumption. The purposes of the study were to investigate the presence of pollutants in cultured fish and to compare the results to the levels in wild freshwater fish species.

Analysis of DDTs and PCBs were performed using gas chromatography system with mass spectrometry detection. DDTs were the predominant organochlorine contaminants in all species, with the *p,p'*-DDE contributing to more than 70% to the total DDTs.

The sum of DDT and its metabolites was determined from 2.81 to 4.28 ng/g wet weight (in seabass and brown trout, respectively) and PCBs concentrations were found below 1.93 ng/g ww. The levels of persistent organochlorine pollutants studied in cultured fish were found lower compared to those in wild fish species from some lakes in Bulgaria.

Key words: fish, PCBs, organochlorine pesticides, Bulgaria

1. INTRODUCTION

Polychlorinated biphenyls (PCBs) and organochlorine pesticides like dichlorodiphenyltrichloroethane (DDT) are known as persistent compounds in aquatic systems and fish accumulate these substances both from the surrounding environment and from their diet (Antunes and Gil, 2004; Bordajandi et al., 2006). They are listed as persistent organic pollutants (POPs) in The Stockholm Convention. The Stockholm Convention (SC) on POPs is a global treaty to commit to human health and environmental protection from chemicals that are stable in the environment for years or even decades (SC, 2001). The POPs share several characteristics in common: are highly persistent; travel long distances through air and water; bio-accumulate in fatty tissue; and even at low levels they are highly toxic. Many of the 12 chemicals covered by the Stockholm Convention are no longer produced, and yet they persist in the environment (SC, 2001). Even though only a limited number of POPs are currently receiving attention, more may be identified through ongoing screening activities. Several studies have shown that food is the major contributor for PCBs and DDTs bioaccumulation (Loizeau et al., 2001; Covaci et al., 2006). Fish and seafood accumulate POPs and they may represent a major exposure route for persistent and bioaccumulative compounds from the aquatic systems into humans (Van Ael et al., 2012; Dorea, 2008). Several 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).

Polychlorinated biphenyls PCB are a group of indicative congeners, which are frequently the most abundant in various environmental matrices (Falandysz et al., 2004). PCBs 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. (Tanabe et al., 1997)

The purposes of the study were to investigate the presence of pollutants in cultured fish and to compare the results to the levels in wild freshwater fish species.

The gilthead seabream (*Sparus aurata*) occurs naturally in the Mediterranean and the Black Sea. The gilthead seabream inhabits seagrass beds and sandy bottoms as well as the surf zone. It is an euryaline species and moves in early spring towards protected coastal waters in search for abundant food and

milder temperatures (trophic migration). In late autumn it returns to the open sea for breeding purposes. It is mainly carnivorous (shellfish, including mussels and oysters), accessorially herbivorous (Froese R. and Pauly D., 2006).

The European seabass (*Dicentrarchus labrax*) has long been reared in traditional aquaculture. Its habitats include estuaries, lagoons, coastal waters, and rivers. It is found in the waters in and around Europe, including the Mediterranean Sea and the Black Sea. European seabass was one of the first types of fish after salmon to be farmed commercially in Europe.

Brown trout (*Salmo trutta fario*) lives in fast flowing, oxygen-rich, cool clear waters with gravel or sandy riverbeds. Brown trout are very faithful to their habitat (i.e. they live only at one spot and do not migrate), leaving it only to reproduce. Depending on size and habitat, they feed mainly on insects, small fish, small crustaceans, snails and other water animals.

The rainbow trout (*Oncorhynchus mykiss*) is a trout and species of salmonid native to cold-water tributaries of the Pacific Ocean in Asia and North America. Rainbow trout are predators with a varied diet and will eat nearly anything they can capture. Rainbow trout feed on larval, pupal and adult forms of aquatic insects. They also eat fish eggs and adult forms of terrestrial insects, small fish, crayfish, shrimp, and other crustaceans.

2. EXPERIMENTAL

2.1. Sampling and sample preparation

Four fish species: gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*), brown trout (*Salmo trutta fario*) and rainbow trout (*Oncorhynchus mykiss*) were collected from fish markets in Varna, Bulgaria in 2011. Samples were immediately transferred to the laboratory in foam boxes filled with ice. 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.

2.2. Chemical analysis

The method used for the preparation and clean-up of the samples has been previously described in detail (Stancheva et al., 2013) and is briefly presented below. Briefly, the edible tissues of fish were homogenized and twenty grams were taken for extraction. 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 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. 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) were used for instrument calibration, recovery determination and quantification of compounds. Measured compounds were p,p'-DDT, p,p'-DDD and p,p'-DDE 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 1 and Fig 2 present chromatograms of massspectrometry determination of DDT and PCB.

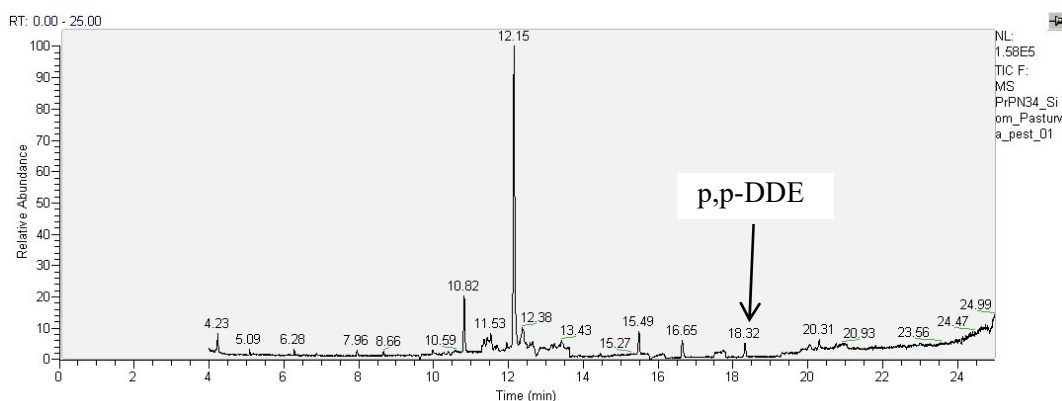


Fig. 1. GC-MS chromatogram of DDTs in Rainbow trout

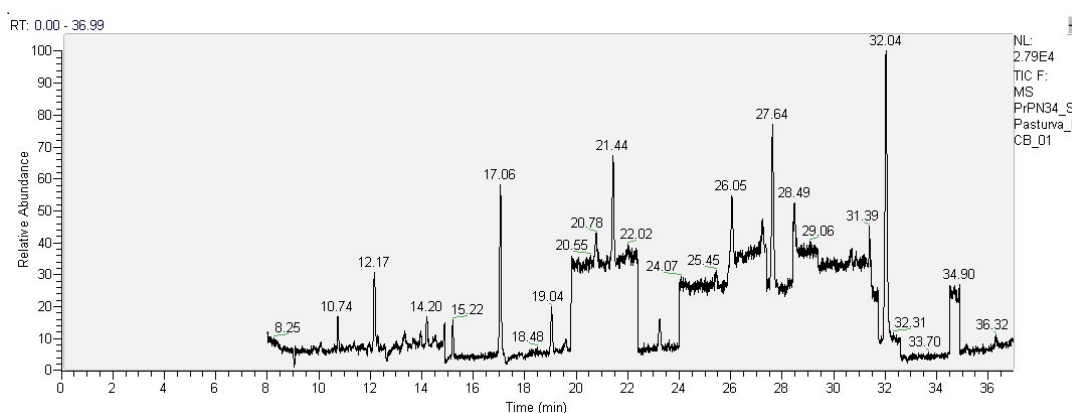


Fig. 2. GC-MS chromatogram of PCBs in Rainbow trout

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. PCBs levels

The set of six PCB congeners (IUPAC № 28, 52, 101, 138, 153 and 180) have been selected by the International Council for the Exploration of the Sea (ICES) for their abundance in environmental samples and have also been recommended by the European Union as indicators of PCB contamination (Giandomenico et al., 2013). The sum of the six indicator PCBs can be used as an appropriate marker for occurrence and human exposure to non dioxin-like PCBs (ndl-PCBs) because this value represents about 50% of the total ndl-PCBs in food (EFSA, 2010). We investigated also the levels of dioxin-like PCBs (non-ortho PCBs 77, 126, 169 and mono-ortho PCBs 105, 118, 156). The dioxin-like PCBs (dl-PCB) show a similar toxicity as polychlorinated dibenzodioxins and polychlorinated furans (Van den Berg et al., 2006).

The concentration of individual PCB congeners determined in fish species are shown in Table 1.

Table 1. Concentration levels of PCBs (ng/g wet weight) determined in fish species from fish market in Varna

PCB	Gilthead sea bream (<i>Sparus aurata</i>)	European sea bass (<i>Dicentrarchus labrax</i>)	Brown trout (<i>Salmo trutta fario</i>)	Rainbow trout (<i>Oncorhynchus mykiss</i>)
PCB 28+31	nd	0.46±0.09	nd	0.21±0.06
PCB 52	nd	nd	nd	nd
PCB 101	nd	nd	nd	nd
PCB 118	nd	nd	0.48±0.10	nd
PCB 153	0.65±0.16	0.50±0.10	0.76±0.19	0.36±0.11
PCB 138	0.54±0.12	0.48±0.11	0.69±0.15	0.22±0.07
PCB 180	0.40±0.08	nd	nd	nd
Total PCBs, ng/g ww	1.59±0.32	1.44±0.29	1.93±0.39	0.79±0.16

nd – not detected

The lowest concentration of the Total PCBs was found in rainbow trout (0.79 ng/g ww) and the highest concentration - 1.93 ng/g ww in brown trout. The differences in concentrations of PCBs may be attributable to various factors such as feeding preferences, the nature of the habitat and lipid contents. The higher levels of PCBs in rainbow trout compared to other fish species may be due to its feeding habit. Rainbow trout is predator specie with high lipid content and probably receive large quantities of organochlorine pollutants through a process of biomagnification.

The pattern of PCBs in muscle tissues of fish was very similar between species and PCBs 153 and 138 (hexachlorobiphenyls) were the dominant congeners (Figure 3). These congeners are usually reported by several authors as dominant in marine fishes (Bayarri et al., 2001; Loizeau et al., 2001; Perugini et al., 2004; Naso et al., 2005; Georgieva et al., 2015).

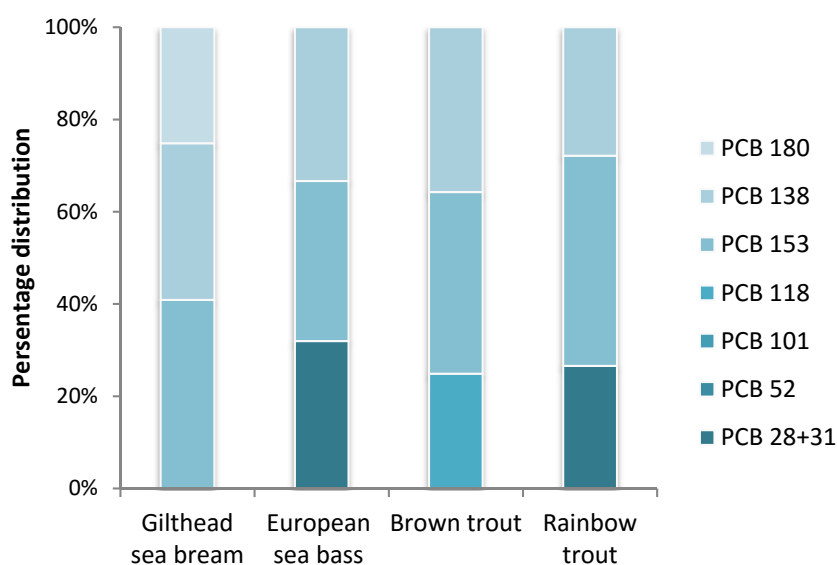


Fig. 3 Percentage distribution of PCBs congeners in cultured fish species

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 Total I-PCBs in all fish species (max values 1.59 ng/g ww in sea bream) did not exceed this limit.

Regarding toxic dl-PCBs, only PCB 118 were found in brown trout, while non-ortho PCBs 77, 126, 169 and mono-ortho PCBs 105, 156 were not found in detectable levels in cultured fish.

The contamination degree with PCBs of cultured fish from fish market in Varna, Bulgaria was found lower than PCB levels found in other studies. PCB concentrations found by Erdogrul 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. Antunnes and Gil, 2004 studied levels of PCBs in cultured sea bass 26 – 179 ng/g dry weight.

3.2. DDTs 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. In the environment DDT metabolizes slowly and the key metabolite DDE (1-1 dichloro-2,2-bis (p-chlorophenyl) ethylene) is also persistent compound (Ferreira et al., 2004). Lipid contents and concentrations of p,p'-DDE, p,p'-DDD, p,p'-DDT found in the samples, are present in Table 2.

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

Fish species	Lipids, %	p,p-DDE	p,p-DDD	p,p-DDT
Gilthead sea bream (<i>Sparus aurata</i>)	4.9±0.5	2.81±0.70	nd	nd
European sea bass (<i>Dicentrarchus labrax</i>)	8.1±0.7	2.86±0.75	nd	nd
Brown trout (<i>Salmo trutta fario</i>)	2.1±0.2	2.11±0.53	1.28±0.32	nd
Rainbow trout (<i>Oncorhynchus mykiss</i>)	13.0±1.2	4.28±1.07	nd	nd

nd – not detected

From the DDT metabolites, DDE was found in the highest concentration in tissues of all the analysed fish samples (Table 2) and representing more than 62% of total DDT. The concentrations of p,p'-DDE varied in the range from 2.11 to 4.28 ng/g wet weight (ww) in brown trout and rainbow trout samples respectively. The p,p'-DDD was found only in brown trout (38%). DDT did not found at detectable levels in fish species investigated. 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., 2015a). This suggests that these pesticides have not been recently used in agriculture after their ban. The observed differences in concentrations may be attributed partly to higher lipid content in fish (2.1% and 13% in brown trout and rainbow trout, respectively). Lipid content of fish species may influence its organochlorine concentrations (Pastor et al., 1996; Bayarri et al., 2001).

The concentrations of DDTs in investigated samples gilthead sea bream, European sea bass, brown trout, rainbow trout were found lower than those reported by Covaci, A. et al., 2006 from Danub Delta (2 847 ng/g lipid weight) and those reported by Erdogrul, Ö., 2005 for carp from the Kahramanmaras, Turkey (median 77.4 ng/g ww) (Erdogrul et al., 2005). 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). The levels of DDTs in cultured sea bass from Portugal were measured from 22 – 194 ng/g dry weight (Antunnes and Gil, 2004).

The concentration levels of POPs were measured in three wild fish species from Lake Varna and Lake Beloslav, Bulgaria in our previous study (Georgieva et al., 2015b). The data were summarized and the Total PCBs and Total DDTs values was presented in order to compare the results obtained to the levels in cultured fish from this study – Figure 4.

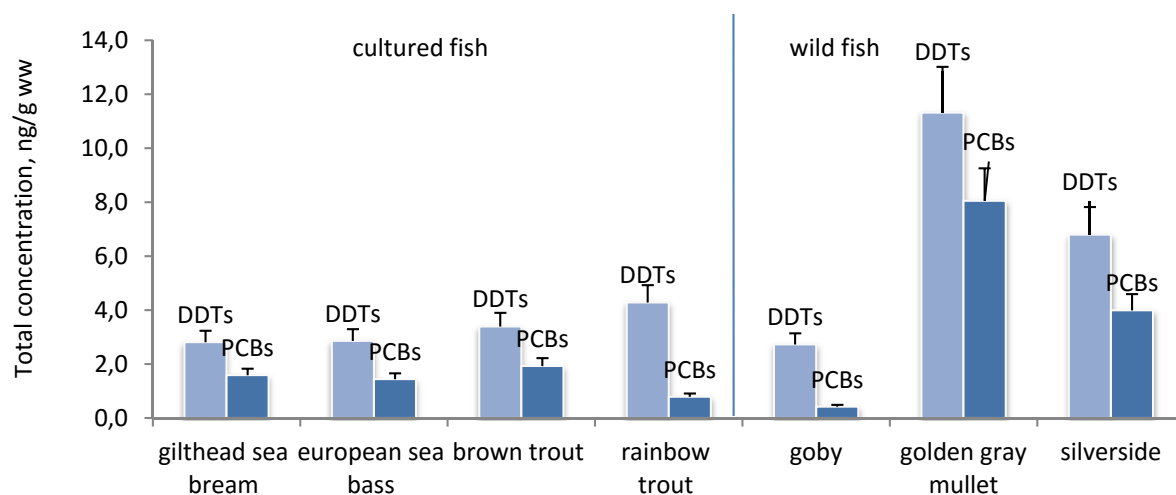


Fig. 4 Comparison of DDTs and PCBs levels, ng/g ww in cultured fish and in wild fish from coastal lakes.

The levels of persistent organochlorine pollutants (PCBs and DDTs) studied in cultured fish were found lower compared to those in wild fish species from Lake Varna and Lake Beloslav, Bulgaria. Total DDTs in cultured fish tissues were found lower than concentration in golden grey mullet 11.3 ng/g ww and silverside – 6.8 ng/g ww. Regarding PCBs, total concentrations were found 8.05 and 3.99 ng/g ww in golden grey mullet and in silverside, respectively.

4. CONCLUSION

The residues of PCBs varied in the range between 0.79 ng/g ww in rainbow trout and 1.93 ng/g ww in brown trout. The very low levels of PCBs observed in fish tissues correspond with the fact that no industrial PCBs production in Bulgaria. The analysis of fish tissues of gilthead sea bream, European sea bass, brown trout, rainbow trout showed a mean total load of DDT pollutants 3.34 ng/g ww, respectively. In all fish samples DDT was present only in the form of its metabolites p,p'- DDE and p,p'- DDD, suggesting previous contamination. The levels of PCBs and DDTs in fish species: gilthead sea bream, European sea bass, brown trout, rainbow trout from the fish markets were found lower than levels measured in the fish species from Lake Varna and Lake Beloslav, Bulgaria.

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