

ORGANOCHLORINE COMPOUNDS (PCBs AND DDTs) IN SEAFOOD FROM THE BLACK SEA, BULGARIA

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Abstract

Several researches have shown that some polychlorinated biphenyl congeners and organochlorine pesticides degrade slowly in the environment and build up in the food chain, causing a wide range of possible adverse effects to humans. Marine organisms are an excellent indicator for pollution in aquatic ecosystems, where trace contaminants are difficult to be analyzed directly.

The aim of the study was to determine concentrations of persistent organochlorine compounds in some marine species from the Black Sea, Bulgaria. Concentrations of polychlorinated biphenyls (PCBs), DDT and its metabolites were determined in rapana (*Rapana venosa*), shrimp (*Crangon crangon*) and mussels (*Mytilus Galloprovincialis*) collected from the Black Sea in summer 2011.

Determination of PCB congeners, DDT and its metabolites DDE and DDD was carried out by gas chromatography coupled to mass spectrometry after clean-up of the fatty extracts. The levels of organochlorine pollutants in rapana and mussels 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 1.78 to 3.27 ng/g wet weight (in rapana and mussels, respectively) and PCBs concentrations were found below 0.12 ng/g ww.

The levels of persistent organochlorine pollutants in mussels, rapana and shrimp were found lower compared to those in seafood from other studies.

Key words: seafood, mussel, DDTs, PCBs, Bulgaria

1. INTRODUCTION

Organochlorine compounds have characteristic of volatility and long-range environment transport, being widely distributed in marine environment as a consequence of uncontrolled spillage of agricultural sources, surface runoff and atmospheric deposition (Turner et al., 1986). Dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) are known as persistent compounds in aquatic systems and seafood 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 (SC, 2001). POPs have a negative effect on the flora and fauna in affected areas (Naccari et al. 2004, Zelníčková et al. 2015).

Rapana, shrimp, mussels and other seafood can be used as a bioindicator to evaluate the toxic effects of chemical pollutants in marine organisms, representing an important tool for biomonitoring environmental pollution in coastal areas (Namieśnik et al., 2010). It is known that these organisms concentrate pollutants to levels above those present in marine water (Verweij et al., 2004; Stefanelli et al., 2004; Carro et al., 2010). Advantages of using seafood to monitor organochlorine pollutants are their wide geographical distribution, easy sampling, sessile lifestyle and rapid accumulation of toxic substances (Okay et al., 2009; Suárez et al., 2013). Seafood accumulate POPs and they may represent a major exposure route for persistent and bioaccumulative compounds from the aquatic systems into humans (Dorea, 2008; Van Ael et al., 2012). Previous studies have shown that food is the major contributor for PCBs and DDTs bioaccumulation (Loizeau et al., 2001; Covaci et al., 2006). Fang (2004) has described concentrations and patterns of organochlorine pesticide residues, and PCBs were analyzed in mussel (*Perna viridis*) samples from 10 coastal sites along the Pearl River Delta, southern China. Contaminant levels (organotins, trace metals, PCBs and PAHs) were measured in tissues of *Mytilus* sp. mussels. PCB levels in cultivated mussels *M. galloprovincialis* from the Galician coast have been studied as biomarkers of pollution (Carro et al., 2005).

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). The main sources of PCBs in the ecosystem are soils, in which more than 90 % of these substances are deposited (Lana et al. 2008; Zelníčková et al. 2015). 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). 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). Because of their wide distribution in air, water, soil and food, PCBs, p,p'-DDT and its metabolites remain a human health concern and have been quantified in the present study.

The aim of the study was to determine concentrations of persistent organochlorine compounds in some marine species from the Black Sea, Bulgaria and to compare the results to the levels in other studies.

2. EXPERIMENTAL

2.1. Sampling and sample preparation

Rapana (*Rapana venosa*), shrimp (*Crangon crangon*) and mussels (*Mytilus Galloprovincialis*) samples were collected manually from three coastal areas of the Black Sea, Bulgaria. The sampling was carried out in late June to early September 2011 in natural populations at a sea depth of 3–4 m. The sampling strategy includes three important regions of Black Sea: North (near cape Kaliakra), Varna (Varna bay) and South (Burgas).

The collected samples rapana, shrimp and mussels were characterized by a similar maximum length and size of analyzed organisms. This approach guaranteed that compared species had similar metabolic conditions, and the influence of physiological differences was less pronounced. Samples were immediately transferred to the laboratory in foam boxes filled with ice.

Analysis were carried out on pooled samples of the individual species collected at each sampling point. The number of individual specimens per pool varied depending on the species, for mussel samples about 60 specimens, for rapana about 40, and for shrimp the number of single specimens per sample ranged from 80 to 100. Whole soft tissue from pooled samples were homogenized using a blender and pools of about 300 g were taken for analysis.

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, twenty grams of homogenized tissue 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 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.

Individual congeners of PCBs and DDTs were quantified using PCB 30 as an internal standard and a mixture of standards (EPA 625/CLP Pesticides Mix 2000 µg/ml - Supelco and PCB Mix 20 - Dr. Ehrenstorfer Laboratory) as calibration standards. The calibration curves of PCBs and DDTs were carried at four concentration levels for compound analyzed covering the range of 1–600 ng/ml. Each concentration level was injected in duplicate and all relative data to each analyzed congener were fitted by linear regression. 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.

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. Blank samples were analysed using anhydrous sodium sulphate processed over the whole analytical procedure to check for interferences or contamination from solvents and glassware. Blanks did not contain traces of contaminants.

3. RESULTS AND DISCUSSION

3.1. DDTs concentrations

Lipid contents and concentrations of p,p'-DDE, p,p'-DDD, p,p'-DDT found in rapana, shrimp and mussel, collected from the Black Sea are presented in Table 1. The results are expressed in ng/g wet weight (ww).

Among the different organochlorine compounds, p,p-dichlorodiphenylethylene (p,p-DDE) is the most abundant. It is a degradation product of p,p-dichlorodiphenyltrichloroethane (p,p-DDT), which can be formed either by UV-irradiation in the atmosphere or by the metabolism of organisms (Sánchez et al. 1993). The metabolism of DDT in organisms is generally accomplished through dehydrodechlorination to DDE (Schmitt et al. 1990). The content of p,p-DDE reported in the soft tissue of rapana, shrimp and mussel is rather low and varied in the range from 1.03 to 2.14 ng/g ww in rapana and mussels, respectively. The highest concentrations of p,p'-DDE in rapana samples were found from the Varna bay, located near the shipyard and port in Varna city (1.17 ng/g ww).

Table 1. Lipid content (%) and concentrations of p,p'-DDE, p,p'-DDD and p,p'-DDT (ng/g ww)

Species	Sampling area	n	Lipids, %	p,p-DDE	p,p-DDD	p,p-DDT
rapana (<i>Rapana venosa</i>)	North	3	0.39±0.05	1.07±0.31	0.65±0.22	nd
rapana (<i>Rapana venosa</i>)	Varna	4	0.2±0.02	1.17±0.36	0.82±0.25	nd
rapana (<i>Rapana venosa</i>)	South	4	0.29±0.03	1.03±0.28	0.55±0.11	nd
shrimp (<i>Crangon crangon</i>)	Varna	3	1.46±0.15	1.85±0.52	nd	nd
mussels (<i>Mytilus Galloprovincialis</i>)	Varna	4	3.35±0.33	2.14±0.58	1.13±0.32	nd

nd – not detected

The p,p'-DDD was found between 0.55 (rapana South) and 1.13 ng/g ww (mussels). DDT did not found at detectable levels in seafood 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., 2015). 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 mussels (3.35%). Lipid content of fish species may influence its organochlorine concentrations (Pastor et al., 1996; Bayarri et al., 2001).

The ratio between the concentration of DDE and total DDTs has been used as an index of the frequency of usage of pesticides whose active ingredient is DDT (Aguilar 1984; Herceg-Romanic et al., 2014). Aguilar (1984) suggested that a ratio greater than 0.6 implies that the system is relatively stable with no new inputs of DDT. In our study DDE/Total DDT ratios varied in the range from 0.61 in rapana to 1.0 in shrimp, suggesting that there are no new inputs of DDT in area of the Black Sea coast of Bulgaria. These results are not surprising, since it is well known that, despite the ban of DDT, there is still sporadic input of this compound into the coastal environments either by atmospheric deposition or resuspension from the sediment. It is known that the main pathway of global distribution of organochlorine pesticides is through atmospheric transport (Sánchez et al., 1993). The transport of organochlorine pesticides from the terrestrial to the marine environment is also important. The concentrations of these pollutants found in seafood from Black Sea coast of Bulgaria seem to have been a consequence of the high input of the contaminants with the Danube River influence (Zhulidov et al., 2003).

Total DDTs concentration (sum of DDE and DDD) varied in the range from 1.58 ng/g ww (rapana South) to 3.27 ng/g ww (mussels) – Figure 1. Shrimp tissues contained the lowest concentrations of DDTs (1.85 ng/g ww), as also noticed by Voorspoels et al. (2004) and by Van Ael et al. 2012. These authors suggested that the low levels in shrimp were partially caused by their more pelagic nature and their feeding habits. Shrimp feed mainly on preys of lower trophic levels, like amphipods or mysids (Van Ael et al. 2012).

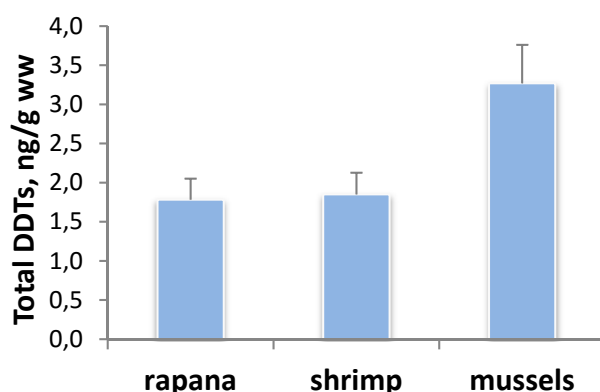


Fig. 1 Total DDT concentration (ng/g ww) in rapana, shrimp and mussels from Black Sea coast of Bulgaria

The highest levels of DDTs in mussels, may be due their physiology characteristics and higher lipid content compared to rapana and shrimp. Bivalves are known for their ability to concentrate lipophilic substances. The mussels are filter feeders and have the ability to bioconcentrate pollutants in their tissues at very high levels, above those present in marine water.

Among the sampling sites, the maximum level of Total DDTs in rapana was found at the Varna sampling site (1.99 ng/g ww), while the minimum value was found at the South (1.58 ng/g ww) – Figure 2. The experimental results for Total DDTs in rapana from different sampling areas showed no significant differences between the North, Varna and South coast sampling area (statistical test - $p > 0.05$).

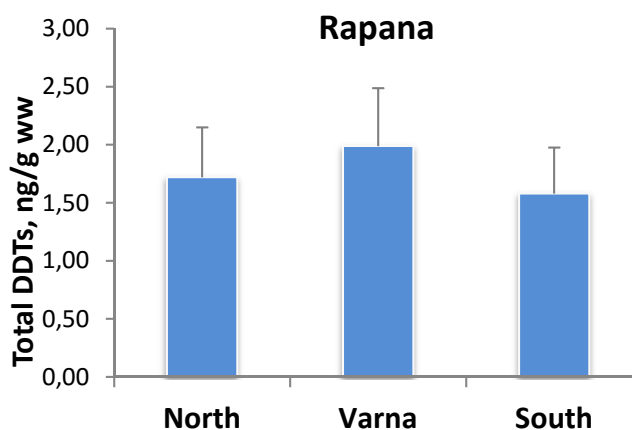


Fig. 2 Comparison of DDTs, ng/g ww in rapana from different sampling areas

Total DDTs in rapana, shrimp and mussels from Black Sea, Bulgaria were lower than level observed by Kurt and Ozkoc (2004) in mussels from Mid-Black Sea, Turkey (1.55-5.52 ng/g ww). Van Ael et al. (2012) studied DDTs in brown shrimp (0.12 – 1.37 ng/g ww) and in blue mussels (average 39.9 ng/g ww) from Scheldt estuary, Belgium. Herceg-Romanic et al. (2014) were found residues of DDTs in cultured mussels from the Ctoatian coast of the Adriatic Sea in the range 0.12 - 2.61 ng/g ww.

3.2. PCBs levels

The six PCB congeners (IUPAC № 28, 52, 101, 138, 153 and 180) are 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). They have been recommended by the European Union as indicators of PCB contamination (Giandomenico et al., 2013). We determined the levels of six dioxin-like PCBs (non-ortho PCBs 77, 126, 169 and mono-ortho PCBs 105, 118, 156). The dl-PCB show a similar toxicity as polychlorinated dibenzodioxins (Van den Berg et al., 2006).

Polychlorinated biphenyls (both Indicator PCBs and dioxin-like PCBs) were found below detectable limits (below 0.12 ng/g ww) in all analyzed samples. The levels found in the present study are in accordance with the results for mussels from the Mid-Black Sea Coast of Turkey, where no PCBs were detected in any samples (Kurt and Ozkoc, 2004). However, Okay et al. in 2009 were measured levels of PCBs in mussels of the Istanbul strait, Turkey during the period of January-February 2007 and their results ranged from 1026 to 35 983 pg/g wet weight. Namieśnik et al., 2010 were determined the levels of PCBs in rapana from Black Sea – 62 - 82 ng/g dry weight.

Our results for Total I-PCBs in all species did not exceed the recommended a maximum level of 75 ng/g wet weight, calculated as the sum of the six I-PCBs (EC, 2011).

4. CONCLUSION

The monitoring of priority organochlorine pollutants in seafood is of paramount importance in order to protect marine ecosystem. DDT and its metabolites (p,p'-DDE and p,p'-DDD) were the major compounds in all the studied samples. The concentration order was DDE > DDD. In our study DDE/Total DDT ratios varied in the range from 0.61 in rapana to 1.0 in shrimp, suggesting that there are no new inputs of DDT in area of the Black Sea coast of Bulgaria. Both Indicator and dioxin-like PCBs were found below detectable limits (below 0.12 ng/g ww) in all analyzed samples. The results obtained in this study indicate that contamination by organochlorine compounds in rapana, shrimp and mussels were lower than levels measured in seafood from neighbor seas - Adriatic Sea and Mediterranean Sea. Importantly, the maximum values of these contaminants were below the EC regulation limits.

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