

Persistent Organic Pollutants in Muscle and Feather of Ten Avian Species from Māzandarān Province of Iran, on the Coast of the Caspian Sea

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Abstract Feather and muscle of 10 avian species ($n = 46$), were analyzed for polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). Muscle contained significantly higher PCB and OCP than liver and feather. Mean muscle and feather PCB was 408.5 ± 134.5 and 32 ± 4.5 ng/g wet weight. Highly chlorinated PCBs were found in muscle and liver while feather had PCBs with less chlorination. Gulls had highest levels of both pollutants. Gull feather PCB and OCP were strongly correlated with their levels in the liver and muscle tissues ($0.6 < r < 0.9$, $p < 0.01$). Analysis of gull feather can be used as a non-invasive method for monitoring organic pollutants.

Keywords Polychlorinated biphenyls and organochlorine pesticides · Liver · Muscle · Feather · Avian · Māzandarān Province of Iran

Persistent organic pollutants (POPs) have been linked to decline of many avian species worldwide. Such declines were associated with decreased chick survival as a result of

poor hatchability and severe eggshell thinning caused by dichlorodiphenyltrichloroethane and dioxin-like compounds in the environment. In 2001, Stockholm Convention on Persistent Organic Pollutants, an international environmental treaty, formally adopted a plan to restrict and eventually eliminate these pollutants that have already had adverse impacts on wildlife and human health and reproduction. Iran has been a member of Stockholm Convention since 2003 and although the use of POPs is strictly forbidden in Iran, intense none-organic agricultural practices in the Caspian Sea area utilize pesticides in large quantities. Such practices are of great local and national concerns.

Environmental contamination by POPs, including significant lindane pollution in eighteen rivers in the coastal provinces of the Caspian Sea, has been reported. In addition, high concentrations of PCBs and OCPs have been detected in the Caspian Seal, fish and birds from this area (Watanabe et al. 1999; Kajiwarra et al. 2003; Rajaei et al. 2010). This trend in environmental degradation must be addressed. To protect and preserve the Caspian Sea ecosystems from further damage, its eco-toxicological status must be further investigated and the extent of OCP and PCB contamination must be elucidated. Many avian species have been used to monitor contaminations of the region. However, ethical concerns have been raised. Use of feather in biomonitoring of contamination has been suggested as a non-invasive biomonitoring method (Jaspers et al. 2006). Our aim was to investigate the relationship between levels of POPs in avian feather and internal tissues in order to coin a useful non-destructive biomonitoring tool for these pollutants in this area. This non-destructive tool will be used in determining progress in area remediation efforts that are intended to protect ecological integrity of the Caspian Sea.

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Materials and Methods

Forty-six injured or killed birds were collected from Māzandarān Province in the winter of 2008 (Fig. 1). A net was used to catch another 10 birds. Birds were identified, and placed in four families: (1) *Phalacrocoracidae*, (2) *Podicipedidae*, (3) *Laridae*, and (4) *Anatidae*; then dissected the same day and muscle, liver, and feather were removed. A mixer was used to homogenize muscle and liver. Homogenized tissues was wrapped in aluminum foil, put in clean plastic bags, and stored at -20°C until analysis. Feather were washed well with distilled water and dried at room temperature.

Organochlorine pesticides including HCHs (α -HCH, β -HCH, γ -HCH), hexachlorobenzene (HCB), *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *p,p'*-DDD, and some congeners of PCB (IUPAC Nos. PCB 28, 52, 101, 118, 138, 153 and 180) were analyzed. Standards were obtained from Ehrenstorfer Inc. (Augsburg, Germany) and chemicals were purchased from Merck Inc. (Darmstadt, Germany). Sample treatment and analysis followed Jaspers et al. (2006) with minor modifications. Briefly, -6 g of soft tissue was homogenized then dried by blending it with anhydrous sodium sulfate. Samples were spiked with internal standard (PCB 143 and ϵ -HCH). Extraction was done with 100 mL hexane/acetone (3:1, v/v) in Soxtec 2050 (Foss) for 2 h. The extract was treated with concentrated sulfuric acid for lipid purification and further cleanup was accomplished on a column filled with 8 g acidified silica gel and desiccated sodium sulfate. The column was eluted with 15 mL hexane and 10 mL dichloromethane, respectively. The eluate was concentrated to 100 μL under

a gentle nitrogen stream. Feather preparation was adapted from the methods of Covaci and Schepens (2001). Prepared feather samples were weighed and spiked with internal standard (PCB 143 and ϵ -HCH) and incubated overnight at 40°C with HCL and mixture of hexane and dichloromethane (4:1 v/v). After liquid Extraction, clean up was performed with cartridge filled with acid silica and anhydrous. The final elute was concentrated to 50 mL and 1 μL of extract was injected into a gas chromatographic (GC) system.

GC analysis was performed using a Dani 1000 gas chromatograph equipped with ^{63}Ni electron capture detector and a DB-5 capillary column (60 m \times 0.25 mm i.d., 0.25 μm film thickness, Macherey- Nagel). Helium, at a flow rate of 2 mL/min, was used as a carrier. The operating conditions were split (1:1) injection mode. Temperature program was: 100°C (1 min), $10^{\circ}\text{C}/\text{min}$ to 240°C (1 min), $3^{\circ}\text{C}/\text{min}$ to 260°C (1 min), $20^{\circ}\text{C}/\text{min}$ to 300°C (10 min). The injection port temperature and detector temperature were 250 and 300°C . Multiple-level calibration curves were created for the quantification. Good linearity ($r^2 > 0.99$) was achieved for tested intervals, including the concentration range of samples. Each analyte was identified by a comparison of its relative retention time to the peaks form the calibration standards. Quantification was based on a comparison with calibration curves in the concentration range of 0.005, 0.01, 0.05, 0.1, 0.3, 0.5 ppm. Spiking was done at 0.2 and 0.4 ppm. Recoveries of spiked PCBs and OCPs in samples which passed through the analytical procedure were between 91% and 105%. Limit of quantification (LOQ) for OCPs ranged between 0.1 and 0.6 ng/g ww; and LOQ for PCBs ranged between 0.1 and 0.8 ng/g ww ($\text{RSD} \leq 13$).

SPSS software (Version 11.5) was utilized. Data were tested for normality using a Kolmogorov–Smirnov test. Data was normally distributed and a nonparametric procedure was used. Kruskal–Walis was used to determine any differences in concentration of OCPs and total PCBs among different species and families and tissues. If significant differences were detected a Mann–Whitney U test was used. Spearman's rank correlation coefficients were used to test for the correlations amongst OCs tissues and feather. Samples that had frequencies of lower than three were removed from statistical analysis and p was set at <0.05 .

Results and Discussion

Organochlorine pesticides and PCBs in feather and muscle (mean ng/g wet weight) of birds from Māzandarān Province of Iran are presented in Tables 1 and 2. Figures 2 and 3 allow a visual comparison between levels in feather



Fig. 1 Māzandarān Province, in the cost of the Caspian Sea, where birds of this study were collected

Table 1 Organochlorine pesticides and PCB congeners in muscle of birds from Māzandarān Province of Iran (mean ng/g wet weight)

Mallard (n = 3)	Common teal (n = 4)	Pintail (n = 3)	Common gull (n = 3)	Little gull (n = 5)	Black-headed gull (n = 8)	Little grebe (n = 3)	Black-necked grebe (n = 3)	Great crested grebe (n = 6)	Great cormorant (n = 8)	
<LOQ	1.20 (0.6–2.2)	0.1 (0.1–1.5)	0.8 (5–10.5)	32 (46–23.8)	8.4 (3.5–17.5)	12 (4–15)	14 (3.5–45.5)	0.6 (4–8.5)	0.15 (0.5–44.5)	p,p'-DDT
<LOQ	0.2 (0.1–6.7)	2 (<LOQ–6.4)	6.6 (0.2–17.8)	0.36 (96.7–88.5)	1.5 (<LOQ–3)	<LOQ	0.5 (0.1–1.5)	0.1 (0.1–19)	0.7 (0.1–1.7)	o,p'-DDT
2 (0.4–3)	0.6 (<LOQ–1.2)	2.2 (0.3–4.8)	2.3 (0.2–4.5)	19.5 (<LOQ–90)	2.20 (0.40–7.80)	7 (5.5–9)	6.50 (<LOQ 32.00)	5.50 (0.20–85.50)	00.6 (<LOQ–16.50)	p,p'-DDD
483.5 (15.5–141)	557 (1–2,199)	150.8 (0.5–450)	2,293 (33–6,783)	3,382 (9–9,040)	16 (3–52)	31 (14–35)	0.44 (2.5–178)	0.35 (1.5–7,639)	24.4 (4–58.5)	p,p'-DDE
<LOQ	0.6 (<LOQ–0.5)	<LOQ	0.5 (<LOQ–0.5)	1.5 (0.6–2.8)	0.5 (<LOQ–1.51)	<LOQ	0.1 (<LOQ–0.2)	1.30 (0.4–2.5)	0.7 (<LOQ–2)	o,p'-DDE
<LOQ	0.3 (<LOQ–1.3)	<LOQ	<LOQ	6.8 (<LOQ–34)	0.7 (<LOQ–25)	<LOQ	14.3 (<LOQ–54)	<LOQ	3.9 (<LOQ–24)	α-HCH
2.4 (0.2–6.6)	2.3 (0.5–3.9)	3.2 (1.5–5.3)	0.4 (<LOQ–7.5)	11.2 (<LOQ–8.8)	8.5 (0.3–20.6)	7 (2–9)	33.8 (<LOQ–13.6)	8.5 (2.5–14)	9.2 (<LOQ–20.5)	β-HCH
<LOQ	0.3 (<LOQ–1)	0.6 (<LOQ–1.8)	4.4 (<LOQ–3.2)	4.4 (<LOQ–6.7)	5.6 (ND–23.4)	31.5 (19–45)	8.7 (<LOQ–45)	0.6 (<LOQ–2.7)	4 (<LOQ–2)	γ-HCH
166.7 (0.3–499)	30 (<LOQ–120)	78.5 (<LOQ–235)	424.5 (0.8–1,271)	260.4 (0.7–689)	2.3 (1.1–4.9)	2 (1–5)	3.38 (1.6–7.9)	212.1 (0.9–501)	9.5 (1.1–46.2)	HCB
7 (2–9.5)	2 (1.5–31)	4 (2–6.5)	6 (4–7)	14.5 (0.8–6)	5 (0.3–11)	9 (3–11)	11 (1.5–17)	3 (2–7)	6 (<LOQ–21)	PCB28
<LOQ	0.3 (<LOQ–11)	0.5 (<LOQ–2)	<LOQ	0.5 (<LOQ–1.5)	2 (<LOQ–11)	<LOQ	<LOQ	<LOQ	0.5 (<LOQ–1.5)	PCB52
<LOQ	0.4 (<LOQ–1.5)	<LOQ	1.5 (<LOQ–4)	1 (0.2–3)	0.5 (0.2–1.5)	0.5 (0.5–1)	0.2 (<LOQ–0.4)	0.7 (<LOQ–1.5)	0.4 (<LOQ–0.8)	PCB101
6.5 (0.2–9.5)	1.5 (<LOQ–5)	0.2 (<LOQ–0.4)	9 (2.5–17.5)	29 (12.5–48)	16 (7–34)	6 (4–9)	12 (4–40)	526 (2–11,056)	16 (4–27)	PCB118
52.5 (10–136)	33 (<LOQ–124)	10 (<LOQ–30)	487 (3–1,423)	1,297 (50–4,098)	18 (8–42)	6 (4–8)	12 (5–39)	7 (4–13)	18 (6–34)	PCB153
8.5 (0.7–12.5)	2 (<LOQ–6)	<LOQ	10 (5–13)	29 (21.5–41.5)	10 (3–20.5)	10 (5–13)	15.00 (2–55)	6 (0.2–9)	14 (6–33)	PCB138
4.5 (0.5–7)	8.5 (0.7–12.5)	0.5 (0.2–0.7)	13 (2–25.5)	44.5 (3–12)	6 (<LOQ–15)	2 (1–3.5)	6 (2–15)	15 (3–25)	7 (3–14)	PCB180

Number of samples (n) is indicated at the top of each column, and the range is under the mean in parentheses

Table 2 Organochlorine pesticides and PCB congeners in feather of birds from Māzandarān Province of Iran (mean ng/g wet weight)

Mallard (n = 3)	Common teal (n = 4)	Pintail (n = 3)	Common gull (n = 3)	Little gull (n = 5)	Black-headed gull (n = 8)	Little grebe (n = 3)	Black-necked Grebe (n = 3)	Great crested Grebe (n = 6)	Great cormorant (n = 8)	
1.4 (<LOQ-2)	1 (<LOQ-3)	0.5 (0.3–0.9)	1.8 (<LOQ-3)	5.8 (<LOQ-13)	6.7 (<LOQ-19)	15 (6–10)	1.3 (<LOQ-1.3)	1 (<LOQ-2.3)	2.3 (<LOQ 7)	p,p'-DDT
7 (3.3–9)	40.5 (2–89.7)	40 (26.3–52.2)	7.9 (<LOQ-19)	50.2 (ND-123)	10.5 (<LOQ-41.5)	1 (0.5–3)	10.3 (<LOQ-43.4)	1 (<LOQ-5.9)	16 (4.1–35.5)	o,p'-DDT
1.3 (0.5–1.7)	3.2 (2.5–3.8)	2.4 (1.8–2.9)	0.7 (<LOQ-1.8)	12.3 (<LOQ-28.9)	2 (<LOQ-8)	5 (<LOQ-15)	3.4 (0.7–7)	1 (<LOQ-2.4)	4 (11–6)	p,p'-DDD
14.3 (8–18)	50.5 (11.5–125)	17 (10–23)	28 (12.8–41)	96 (44–148)	99 (11–370)	13 (3–8)	69 (5.2–267)	89 (5.2–31)	21 (3.3–36)	p,p'-DDE
0.2 (<LOQ-0.01)	2 (0.2–4)	0.04 (<LOQ-0.1)	0.4 (0.1–0.8)	12 (<LOQ-58)	0.5 (<LOQ-1)	<LOQ	0.3 (<LOQ-1)	0.2 (<LOQ-1)	0.5 (<LOQ-1)	o,p'-DDE
ND	1 (<LOQ-2.4)	1 (<LOQ-3.2)	6 (<LOQ-12)	<LOQ	4.70 (ND-20)	<LOQ	8.4 (<LOQ-25.3)	<LOQ	1 (<LOQ-0.4)	α-HCH
15 (14–15.4)	25 (1.4–66)	5 (0.8–12)	21 (<LOQ-58)	17 (<LOQ-27)	9 (<LOQ-26)	2.5 (1–5)	9 (<LOQ-22.4)	4 (<LOQ-10)	13 (2.4–28)	β-HCH
6 (<LOQ-18)	21.5 (3.5–40)	12 (3.5–19)	7.5 (<LOQ-12)	4 (<LOQ-14)	11 (ND-45)	4.5 (<LOQ-7)	26 (2.8–93)	7 (<LOQ-23)	7 (<LOQ-29)	γ-HCH
0.4 (0.4–0.5)	6 (2–10)	2 (1–4)	4.5 (3–7)	1 (<LOQ-3)	1.6 (<LOQ-8)	39 (10–40)	4 (1.9–12)	2 (1–4)	2 (<LOQ-5.5)	HCB
29.7 (6–42)	26 (10–46)	9.6 (7–14)	8 (3–19)	59 (<LOQ-99)	48 (<LOQ-107)	8 (3–10)	47 (7–125)	17 (1–38)	22 (4–47)	PCB28
48 (<LOQ-72)	9 (<LOQ-24)	19 (3–46)	12 (<LOQ-34)	41 (<LOQ-141)	40 (1–82)	16 (5–20)	26 (<LOQ-80)	13 (<LOQ-63)	17 (<LOQ-62)	PCB52
<LOQ	3 (1–5)	0.7 (0.2–1.10)	0.2 (<LOQ-0.7)	0.7 (<LOQ 2.2)	0.9 (<LOQ-1.6)	<LOQ	1 (<LOQ-3)	1.3 (0.7–3)	0.5 (<LOQ-1.6)	PCB101
1.2 (<LOQ-1.9)	2 (<LOQ-3.5)	1 (0.9–1.2)	1.4 (0.6–1.8)	11.8 (5–19.5)	5 (1.5–8.6)	1 (0.5–1)	2.7 (<LOQ-8.6)	3.3 (0.9–9)	3.7 (1–6.5)	PCB118
2 (<LOQ-3)	3 (1–5)	2 (0.4–2.3)	2 (0.2–3)	14.2 (6–31)	4 (1.5–7)	<LOQ	4 (1.3–11)	1.6 (<LOQ-4)	7 (1.4–15)	PCB153
4 (0.2–5.5)	6.5 (<LOQ-12)	2 (0.6–3.2)	2.5 (2–3)	25 (2–3)	7 (3–16)	2.5 (1–5)	7 (1.4–25)	5 (2–12)	2 (<LOQ-4)	PCB138
ND	3 (ND-13)	ND	36 (ND-80)	27 (6–60)	18 (3–51)	<LOQ	6 (<LOQ-30)		24 (<LOQ-111)	PCB180

Number of samples (n) is indicated at the top of each column, and the range is under the mean in parentheses
LOQ limit of quantification, ND not detected

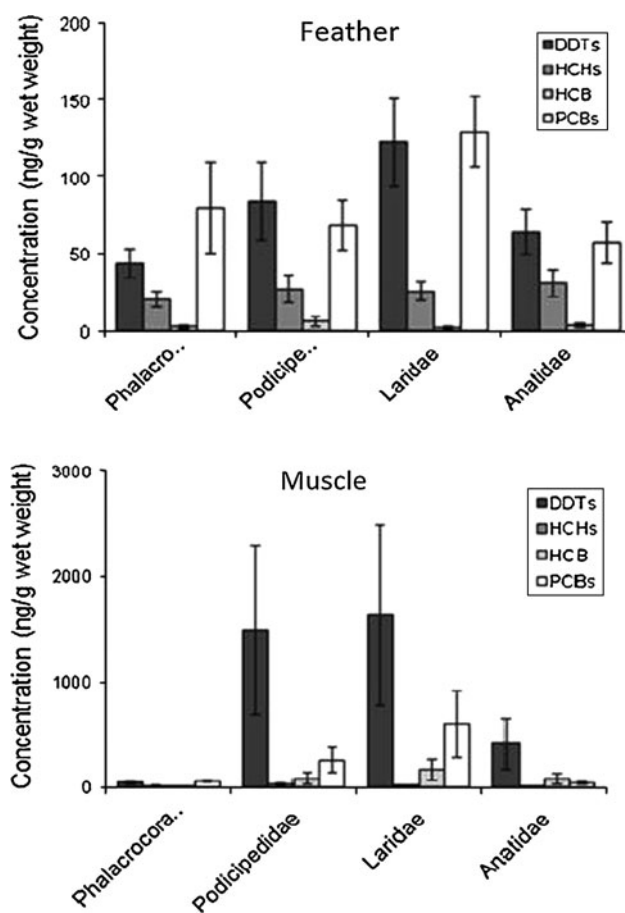


Fig. 2 Comparison of organochlorine compounds in feather and muscle of four families of water birds from Māzandarān Province of Iran

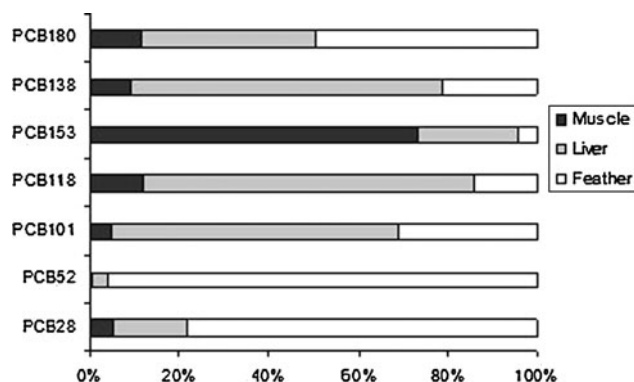


Fig. 3 Over all comparison between levels of PCBs congeners in liver, muscle and feather of 10 avian species of water birds from Māzandarān Province of Iran

compared to the soft tissues (muscle and liver). Liver data was previously published (Rajaei et al. 2010). PCB and OCP were significantly higher in the muscle ($p < 0.05$); but feather and liver had similar levels of both pollutants. Highest and lowest OCPs in the muscle were DDTs

(1089.5 ± 380 , 89% of total) and HCHs (20 ± 3.8 , 1.6% of total); and in feather they were DDTs (87 ± 13.2 , 53% of total) and HCB (3.5 ± 1 , 1% of total). DDE and β -HCH were the most abundant DDT, and most abundant HCHs. This profile of accumulation suggests old rather than recent use of these organics.

Bioaccumulation of POPs in birds and other organisms depend on age, size, type and lipid content of the organism. In birds, molting and the ability to detoxify pollutants also play a significant role in body toxicant levels (Fossi et al. 1995). During molting, levels of some pollutants in tissues drop as they are sequestered in the feathers that are molted. When molting is completed and the birds begin to feed on contaminated food, once again, levels of pollutants in the internal organs will raise until the next molting when the process of detoxification by molting is repeated.

Lipids like triglycerides and phospholipids are both found in birds. They differ in structure and function and they are differently distributed in the body. Differences in the type of lipids found in an organ can affect the types of POPs accumulated in that organ (Boumphrey et al. 1993).

We found muscle and liver to have similar profiles for PCB accumulation but this profile was different in the feather. Highly chlorinated PCB congeners like, 138 (11.5 ± 2), 153 (261 ± 113), and 118 (12 ± 2) ng/g wet weight, were found in the muscle. Feather contained less chlorinated congeners including 52 (25.5 ± 5) and 28 (32.5 ± 5). Similar results have been reported in the literature and it has been argued that lipids in the preen gland, that end up on the feathers, have less chance of undergoing metabolism before secretion; therefore they contain lower chlorinated PCB congeners (Yamashita et al. 2007). Dauwe et al. (2005) reported that although temporal differences may cause seasonal changes in lipid reserves, POPs in feathers and fat showed significant positive correlations in samples collected during the breeding season and feathers appear shows potential to be a new, nondestructive biomonitor for PCBs and DDTs in avian wildlife.

We examined the differences between species and families. OCPs and PCB varied widely since our birds belonged to different families and occupied varying trophic levels. From highest to lowest, OCs were found in Laridae > Podicipedidae > Anatidae > Phalacrocoracidae families. Laridae and Podicipedidae were significantly different than others ($p < 0.05$). As previously shown, we found that trophic level is important in bioaccumulation of toxicants. Black headed Gull, which forages on agricultural lands and near drainage channels, had high OCPs. Scavenging habit of gulls may be the cause of high exposure to xenobiotics. Small number of cytochrome P450-associated enzyme and resulting low capacity for OCs biotransformation may also be

responsible for large accumulation of OCs in gulls (Henriksen et al. 2000). The Great Crested Grebe, another fish eater, also has high capacity to bioaccumulate OCs. But, OC levels in Gulls and Great Crested Grebes were significantly different in this study. In migratory birds, accumulation of organochlorines depends on the degree of pollution in the area of collection, and also on pollution in stopover sites and breeding grounds. Many birds that winter in Iran have their breeding ground in Russia. Gulls that winter in the Caspian, summer in western Siberia where high levels of DDTs have been reported in Ringed Seals; suggesting that DDTs are widely distributed in this region (Nakata et al. 1998). Elevated OC have also been reported in migratory birds that breed near Lake Baikal and winter in southern Asia. In contrast to gulls, Great Crested Grebe winters exclusively in the Caspian and showed lower OC contamination. The Great Cormorant winters partly in the Caspian and travels down to the Persian Gulf area for some of the winter. *Anatidae* that feed on lower trophic levels had lower OCPs and PCBs, verifying that feeding on lower trophic levels leads to lower PCB and OC contamination. Significant correlations were found between feather POPs (OCs and PCBS) and POPS in the soft tissue of gull and the Great Cormorant of this study ($r > 0.9$).

In closing, here we report organochlorine pesticide in muscle and feather of ten species of sea birds from the Caspian region are reported. Mean muscle PCBs and OCPs was significantly higher than liver and feather. Significant correlations were found between feather POPs (OCs and PCBS) and POPS in the soft tissue of gull and the Great Cormorant indicating that testing feather is a suitable a non-destructive method for organic contamination monitoring.

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