Quality Assurance and Safety of Crops & Foods, September 2013; 5 (3): 243-251

Wageningen Academic Publishers

Organochlorine pollutants in fish from the Bulgarian region of the Black Sea

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Received: 22 December 2011 / Accepted: 21 November 2012

RESEARCH PAPER

Abstract

Persistent organochlorine pollutants like polychlorinated biphenyls (PCBs) and dichloro diphenyl trichloroethane residues (DDTs) can still be a problem for the aquatic environment and human health. The levels of DDTs and PCB congeners were determined in fish from the Bulgarian Black Sea coast. Four fish species with different feeding behaviour, goby (*Neogobius cephalargoides),* sprat (*Sprattus sprattus sulinus*), horse mackerel *(Trachurus Mediterraneus ponticus)* and shad *(Alosa pontica pontica)* were sampled from the Bulgarian Black Sea coast during 2007-2010. The DDT and PCB residues were measured in clean fish extracts by gas chromatography with mass detection. The main metabolite p,p'-dichloro diphenyl dichloroethylene was the most frequently detected compound in all fish species and was present in much higher concentrations than the other DDTs (ranging from 119.32 to 1,324.44 ng/g fat). PCBs were found in all fish species at concentrations ranging between 135.1 ng/g fat in horse mackerel and 990.8 ng/g fat in goby (calculated as the sum of 15 investigated congeners). The levels of DDTs and PCBs in fish from Bulgarian Black Sea coast were comparable to those found in fish species from the Black Sea and from neighbouring seas (the Marmara Sea, the Aegean Sea and the Mediterranean Sea).

Keywords: Black Sea, Bulgaria, DDT, fish, PCB

1. Introduction

Pollution by persistent organic compounds, such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) like dichloro diphenyl trichloroethane residues (DDTs), has spread all over the world as evidenced by their detection both in humans and wildlife. (Borghesi *et al.*, 2008; Smith and Gangolli, 2002). PCBs and OCPs are generally found in the environment as complex mixtures, their presence often reflecting local anthropogenic impacts. These lipophilic contaminants are very persistent, widely distributed in the environment and exhibit the potential for bioaccumulation (Thomson and Rose, 2011). In marine organisms, the uptake of chlorinated compounds occurs directly from the sea and through the food chain. (Serrano *et al.*, 2008, Storelli and Marcotrigiano, 2006; Szlinder-Richert *et al.*, 2008).

PCBs are commonly used in many industrial activities since they make up the composition of some electrical equipment and ship paint. DDT is a chlorinated pesticide widely used in the past to control the spread of insects and other agricultural pests. In the environment DDT metabolised slowly and the metabolite dichloro diphenyl dichloroethylene (DDE) is a particularly persistent compound (Weijs *et al.*, 2010). In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in the endocrine, reproductive and nervous system (Langer *et al.*, 2003).

OCPs and 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 (Kurt and Ozkoc, 2004; Maldonado and Bayona, 2002). Although the manufacture and use of these compounds is banned or highly restricted, residues of polychlorinated compounds continue to be detected in the blood and breast milk of humans (Ennaceur *et al.*, 2008). PCBs and organochlorine pesticides were measured in sediments collected from the Danube Delta and it was found that the Danube River is a potential source of contamination to the Black Sea (Covachi *et al.*, 2006; Fillman *et al.*, 2002; Okay *et al.*, 2009). Fish are a suitable indicator for the pollution in aquatic ecosystems, where trace contaminants are difficult to analyse directly.

Human exposure to different environmental contaminants (like PCBs and DDTs) is possible by several routes: inhalation of air, dermal absorption and food consumption. Dietary intake is the major route of uptake, representing more than 90% of the daily exposure to these compounds (Bayarri *et al.*, 2001). Foodstuff of animal origin, such as fish and seafood, is recognised as one of the main contributors (European Commission, 2000). Populations at higher risk than most are those subsisting largely on fish and other marine organisms. There are only a few data available on residue concentrations of DDTs in fish and mammals from the Black Sea (Kalyoncu *et al.*, 2009; Weijs *et al.*, 2010) and particularly from the Bulgarian Black Sea coast (Stoichev *et al.*, 2007).

The aim of this study was to determine the levels of DDT (including its metabolites p,p'-DDE and p,p'-dichloro diphenyl dichloroethane (p,p'-DDD)) and PCB congeners in fish species from the Bulgarian Black Sea coast and to monitor the accumulation of these pollutants during 2007-2010.

2. Materials and methods

Sampling and sample preparation

The fish species were selected according to their characteristic feeding behaviour and importance to human consumption in Bulgaria: goby (*Neogobius cephalargoides),* sprat (*Sprattus sprattus sulinus*), horse mackerel *(Trachurus Mediterraneus ponticus)* and shad *(Alosa pontica pontica).* Goby is a non-migratory species that feeds mainly on benthic organisms. Sprat is a small, pelagic marine fish that feeds on planktonic crustaceans. Horse mackerel is a pelagic, summer spawning fish with Mediterranean origin; it feeds on different small fish (anchovy, sprat), crustaceans and worms. Shad is a pelagic fish species; it migrates to middle reaches of large rivers for spawning. It feeds on a wide variety of zooplankton and small fish.

Samples were collected from different parts of the Bulgarian Black Sea coast during 2007-2010. The map of the study area is presented in Figure 1. The sampling strategy allowed the entire Bulgarian Black Sea coast to be covered and includes three important fishing regions: the north coast (near cape Kaliakra, Krapec and Balchik), Varna Bay and the south coast (Bourgas, Nessebar). 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 number of fish collected in every sample at each location varied between 30 and 40 for sprat and goby, between 15 and 25 for horse mackerel, and between 5 and 10 for shad. The length and weight of each specimen were measured and the fish were rinsed with distilled water to remove sand and impurities. The edible portion of the same species was homogenised using a blender; pools of about 300 g were made with fillets taken from several individual fish.

Analytical methods

The analytical method for determination of residues of OCP and PCB was based on BDS EN 1528:2001 of the Bulgarian Institute for Standardization (Sofia, Bulgaria). The edible tissues of fish were homogenised 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. PCB and DDTs were extracted with hexane/dichloromethane (3/1, v/v) in a Soxhlet apparatus. 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). 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 a GC FOCUS (Thermo Electron Corporation, Austin, TX, USA) using a POLARIS Q Ion Trap mass spectrometer and equipped with an AI 3000 autosampler (Thermo Electron Corporation, Milan, Italy). The experimental MS parameters were the following: the ion source and transfer line temperatures were 220 °C and 250 °C, respectively. The splitless injector temperature

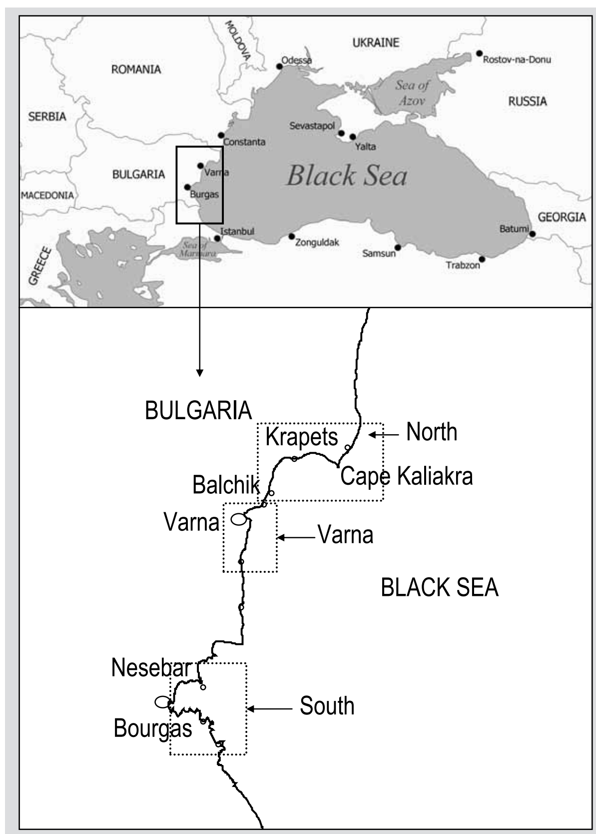

Figure 1. Black sea map and sampling area (north coast, Varna Bay and south coast).

was 250 °C. For DDT determination the GC 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 used 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 (Thermo Electron Corporation) 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 ion trap mass spectrometry (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 pesticide mix 2,000 μg/ml purchased from Supelco (Bellefonte, PA, USA) and PCB Mix 20 purchased from Dr. Ehrenstorfer Laboratory, Augsburg, Germany) were used for instrument calibration, recovery determination and quantification of compounds. Measured compounds were: dichlorodiphenyl-trichloroethane (p,p'-DDT) including 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 no. 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 170 and 180).

The limit of detection (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 relative standard deviation value was less than 16% (determined with five times repeatedly).

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) purchased from the Institute for Reference Materials and Measurements of the European Commission. Recoveries of DDTs from the certified reference material BCR-598 were: 97.5% for DDE, 103.4% for DDD and 85.4% for DDT. PCB recovery was in the range 85-119% for individual congeners. All calculations were carried out using MS Excel for Windows on the basis of linear equation.

Statistical analysis

Statistical analysis was carried out on the lipid-normalised organochlorine concentrations. DDTs and PCBs concentrations were $\log_{10}$-transformed to approximate a normal distribution of the data. The statistical analysis of the data was based on the comparison of average values by a t-test, a significance level of $P<0.05$ was used. All statistical tests were performed using SPSS 16 software (SPSS Inc., Armonk, NY, USA).

3. Results and discussion

DDT and its metabolites

The experimental results for goby, sprat, horse mackerel and shad from different sampling sites showed no significant differences between the northern and southern coast sampling area ($P>0.05$). The fish species studied do not present any trend in the geographical variations and cannot be used to assess the sources of pollution. The lipid content and average annual concentrations of DDT and its metabolites DDE and DDD in the edible parts of investigated species from the Black Sea coast of Bulgaria are shown in Table 1.

Lipid determination was performed on an aliquot of the extract (1/5) before clean-up. The lipid percentage ranged from 2.2±1.2% in goby to 22.8±6.7% in shad. The whole-body lipid percentage in sprat was 6.4±1.7%. (Table 1). The lipid content of fish tissue is influenced by several factors, such as sex, age, species, nourishment and spawning status (Voorspoels *et al.*, 2004).

The main metabolite p,p'-DDE was the most frequently detected compound in the marine species investigated and was present in much higher concentrations than the other DDTs (ranging from 119.32 to 1,324.44 ng/g fat). p,p'-DDD concentrations was found in all samples at levels ranging from 75.53 (horse mackerel in 2010) to 507.32 ng/g fat (goby in 2009). p,p'-DDT was found at annual concentrations between 31.19 and 400.98 ng/g fat. In 26.6% of the goby samples p,p'-DDT was not found at detectable levels.

Statistical analysis of data showed no statistically significant difference between the levels of DDTs in the years of study ($P>0.05$). The highest concentrations of ∑DDTs had been measured in 2009 (2,232.94 ng/g fat) in goby samples and the lowest concentration was found in horse mackerel in 2010 (226.04 ng/g fat). In sprat and shad muscle samples the levels of ∑DDTs ranged between 857.20 and 1,346.51 ng/g fat and between 744.09 and 1,235.22 ng/g fat, respectively.

DDT was presented mainly in the form of its metabolites p,p'-DDE and p,p'-DDD. This suggests that these pesticides have not been used recently in agriculture after their ban. 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 authors (Coelhan *et al.*, 2006; Covachi *et al.* 2006; Stoichev *et al.*, 2007). Thus, p,p'-DDE was the principal form of DDT in all the species studied, constituting between 49.0% and 75% of the total DDTs (Table 1). These findings

Table 1. Lipid content (% and standard deviation) and concentrations of DDT and its metabolites (mean and range, ng/g fat).

Fish species	Year	Lipids (%)	DDE (ng/g fat)	DDD (ng/g fat)	DDT (ng/g fat)	∑DDT (ng/g fat)
Goby (*Neogobius cephalarges*)	2007	2.90±1.1	556.67 (521.50-611.49)	133.72 (45.93-203.33)	115.00 (nd-345.00)	805.39 (657.41-1,085.36)
	2008	2.66±0.9	807.58 (324.77-2,020.2)	212.00 (93.56-423.57)	135.56 (nd-389.78)	1,155.15 (515.77-2,833.9)
	2009	1.15±0.3	1,324.64 (630.91-1,881.46)	507.32 (328.47-794.27)	400.98 (272.22-688.23)	2,232.94 (1304-2,923.63)
	2010	1.06±0.5	881.57 (621.00-1,142.14)	266.69 (213.40-319.98)	34.09 (nd-68.18)	1,182.35 (834.40-1,530.30)
Sprat (*Sprattus sprattus*)	2007	4.74±1.5	741.81 (670.22-813.39)	421.95 (384.98-458.91)	151.87 (123.84-179.90)	1,315.63 (1,235.11-1,396.14)
	2008	7.14±2.6	528.73 (670.22-813.39)	239.02 (147.44-330.60)	89.45 (69.43-109.47)	857.20 (518.21-1,196.19)
	2009	7.40±2.9	727.82 (692.93-762.71)	382.06 (325.95-438.18)	133.07 (52.15-213.99)	1,242.96 (1,140.81-1,345.11)
	2010	3.99±0.6	829.92 (319.66-1,720.91)	388.72 (177.07-805.19)	127.87 (53.39-271.64)	1,346.51 (550.11-2,798.74)
Horse mackerel (*Trachurus mediterraneus ponticus*)	2007	11.46±1.7	286.32 (174.01-453.13)	130.32 (86.34-196.50)	52.84 (nd-88.86)	469.47 (260.35-738.49)
	2008	7.36±3.2	404.20 (148.47-566.96)	205.65 (93.84-298.93)	87.94 (nd-185.18)	697.79 (241.88-1,051.07)
	2009	16.84±5.9	295.62 (171.59-546.15)	237.14 (239.40-388.17)	64.61 (938.35-121.87)	597.37 (418.27-1,056.19)
	2010	10.51±1.3	119.32 (99.87-141.17)	75.53 (54.81-102.050	31.19 (21.68-41.62)	226.04 (197.86-284.83)
Shad (*Alosa pontica pontica)*	2007	14.41±4.8	566.72 (130.66-1,002.79)	269.01 (nd-164.36)	82.18 (nd-164.36)	917.91 (130.66 -1,705.16)
	2008	25.45±10.7	426.50 (199.33-653.68)	234.65 (11.36-352.95)	82.93 (49.58-11.28)	744.09 (365.27-1,122.90)
	2009	27.51±3.5	587.36 (550.84-612.64)	258.81 (175.14-316.56)	120.82 (105.31-133.10)	966.99 (893.09-1,039.53)
	2010	18.13±2.7	804.39 (639.33-1,101.31)	330.22 (195.97-495.23)	100.60 (77.29-129.33)	1,235.22 (963.70-1,725.87)

nd = not detected.

are not surprising considering the high chemical stability and hydrophobicity of p,p'-DDE and its long half-life and persistence in both abiotic and biotic components of the aquatic ecosystems (Perugini *et al.*, 2004).

The average concentrations of individual DDTs were based on the total DDT to calculate the percentage ratio of the metabolites (DDE and DDD) in the total DDT pollution (∑DDT) in the analysed fish species (Figure 2). DDT profiles in goby were as follows: 70.0% p,p'-DDE, 21.6% p,p'-DDD and 8.4% p,p'-DDT.

In 26% of the goby samples and 20% of the horse mackerel samples p,p'-DDT was not found in detectable levels. These findings are not surprising considering the high chemical

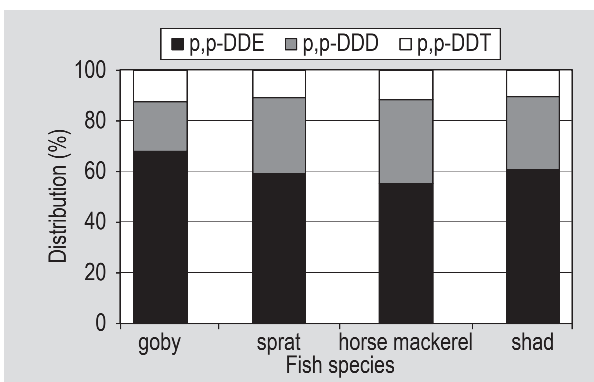

Figure 2. Distribution pattern of DDTs in fish species coming from the Black Sea. Each compound refers to the total DDT pollution.

stability and hydrophobicity of p,p'-DDE and its long half-life and persistence in both abiotic and biotic components of the aquatic ecosystems (Perugini *et al.*, 2004). The highest DDE values, found in goby (average 68% of total DDTs), may be due to the greater metabolic efficacy of the benthic organisms. Pelagic fish are also able to metabolise DDT but their metabolic ratio values are lower than those reported in benthic organisms (Perugini *et al.*, 2004).

Levels of DDTs in goby, sprat, horse mackerel and shad (see Table 2 and 3) were found in comparable levels to those measured in similar fish species from neighbouring seas (Marmara Sea, Adriatic Sea, Mediterranean Sea and Belgian North Sea) (Coelhan *et al.*, 2006; Kalyoncu *et al.*, 2009; Voorspoels *et al.*, 2004). In two studies (Tanabe *et al.*, 1997 and Coelhan *et al.*, 2006) total DDT concentrations were given as 3,800 and 455.09 ng/g fat, respectively, for horse mackerel samples from the Black Sea and the Marmara Sea.

Polychlorinated biphenyls levels

The fifteen congeners of PCB were analysed, including the set of 7 indicators PCBs (IUPAC no. 28, 52, 101, 118, 138, 153 and 180) recommended by the European Union for assessing the pollution by PCBs (Commission of the European Communities, 1999) and also the 'dioxin-like' PCBs (77, 105, 118, 156, 126, 169) used in order to estimate the toxic equivalent concentrations (TEQs) of

Table 2. Polychlorinated biphenyls (PCBs; ng/g fat) concentration levels (mean values and range) determined in fish species goby and sprat collected from the Black Sea.

	Goby (*Neogobius cephalarges*)				Sprat (*Sprattus sprattus*)			
PCB congeners	2007	2008	2009	2010	2007	2008	2009	2010
28*+31	29.1 (nd-50.5)	40.9 (nd-84.6)	55.9 (nd-142.2)	103.2	78.0 (51.4-104.5)	49.2 (39.1-59.2)	47.1 (41.5-52.8)	29.9 (nd-89.6)
52*	13.4 (nd-40.3)	61.0 (31.4-87.7)	37.9 (nd-117.3)	nd	78.4 (53.8-102.9)	46.4 (39.0-53.8)	49.0 (41.5-56.6)	13.5 (nd-27.1)
101*	73.3 (33.8-123.5)	76.0 (38.6-162.0)	77.5 (nd-131.7)	nd	91.2 (65.9-116.5)	56.2 (38.3-74.1)	59.3 (43.9-74.7)	15.2 (nd-38.9)
77	nd	nd	nd	nd	nd	nd	nd	nd
118*	40.6 (nd-78.8)	99.6 (52.9-168.0)	117.6 (nd-210.8)	nd	116.5 (80.9-152.2)	70.0 (50.6-89.5)	76.2 (56.6-95.8)	21.9 (nd-51.0)
153*	212.8 (115.7-283.9)	297.8 (153.8-490.3)	289.2 (94.1-431.7)	186.5 (140.0-233.1)	160.8 (105.8-215.8)	112.4 (67.2-157.7)	148.2 (95.3-201.0)	112.5 (50.1-237.2)
105	27.7 (nd-48.2)	69.4 (40.1-88.4)	27.7 (nd-138.5)	nd	86.7 (56.3-117.2)	48.8 (37.9-59.8)	52.9 (42.2-63.6)	11.6 (nd-27.8)
138*	199.6 (99.4-260.8)	213.4 (114.7-333.7)	248.8 (98.2-335.5)	69.8 (66.0-73.5)	174.6 (113.7-235.5)	89.4 (67.1-111.7)	133.0 (81.7-184.3)	87.3 (34.0-190.6)
126	nd	nd	nd	nd	nd	nd	nd	nd
128	14.9 (nd-44.8)	13.6 (nd-68.0)	nd	nd	21.1 (nd-42.2)	nd	22.6 (nd-45.2)	10.9 (nd-26.1)
156	nd	nd	nd	nd	nd	nd	nd	nd
180*	69.2 (nd-153.2)	96.5 (46.2-125.8)	121.3 (nd-292.4)	51.6 (nd-103.3)	81.7 (52.8-110.7)	52.2 (36.6-67.9)	57.8 (38.9-76.7)	86.6 (34.5-158.2)
169	nd	nd	nd	nd	nd	nd	nd	nd
170	36.0 (nd-73.1)	22.6 (nd-62.5)	nd	61.0 (nd-122.0)	nd	nd	38.4 (nd-76.7)	79.4 (35.9-132.5)
∑PCBs	716.7 (453-1,096)	990.8 (524.1-1,593)	975.9 (380.4-1,569)	472.2 (309.5-634.8)	889.0 (622.8-1,155)	524.7 (375.7-673.7)	684.5 (441.4-927.5)	468.6 (202.6-978.8)
∑Indicator PCBs*	638.1 (383.4-930.2)	885.2 (484.0-1,379.9)	948.2 (380.4-1,430.8)	411.2 (309.5-512.8)	781.2 (524.3-1,038.1)	475.8 (337.7-741.9)	570.6 (399.3-741.9)	366.8 (153.2-792.4)

nd = not detected; * = indicator PCB.

Table 3. Polychlorinated biphenyls (PCBs; ng/g fat) concentration levels (mean values and range) determined in fish species horse mackerel and shad collected from the Black Sea.

	Horse mackerel (*Trachurus mediterraneus ponticus*)				Shad (*Alosa pontica pontica*)			
PCB congeners	2007	2008	2009	2010	2007	2008	2009	2010
28*+31	8.3 (nd-24.9)	52.9 (23.8-72.9)	30.0 (16.2-41.6)	25.7 (23.1-28.9)	15.1 (nd-30.2)	34.5 (30.7-38.4)	23.6 (15.9-27.7)	20.6 (nd-38.4)
52*	26.5 (24.0-28.7)	48.7 (19.7-66.1)	26.4 (13.2-40.4)	3.0 (nd-4.7)	37.1 (26.1- 48.2)	39.3 (33.0-45.5)	20.5 (9.1-29.6)	28.4 (18.7-43.0)
101*	24.1 (19.9-30.7)	50.5 (19.7-66.2)	33.6 (18.8-59.5)	nd	52.5 (20.9-84.2)	52.9 (38.7-67.0)	21.5 (7.9-34.6)	46.1 (23.4-84.5)
77	nd	nd	nd	nd	10.1 (nd-20.2)	nd	nd	nd
118*	35.1 (27.7-43.9)	63.8 (25.9-78.7)	47.1 (24.6-81.1)	nd	82.0 (27.9-136.1)	84.7 (58.6-110.8)	32.7 912.8-50.6)	86.3 (39.3-171.0)
153*	53.1 (48.6-57.9)	119.1 (71.3-137.0)	122.7 (74.9-199.2)	29.4 (20.5-39.7)	118.8 (49.4-188.2)	114.0 (80.0-148.0)	51.3 (24.1-70.5)	148.9 (83.9-275.1)
105	28.6 (24.4-31.9)	52.9 (22.2-69.3)	31.8 (16.3-47.9)	0.4 (nd-1.7)	31.1 (nd-62.2)	45.9 (35.2-56.6)	17.2 (5.8-32.2)	30.9 (13.0-62.1)
138*	48.5 (42.9-56.9)	103 (48.6-128.3)	107.9 (62.7-181.6)	24.2 (14.5-39.0)	114.8 (50.0-179.5)	104.6 (72.1-137.2)	42.5 (20.7-69.6)	108.4 (57.1-200.1)
126	nd	nd	nd	nd	nd	nd	nd	nd
128	nd	nd	26.3 (14.0-38.6)	nd	18.8 (nd-37.6)	27.4 (22.8-32.00)	6.6 (nd-19.90)	3.4 (nd-10.3)
156	nd	nd	nd	nd	10.4 (nd-20.7)	10.5 (nd-20.9)	nd	nd
180*	28.9 (24.1- 32.7)	58.4 (32.9-69.8)	53.5 (29.9-81.5)	27.3 (nd-44.2)	42.4 (35.3-49.4)	35.5 (27.4-43.6)	22.9 (14.8-29.2)	50.6 (30.5-84.5)
169	nd	nd	nd	nd	nd	nd	nd	nd
170	nd	4.9 (nd-19.5)	26.6 (16.5-34.2)	25.0 (nd-34.3)	14.3 (nd-28.6)	13.8 (nd-27.6)	5.1 (nd-15.2)	36.1 (24.2-57.2)
∑PCBs	253.1 (236.5-282.6)	554.4 (283.4-661.6)	505.8 (287.1-805.6)	135.1 (118.2-150.0)	547.4 (209.6-885.1)	563 (398.6-727.5)	244.0 (126.6-359.3)	559.8 (326.9-1015.8)
∑Indicator PCBs*	253.1 (210.7-250.7)	496.6 (241.8-597.3)	421.2 (240.3-684.9)	109.7 (82.3-150.0)	462.7 (209.6-715.8)	465.5 (340.6-590.4)	215.1 (105.3-307.1)	489.3 (285.0-896.5)

nd = not detected; * = indicator PCB.

PCB exposure, using the toxic equivalency factors (TEFs) for fish developed by the World Health Organisation (Van den Berg *et al.*, 1998). The concentrations of the individual substances in a sample are multiplied by their respective TEF and subsequently summed to give total concentration of dioxin-like compounds expressed in TEQs. The present TEF scheme and TEQ methodology are primarily intended for estimating exposure and risks via oral ingestion (e.g. by dietary intake). On the other hand, the existence of indicator PCBs is important for the prediction of degree of lipophilic contamination although their toxicity is less than that of dioxin-like PCBs.

The concentration ranges of the annual means of individual PCBs congeners measured in fish species are shown in Table 2 and 3. PCBs were found in all the marine species at concentrations ranging between 135.1 ng/g fat in horse mackerel and 990.8 ng/g fat in goby (calculated as the sum of all the investigated congeners). The statistical test on ∑PCBs levels indicated that the differences among average annual concentrations were not statistically significant (P>0.05). The sum of the seven target congeners constituted between 75.2% and 98.2% of the PCBs.

The most abundant PCB congeners in all fish species were the indicator PCBs constituting more than 80% of the total amount of PCBs. The PCB pattern found in fish showed a predominance of PCB 153 followed by PCB 138, 180 and 118 for indicator PCBs. 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 (Naso *et al.*, 2005), in the Adriatic Sea (Bayarri *et al.*, 2001; Perugini *et al.*, 2004) and in the Marmara Sea (Coelhan *et al.*, 2006). Although there were differences among the marine species, PCB patterns were always dominated by a large contribution from the hepta-, hexa-, and pentachlorinated PCBs 180, 153, 138 and 118. PCB 153 was the dominant congener in all investigated species (Tables 2 and 3). The aforementioned congeners are the most abundant in commercial PCB mixtures, such as Aroclor 1254 and 1260, which are the most commonly used in European countries (Naso *et al.*, 2005). The 153, 138, 180 and 118 congeners turned out to be the most abundant, in part due to their high lipophilicity, stability and persistence which facilitates the adsorption in sediments and the accumulation in the aquatic ecosystem.

The experimental results indicate that PCB contamination of fish from the Bulgarian Black Sea coast is comparable to the results from the Marmara Sea, where average concentrations (sum of seven PCB congeners) were found in the range from 209.36 to 508.71 ng/g fat (Coelhan *et al.*, 2006). The levels of indicators PCBs found in the present study are lower than the results of fish species from the Gulf of Naples, the Mediterranean Sea (1,005.3-17,259.0 ng/g lipid weight) reported by Naso *et al.* (2005). Total PCB levels in goby from the Belgian North Sea were found in the range 860-3,200 ng/g lipid weight (Voorspoels *et al.*, 2004). The low levels of PCBs observed in fish tissues correspond with the fact that there is no industrial production of PCBs in Bulgaria.

The toxic 'dioxin-like' congeners (coplanar mono- and non-ortho PCBs 77, 105, 118, 156, 126 and 169) showed the lowest levels, especially the non-ortho congeners (PCBs 77, 126 and 169) with concentrations below the LOD for most of the samples. Coplanar PCBs are used in order to estimate the toxicity potential of PCB exposure. In our study, TEQs of the 6 'dioxin-like' congeners were calculated to be 0.25 pg TEQ/g wet weight (w.w.) for goby and 0.68, 0.64 and 2.73 pg TEQ/g w.w. for sprat, horse mackerel and shad, respectively. The comparison of our TEQ values in fish with those mentioned in the literature showed comparable levels for TEQs in sardine from the Spanish Atlantic southwest coast (0.75 pg/g w.w.) (Bordajandi, *et al.* 2006) and lower levels in our study than those measured in salmon from the Baltic Sea (12.6 pg/g w.w.) (Isosaari *et al.*, 2006). The European Union has not set a limit for PCBs in fish, but there has been a proposal for a limit of 8 pg TEQ/g w.w. in muscle meat of fish for the sum of dioxins and dioxin-like PCBs (Commission of the European Communities, 2006).

The PCB and DDT concentrations changed according to species and, in particular, if we evaluated them on wet weight. They would accumulate in the lipid fractions and the fish with a high fat content would be the most contaminated. A comparison of ∑DDTs and ∑PCBs (ng/g w.w.) found in fish samples during the study period (2007-2010) is shown in Figure 3. DDTs are prevalent contaminants in all investigated fish species. The order in terms of DDTs concentrations (ng/g w.w.) was found to be: shad > sprat > horse mackerel > goby. For PCBs the order is: shad > horse mackerel >sprat > goby. The greatest PCB concentrations were found in shad (69.2-140.25 ng/g w.w.), which is the species with the highest fat content. The lowest PCB concentrations were observed in goby samples (15.9 ng/g w.w.).

The differences in feeding preferences and lipid content of goby, sprat, horse mackerel and shad justify the large range of observed DDT levels (from 15.56 to 282.27 ng/g w.w. in goby and shad, respectively). Actually, in marine organisms, habitat, physiologic factors, lipid content, geographical origin and feeding behaviour are significant aspects that explain pollutant storage and pollutant elimination. The organochlorine pollution of the Black Sea ecosystem is possibly due to influences of freshwater flows from the Danube River into the Black Sea (Zhulidov *et al.*, 2003). The hypothesis of Zhulidov *et al.* is confirmed by the presence of the highest concentrations of organochlorine pollutants in shad (99.68 ng/g w.w.), which is a migratory species that enters the Danube River for spawning. The levels of PCBs found in the present study are in accordance with the results of marine fish from the northern Adriatic Sea (33.6-177.0 ng/g w.w.) determined by Bayarri *et al.* (2001).

4. Conclusions

The main metabolite p,p'-DDE was the most frequently detected compound in the marine species investigated and was present in much higher concentrations than the other DDTs (ranging from 119.32 to 1,324.44 ng/g fat). In all investigated samples the residues of DDT were found in the

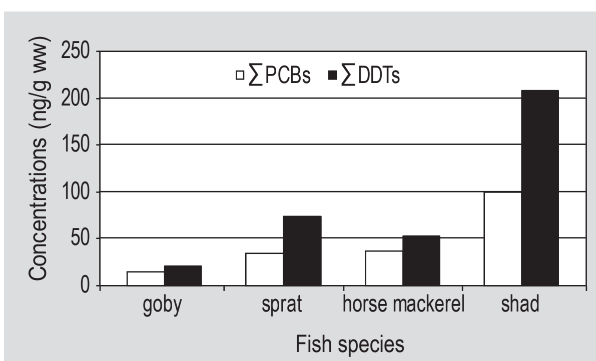

Figure 3. Comparison of ∑DDTs and ∑PCBs (ng/g wet weight) in fish samples from the Black Sea.

order DDE > DDD > DDT, showing the lack of significant fresh input of p,p'-DDT from the Bulgarian coast during 2007-2010 and reflecting the fact that countries around the Black Sea banned the use of DDT in the early 1970s. DDTs are prevalent contaminants in all fish species. PCBs were found in all fish species at concentrations ranging between 135.1 ng/g fat in horse mackerel and 990.8 ng/g fat in goby (calculated as the sum of all the investigated congeners). The most abundant PCB congeners in all fish species were the indicator PCBs constituting more than 80% of the total amount of PCBs. The levels of DDTs and PCBs in fish from the Bulgarian Black Sea coast were comparable to those found in fish from the neighbouring seas (the Marmara Sea, Aegean Sea and Mediterranean Sea).

Acknowledgments

This study was financed by the National Science Fund, Ministry of Education and Science of Bulgaria (project DVU 440/2008).

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