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# Organochlorine Pesticide and Polychlorinated Biphenyls Levels in Fish and Mussel in Van Region, Turkey

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**Abstract** Forty-seven fish (24 endemic *Alburnus tarichi,* Güldenstädt, 1814; 8 *Capoeta capoeta*, Guldenstaedt, 1772; 15 mirror carp *Cyprinus carpio,* L., 1758) and 13 mussel (*Unio stevenianus,* Krynicki, 1837) samples, with 10 specimens per sample, were collected from Van Lake, Turkey, and rivers flowing into it. Gamma-HCH was detected in 21 *Alburnus tarichi* samples (56.57 ng/g ± 22.18 ng/g) and in two *Capoeta capoeta* samples (27.6 ng/g and 36.45 ng/g). Beta-HCH was detected in 8 *Alburnus tarichi* samples (24.95 ng/g ± 4.42 ng/g) and in two mussel samples (101.25 ng/g and 129.44 ng/g). HCB was found in one *Alburnus tarichi* sample (14.4 ng/g) and one mussel sample (181.25 ng/g). The compound 4,4′-DDE was detected in 21 *Alburnus tarichi* samples (87.13 ng/g ± 32.23 ng/g), in 9 mirror carp samples (304.82 ng/g ± 100.76 ng/g) and one mussel sample (149.31 ng/g). PCB 28 was detected in one *Alburnus tarichi* (19.46 ng/g) sample and PCB 101 was found in one *Capoeta capoeta* (60.16 ng/g) sample. PCB 118 was detected in one mirror carp sample (277.5 ng/g) and in two *Capoeta capoeta* samples (43.77 and 54.38 ng/g). PCB 128 was detected in only one *Capoeta capoeta* sample (141.48 ng/g). It is concluded that (i) efforts should be made to reduce contamination of aquatic environments by these compounds and that (ii) their levels in fishery products from Van Lake and connected streams should be monitored and publicly reported on a regular basis.

**Keywords** Organochlorine pesticides · Polychlorinated biphenyls · Fish · Mussel

Organochlorines (OCs) constitute a family of persistent, lipid-soluble compounds that include industrial chemicals e.g. polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB); pesticides e.g. DDT, methoxychlor and mirex; and the by-products of combustion and various industrial processes e.g. polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs) (Ayotte et al. 2005). Polychlorinated biphenyls consist of 209 congeners differing in the number and position of chlorine atoms on the two coupled biphenyl rings (Storelli et al. 2003). Because of their high chemical stability, lipophilicity and persistence, these chemicals tend to bioconcentrate in food chains and persist in the environment for many years, representing a definite health hazard for both wildlife and humans (Naso et al. 2005). Fish are an excellent indicator organism for pollution in aquatic ecosystems, where trace contaminants are difficult to analyze directly. They also generally possess a low metabolism of OCs and should reflect the level of pollution in the aquatic environment (Coelhan et al. 2006).

In humans, the diet is the main source of intake, whereas inhalation and dermal absorption are less important. Contaminated fish, shellfish and animal fats are the main contributors to PCB and OCP intake in humans (Perugini et al. 2004). The United Nations Environment Program (UNEP) identified 12 persistent organic pollutants (POPs),

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including some PCBs and OCPs, as priority pollutants due to their impact on human health and the environment (Malakhova and Voronov 2008). In Turkey, the use of organochlorine pesticides (OCPs) began in 1945, with large quantities having been used during the 1960s and 1970s. Since 1983, the usage of OCPs has been severely restricted or banned (Erdogrul et al. 2005). To determine contamination levels in one environment, the aim of the present study was to investigate the residue levels of selected OCP and PCB compounds in fish and mussels collected from Van Lake and rivers flowing into Van Lake in Turkey.

## Materials and Methods

Forty-seven fish (24 endemic *Alburnus tarichi,* Güldenstädt, 1814; 8 *Capoeta capoeta,* Guldenstaedt, 1772 and 15 mirror carp *Cyprinus carpio,* L., 1758) and 13 mussel (*Unio stevenianus,* Krynicki 1837) samples (each sample consisted of 10 specimens) were collected from Van Lake, Turkey and connected rivers in 2008.

The method described in Bordet et al. (2002) was used to analyse organochlorine pesticide and polychlorinated biphenyl levels. For fat extraction, 25 mL of n-hexane (Merck, Germany) was added to 20 g of homogenized fish flesh. The sample was mixed and centrifuged (Nuve, Turkey) for 5 min at 1,500 rpm. The upper phase was decanted and extraction was repeated with 25 mL of n-hexane. The two extracts were mixed together. Solvent was evaporated to 1 mL in a rotary evaporator (Buchi, Switzerland) at 35°C and evaporation was finalised with a gentle stream of nitrogen. Oil quantities of samples were measured gravimetrically. OCPs (alpha-HCH, beta-HCH, gamma HCH, HCB, aldrin, 2,4′-DDE, 4,4′-DDE, 2,4′-DDT, 4,4′-DDT, Dr.Ehrenstorfer, Germany) and PCBs (28, 70, 74, 81, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187, Dr.Ehrenstorfer, Germany) were extracted from fat by cryogenic extraction. Half a gram (0.5 g) of fat extract was placed in a centrifuge tube, a 3 mL mixture of acetonitrile (Merck, Germany)—methylene chloride (Merck, Germany) (75 + 25, v/v) was added, and they were mixed vigorously with a vortex (Velp Scientifica, Spain). The mixture was centrifuged for 20 min at 3,000 rpm and −9°C. The upper layer of supernatant was retained. The bottom layer was then slowly heated to melt fat and extraction was repeated with 3 mL of the same solvent mixture. The organic phase was evaporated under nitrogen at 35°C to a volume of 2 mL (solution A).

For clean-up, a C18 SPE cartridge (Phenomenex, USA) was twice conditioned with 5 mL of petroleum ether (Merck, Germany), 5 mL of acetone (Merck, Germany), and 5 mL of methanol (Merck, Germany) and eluted to meniscus. Two mL of solution A was loaded into the cartridge, retained for 3 min, and eluted to meniscus. After that, the analyte was eluted with 10 mL of acetonitrile at a rate of 1 drop/3s. The elutant was evaporated at 35°C with 100 μL of dodecane (Merck, Germany) and then diluted in n-hexane (solution B). A florisil SPE cartridge (Phenomenex, USA) was conditioned with 10 mL of n-hexane and eluted to meniscus. Solution B was loaded into the cartridge, retained for 3 min, and eluted with a 10 mL mixture of petroleum ether–diethyl ether (Merck, Germany) (98 + 2, v/v; 1 drop/s) and a 12 mL mixture of petroleum ether–diethyl ether (85 + 15, v/v; 1 drop/3s), successively. The two fractions were mixed and completely evaporated with 100 μL of dodecane at 35°C under nitrogen. The final extract was dissolved in 2 mL of n-hexane (solution C).

Analysis of samples was performed with a gas chromatograph equipped with an electron capture detector (GC-ECD; Schimadzu GC 17A, Japan). A capillary column 60 m × 0.32 mm i.d. and a 25 μm thick film of phenyl-methyl silicon (Teknokroma, Spain) was used. The flow rate of the nitrogen carrier gas was 1.5 mL/min. Column oven conditions were as follows: Initial temperature 100°C, held for 3 min; 10°C/min ramp to 200°C, held for 3 min; 3°C/min ramp to 225°C, held for 3 min; 2°C/min ramp to 270°C, held for 3 min and 1°C/min ramp to 275°C, held for 10 min. A one microliter, splitless injection was carried out at 260°C, the detector temperature was 280°C and analysis time was 67.83 min.

## Results and Discussion

The retention times of the PCB and OCP compounds were determined. Calibration curves of the compounds were drawn from five standard dilutions and the correlation coefficients were between 0.993 and 0.999. Recovery percentages of PCB and OCP compounds were between 68% and 97% and 69% and 117%, respectively. Limits of detection (LOD) were from 0.31 to 1.13 ng/g and from 0.01 to 0.59 ng/g for PCBs and OCPs, respectively. LOD values and recovery percentages of PCB and OCP compounds are shown in Table 1 and 2, respectively.

No samples were positive for residues of PCB-70, 74, 81, 99, 105, 138, 153, 156, 170, 180, 183 and 187, and no mussel samples were positive for PCB residues. Concentrations of PCBs in fish samples are shown in Table 3.

No samples were positive for residues of alpha-HCH, aldrin, 2,4′-DDE, 2,4′-DDT and 4,4′-DDT. Concentrations of OCPs in positive samples are presented in Table 4.

Perugini et al. (2004) reported that the concentrations of PCBs were higher than OCPs in the tissues of marine organisms in Italy. The highest concentrations of total PCBs (1415 ng/g lipid) and total OCPs (507 ng/g lipid)

**Table 1** LOD values and recovery percentages of PCBs

| Compound | LOD (ng/g) | Rec. % (±SD) | Compound | LOD (ng/g) | Rec. % (±SD) |
|---|---|---|---|---|---|
| PCB 28 | 0.72 | 86 (±14) | PCB 128 | 0.58 | 83 (±4) |
| PCB 70 | 0.53 | 84 (±7) | PCB 138 | 0.45 | 88 (±7) |
| PCB 74 | 0.91 | 83 (±6) | PCB 153 | 1.13 | 86 (±10) |
| PCB 81 | 0.69 | 68 (±10) | PCB 156 | 0.70 | 76 (±5) |
| PCB 99 | 0.87 | 92 (±7) | PCB 170 | 0.59 | 97 (±15) |
| PCB 101 | 0.43 | 89 (±7) | PCB 180 | 0.31 | 80 (±5) |
| PCB 105 | 1.88 | 86 (±5) | PCB 183 | 0.34 | 83 (±2) |
| PCB 118 | 0.78 | 88 (±6) | PCB 187 | 0.38 | 84 (±2) |

**Table 2** LOD values and recovery percentages of OCPs

| Compound | LOD (ng/g) | Rec. % (±SD) | Compound | LOD (ng/g) | Rec. % (±SD) |
|---|---|---|---|---|---|
| Alpha-HCH | 0.02 | 69 (±3) | 2,4′DDE | 0.04 | 72 (±9) |
| Beta-HCH | 0.03 | 77 (±6) | 4,4′DDE | 0.02 | 87 (±15) |
| Gamma-HCH | 0.02 | 76 (±2) | 2,4′DDT | 0.59 | 106 (±11) |
| HCB | 0.02 | 69 (±6) | 4,4′DDT | 0.40 | 117 (±15) |
| Aldrin | 0.01 | 69 (±5) | | | |

were detected in pilchards. All samples contained different concentrations of PCBs, and among those the congeners 153 and 138 were in the highest concentrations. Among the OCPs, except for the cuttlefish, 4,4′-DDE and 4,4′-DDD were in the highest concentrations. None of the samples analysed exceeded the tolerance limits established by Italian legislation.

Six indicator PCBs, 12 dioxin-like PCBs (dl-PCBs) and 17 polychlorinated dibenzo-p-dioxin/furan (PCDD/F) congeners were investigated in sediment and mussel samples collected along the coastline of the Istanbul Strait and an island in the Marmara Sea in Turkey by Okay et al. (2009). Samples contained different concentrations of PCBs and among those, congeners 153, 75, 105 and 118 in sediments and congeners 153, 138 and 118 in mussels were most abundant. The concentrations of total PCBs and PCDD/Fs in sediments ranged from 17.9 to 539 746 pg/g dry matter (dm) and from 2.04 to 60.5 pg/g dm, respectively. None of the sediment and mussel samples analysed exceeded the limits stated in the sediment quality guidelines and safe values for seafood intended for human consumption, respectively, set by the European Community (EC).

The contamination of 12 species of fish, shellfish and crustaceans was investigated by Stefanelli et al. (2004) in Italy. Samples collected from October to December 1997 from local fishermen were representative of northern, central and southern Adriatic Sea catches. All samples contained PCBs at various levels. Among those, the PCBs 170, 180, 187, 153, 138, 118, 101 and 52 were the most frequently detected, and were also at the highest concentrations which ranged from 12.42 to 88.17 ng/g. Among the OCPs, essentially only 4,4′-DDE and 4,4′-DDD were detected, with the former appearing at levels up to 25 ng/g wet weight.

The residue levels of OCPs in sediment, mussel and seawater samples were investigated in Turkey by Ozkoc et al. (2007). Samples were collected three times from 2001 to 2003 at nine sampling stations along the mid-Black Sea coast. DDT and its metabolites were detected at concentrations significantly above the detection limits. The highest concentrations of DDT metabolites measured in sediment and mussel samples were 35.9 ng/g and 14 ng/g wet weight, respectively. Considerable quantities of aldrin, dieldrin, endrin, heptachlor epoxide, lindane, endosulfan sulphate and HCB were also detected in the samples. The biota sediment accumulation factor (BSAF) estimated for DDT and its metabolites in mussels was 2.9, which was approximately 70% higher than the benchmark of 1.7. Despite the high BSAF values observed for those compounds, their levels in mussels were significantly below the international legal limits recommended by the Food and Agriculture Organization (FAO) of the United Nations (UN) (Ozkoc et al. 2007).

Heavy metals and both PCB and OCP residues were investigated in marine organisms in Italy by Marcotrigiano and Storelli (2003). In all species examined, higher PCB, OCP and HCB concentrations were detected in the liver and digestive glands than in muscle, in line with the higher lipid contents of those organs. In the liver and muscle tissue of fish, the accumulation order of the different contaminants was PCB = DDTs > HCB. Among DDTs in

**Table 3** Concentration of PCBs on oil and wet weight basis of positive samples (ng/g) from fishery products from Van Lake and connected rivers, Turkey

| | *Alburnus tarichi* | | | *Capoeta capoeta* | | | Mirror carp | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | Con.[a] | Con.[b] | n | Con.[a] | Con.[b] | n | Con.[a] | Con.[b] |
| PCB 28 | 1 | 19.46 | 0.53 | – | <LOD | <LOD | – | <LOD | <LOD |
| PCB 101 | – | <LOD | <LOD | 1 | 60.16 | 0.72 | – | <LOD | <LOD |
| PCB 118 | – | <LOD | <LOD | 2 | 43.77–54.38 | 0.79–0.84 | 1 | 277.5 | 0.56 |
| PCB 128 | – | <LOD | <LOD | 1 | 141.48 | 2.19 | – | <LOD | <LOD |
| Lipid (%) | 24 | 3.68 ± 1.96 | | 8 | 1.42 ± 0.40 | | 15 | 0.33 ± 0.24 | |

[a] Concentration on oil weight basis

[b] Concentration on wet weight basis

**Table 4** Concentration of OCPs on oil and wet weight basis of positive samples (ng/g) from fishery products from Van Lake and connected rivers, Turkey

| | *Alburnus tarichi* | | | *Capoeta capoeta* | | | Mirror carp | | | Mussel | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Con.[a] | Con.[b] | n | Con.[a] | Con.[b] | n | Con.[a] | Con.[b] | n | Con.[a] | Con.[b] |
| Beta-HCH | 8 | 24.95 ± 4.42 | 0.76 ± 0.32 | – | <LOD | <LOD | – | <LOD | <LOD | 2 | 101.25–129.44 | 0.41–0.52 |
| Gamma-HCH | 21 | 56.57 ± 22.18 | 2.02 ± 1.22 | 2 | 27.60–36.45 | 0.50–0.56 | – | <LOD | <LOD | – | <LOD | <LOD |
| HCB | 1 | 14.40 | 0.56 | – | <LOD | <LOD | – | <LOD | <LOD | 1 | 181.25 | 0.36 |
| 4,4′-DDE | 21 | 87.13 ± 32.23 | 2.81 ± 1.07 | – | <LOD | <LOD | 9 | 304,82 ± 100.76 | 0.72 ± 0.13 | 1 | 149.31 | 0.60 |
| Lipid (%) | 24 | 3.68 ± 1.96 | | 8 | 1.42 ± 0.40 | | 15 | 0.33 ± 0.24 | | 13 | 0.25 ± 0.08 | |

[a] Concentration on oil weight basis

[b] Concentration on wet weight basis

muscle tissue, the compound present in the highest proportion was 4,4′-DDT (81.6%). The same compound was also in the highest proportion in the liver (89.7%). In bivalve molluscs, PCB, 4,4′-DDE and HCB levels were very low. In crustaceans too, PCBs and 4,4′-DDE were at low levels, while the HCB concentration was below the LOD in all the samples examined.

OCP (aldrin, alpha-endosulfan, beta-endosulfan, 2,4′-DDT, and 4,4′-DDE) and PCB (PCB-28 and PCB-52) levels were screened in cultured fish in cages on the Aegean coast by Koc et al. (2008) in Turkey. 4,4′-DDE was found in 2.63% of the 114 samples. Contamination levels varied with species, ranging from 18 to 200 ng/g on a wet weight basis.

The Ministry of Agriculture and Rural Affairs (MARA) monitors residues in live animals and foods of animal and plant origin in Turkey. For those purposes, MARA prepares the annual National Residue Monitoring Plan and checks farm and processing facilities through sampling and analysis (MARA 2010). In the Turkish Food Codex and European Commission Directive, MRLs are stated for dioxin and dl-PCBs in fish flesh. According to these regulations, the maximum limit for dioxin and dl-PCBs is 8 pg/g of wet weight TEQ (toxicity equivalence) of fish flesh (EC 2006; MARA 2008a).

In the current study, only two *Capoeta capoeta* (0.79–0.84 ng/g wet weight) and one mirror carp (0.56 ng/g wet weight) samples were positive for a dl-PCB (PCB-118). According to the Turkish Food Codex and European Commission Directive (1881/2006), toxic equivalency factor (TEF) value of PCB-118 is 0.0001. Therefore, calculated TEQ values were 0.079–0.084 pg/g for Capoeta capoeta and 0.056 pg/g for mirror carp samples and those values were approximately 100 times lower than MRLs. The other investigated dl-PCBs (PCB-81, –105, and –156) were also lower than the MRLs.

In the Turkish Food Codex and European Commission Directive, the established MRLs for OCPs for different vegetable and animal foods are stated. However, the MRLs have not been determined for fish and the other seafood yet (EC 2005; MARA 2008b). Although beta-HCH, gamma-HCH and 4,4′-DDE were detected in most of the *Alburnus tarichi* samples, the residue amounts were at a low level on a wet weight basis. The compound 4,4′-DDE was also found in 9 mirror carp samples but the residue levels were low. HCB was found in only 1 *Alburnus tarichi* and 1

mussel sample. Taking into account the wet weight basis for determination, the residue levels were quite low.

In the present study, 47 fish samples and 13 mussel samples (10 specimens/sample) were analyzed for residues of 9 OCPs and 16 PCBs. It is concluded that the residue levels in the analysed fishery product samples appear to represent a minimal risk to public health. However, the levels of persistent organic pollutants should be monitored continuously by similar studies for the protection of public and environmental health.

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