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PCBs and Organochlorine Pesticides in Ducks of Fereydoon-kenar Wildlife Refuge in Iran

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Abstract Levels of organochlorine pesticides (OCPs) and PCB were determined in three duck species: Pintail (*Anas acuta*), Common Teal (*Anas creaca*), and Mallard (*Anas platyhynchos*). Both persistent organochlorines were more abundant in muscle than in the liver; Mallard had the highest levels of PCBs [84.0 ± 39.0 ng/g wet weight (ww)], and Common Teal showed the highest levels of DDTs (561.0 ± 220.0 ng/g ww). This exposure level is more than two orders of magnitude below provisional tolerable daily intake (PTDI) (0.01 mg/kg bw for DDT and its associated compounds) of The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).

Keywords Organochlorine · PCB · Fereydoon-kenar Wildlife Refuge · Iran

Recent population decline(s) in several species of ducks including Pintail (*Anas acuta*), Common Teal (*Anas creaca*), and Mallard (*Anas platyhynchos*) in Fereydoon-kenar region of the Caspian Sea has alerted the public, the Environmental Protection Agency, as well as wetland conservation and rehabilitation advocacy groups in Iran (Rahbar 2002). Causes of such declines are unknown, but concerns have been raised that potential exposure to contaminants may be the culprit. Since the late 1940s several avian population collapses have occurred. For example, populations of bald eagles (*Haliaeetus leucocephalus*) and other fish-eating water birds such as Double-crested Cormorants (*Phalacrocorax auritus*) declined in the Great Lakes. Such declines were not linked to microbial pathogens or habitat modifications. Instead, they were associated with decreased chick survival as a result of poor hatchability and severe eggshell thinning. Such adverse reproductive effects were correlated with exposure to contaminants like DDT and dioxin-like compounds and were thought to contribute to several avian population declines.

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 (Choi et al. 2001). Although use of these persistent pollutants are strictly forbidden, intense none-organic agricultural practices in the area near the Caspian Sea utilizes pesticides in large quantities and such practices are of great local and national concern (Heidari 2003). Fereydoon-kenar Wildlife Refuge is located near a port by the same name at 52°32′E and 36°40′N. The area is made up of a shallow body of fresh water which is now only covering a relatively small area (54 hectare- or 540,000 m^2) near the coast of the Caspian Sea (Fig. 1). Historically this area was much larger but rice paddies have encroached upon them leaving only a small section of these valuable ecosystems. In 1975 this area was set aside as a Wildlife Refuge for resident and migrating birds. A forest surrounds Fereydoon-kenar creating a more secluded area for the birds. As many as 150 species of birds have been reported from this area.

Multiple reasons compel us to study ducks of this region, including the need to maintain diversity and public

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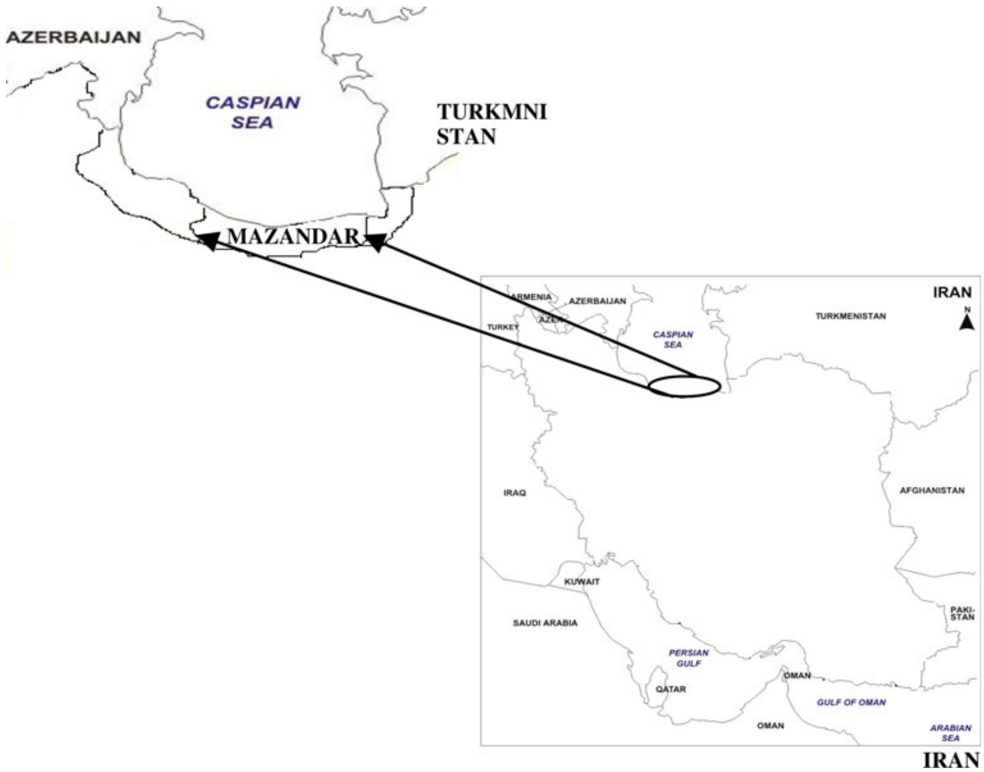

Fig. 1 Location of Fereydoon-kenar Wildlife Refuge in Mazandaran province of Iran where avian species including: Pintail (*Anas acuta*), Common Teal (*Anas creaca*), and Mallard (*Anas platyhynchos*) were collected during winter of 2008

and ecosystem health in this area. Several species of ducks (including subjects of this study) are readily hunted for food and constitute a significant part of the local diet. Public exposure to toxicants is therefore a concern. Our aim was to investigate levels of persistent organic pollutants in muscle and liver of Pintail, Common Teal, and Mallard to determine their suitability for human consumption, and to collect baseline data as a first step in compiling a databank to be used later to detect changes in environmental levels of these pollutants.

Materials and Methods

During the winter of 2008, using nets, we collected 15 aquatic birds from Fereydoon-kenar Wildlife Refuge: Pintail (*Anas acuta*) (n = 5), Common Teal (*Anas creaca*) (n = 5), and Mallard (*Anas platyhynchos*) (n = 5). Birds were dissected the same day and liver and breast muscles were removed. Collected soft tissues were then homogenized separately using a mixer. Homogenized tissue was wrapped in aluminum foil, put in clean plastic bags, and stored at −20°C until analysis.

Organochlorine pesticides including HCHs (α-HCH, ß-HCH, γ-HCH), hexachlorobenzene (HCB), *o,p′*- DDT, *p,p′*-DDT, *o,p′*-DDE, *p,p′*-DDE, *p,p′*-DDD, and target 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. (Darmastadt, Germany). Sample treatment and analysis followed Jasper et al. (2006) with minor modifications. Briefly, soft tissue (approximately 2–4 g of liver or 6 g of muscle) was homogenized then dried by blended with anhydrous sodium sulfate. Samples were spiked with internal standard (PCB 143 and ε-HCH) for analysis. Further, extraction was carried out 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. One µl of extract was injected into a gas chromatograph (GC) system. GC analysis was performed using a Dani 1000 gas chromatograph (Monza, Italy) equipped with 63Ni electron capture detector and a DB-5 capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness, Macherey-Nagel). Helium was used as the carrier gas at a flow rate of 2 ml/min. The operating conditions were split (1:1) injection mode. Temperature program was as follows: 100°C (1 min), 10°C/min to 240°C (1 min), 3°C/min to 260°C (1 min), 20°C/min to 300°C (10 min). The injection port temperature and detector temperature were 250 and 300°C respectively. Multiple-level calibration curves were created for the quantification.

Good linearity ($r^2 > 0.99$) was achieved for tested intervals that included the whole concentration range found in the 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 0.01, 0.05, 0.1, 0.3, 0.5 ppm. Spiking was done at two levels 0.2 and 0.4 ppm. Recoveries of spiked PCBs and OCPs into samples which passed through the analytical procedure were between 95 and 100%. Limit of quantification (LOQ) for OCPs ranged between 0.2 and 0.6 ng/g ww; and LOQ for PCBs ranged between 0.1 and 0.5 ng/g ww (RSD $\leq$ 13).

Data were tested for normality using a Kolmogorov–Smirnov test. Differences in concentration of OCPs and total PCBs among different species were evaluated by one-way analysis of variance (ANOVA). When significant differences were observed among the different species, the Tukey–Kramer multiple-comparison test was applied to determine which means were significantly different. SPSS software (Version 11.5) was utilized for Statistical analysis and $p < 0.05$ was set to indicate significance.

Result and Discussion

Table 1 indicates concentrations of OCPs and PCBs in the liver and muscle of the species we studied. Amongst OCPs; DDTs were present at the highest level (Figs. 2, 3). In muscle, levels of DDTs ranged from < LOQ to 2,207.0 ng/g ww, and liver DDTs were between < LOQ and 127.0 ng/g ww. *p,p′*-DDE occurred at the highest frequency (range <LOQ −2,199.0 ng/g ww) in all birds. Such high levels of *p,p′*-DDE in birds has been attributed to stability of this compound in the living tissue as well as its high environmental persistence. DDD and DDT were also detected, in low concentrations, in our samples. Further more, DDE/DDT ratio was high, which suggests DDE is still present in this habitat at high concentrations. A study of organochlorine content of women's milk and fish from the same area indicates similar recent exposure to these toxicants (Hosseini et al. 2008; Dahmardeh Behrooz et al. 2009). HCHs mean concentration in muscle were (2.5 ± 2.0 ng/g ww) and in liver (9 ± 3.0 ng/g ww). Low concentrations of HCH are seen in this study which may be attributable to the fact that HCHs do not seem to bioaccumulate to any great extent in birds (Olafsdottir et al. 2001). Simply put, the sheer presence of HCHs in the birds of Fereydoonkenar Wildlife Refuge is indicative of HCHs extensive usage of these compounds in this area, which has also been reported to be present in high concentrations in the local rivers (Heidari 2003).

Common Teal had the highest levels of DDTs in the muscle (561.0 ± 320.0 ng/g ww) which contributed to over 90% of the total OCPs, while Pintail had the lowest concentrations of DDTs in muscle (156.0 ± 140.0 ng/g ww) and contributed to only 65% of the total OCPs. DDT levels in the liver exhibited a reversal of that in the muscle for Pintail and Common Teal. Pintail had the highest amount of liver DDT while Common Teal had the lowest levels of DDTs in the liver. Overall, Fig. 3 shows that PCBs and OCPs concentrations were higher in muscle than in liver (with the mean concentration of muscle OCPs at 168.0 ± 99.0 ng/g ww, and in liver 14.0 ± 5.0 ng/g ww) and with PCB levels in muscle and liver at 57.0 ± 13.0 and 30.0 ± 7.0 ng/g ww respectively.

Presence of these compounds in the muscle of our birds followed this pattern: DDTs > HCB > HCH; which is similar to studies of Cleemann et al. (2000) and Naso et al. (2003). In liver, on the other hand, we found the pattern to be DDTs > HCH > HCB. Similar patterns in liver have been reported by Braune et al. (2007) and Bouwman et al. (2008). Figure 3 indicates significant differences in DDTs and HCB concentration between the tissues ($p < 0.05$) but there were no such difference in PCBs and HCHs concentrations. Tissue levels of contaminants highly depend on how long ago the toxicant was ingested (Hobson 1993; Jimenez et al. 2005). For example, liver contaminant levels often indicate pollutants present in the diet during the last week of the animals life; but muscle contaminant levels are usually indicative of what the animal consumed 4–6 weeks earlier (Jimenez et al. 2005). In addition, bird's differing ability to biotransform toxicants at different rates in different tissues can also play a part here (Fossi et al. 1995; Boumphrey et al. 1993).

Differing levels of toxicant in species can also be the result of their varying dietary preferences. Ducks consume variable proportions of animal and plant materials (Fang et al. 2007). Pintail chiefly consumes material in the bottom muddy areas. Relatively longer neck of Pintail, which is an adaptation for bottom feeding, may be providing it with an advantage over other ducks with shorter necks in the same habitat; enabling Pintail to feed on sources unavailable to others. Diverse diets can therefore contribute to varying levels of toxicant in different species. Cramp and Simmons (1997) present correlations between diet and feeding behavior to changes in habitat. In general, ducks consistently show preference for fairly spacious, shallow aquatic habitats in lowlands, grassland, or prairies which are most often eutrophic, and at least moderately productive. During the winter time ducks in this area, show preferences for coastal habitats, and inland waters and the feed predominantly in rice paddies and near by farmlands.

Even migratory routes can influence levels and patterns of pollutants including organohalogenated compounds in tissues of different species (Droge et al. 2008). Since many migratory routes are possibilities, it is difficult to

Table 1 Concentrations of OCPs, and PCBs (mean and range, ng/g ww), in liver and muscle tissues of birds collected in Fereydoon-kenar Wildlife Refuge in Mazandaran province of Iran

	Liver			Muscle		
	Pintail	Common Teal	Mallard	Pintail	Common Teal	Mallard
α-HCH	<LOQ	<LOQ	<LOQ	<LOQ	3.0 (1.5–5.5)	0.5 (<LOQ-2.0)
β-HCH	0.5 (<LOQ-2.5)	7.0 (<LOQ–23.5)	1.0 (<LOQ-3.5)	0.5 (<LOQ-1.5)	2.5 (1.0–3.0)	0.5 (<LOQ-1.0)
δ-HCH	0.5 (<LOQ-1.0)	13.0 (2.5–31.5)	7.5 (1.5–12.5)	<LOQ	2.5 (2–6.5)	<LOQ
HCB	0.5 (<LOQ-1.0)	<LOQ	21 (5–43)	78.5 (<LOQ-235)	30 (<LOQ-120)	166.0 (<LOQ-499.0)
DDE[a]	39.5 (2.5–89.0)	6.5 (<LOQ-29.0)	11 (0.5–18)	150.0 (0.5–449.0)	557 (2–2,199.0)	483.5 (10.5–1,419.5)
DDD	14.0 (3.0–30)	1.0 (<LOQ-2.5)	1.0 (<LOQ-2)	2.0 (0.5–5)	0.5 (<LOQ-1.5)	2.0 (0.5–3.0)
DDT[b]	16.0 (8.5–28.0)	5.0 (<LOQ-12.5)	7.0 (<LOQ-11.0)	3.0 (<LOQ-7.5)	3.0 (1.5–7.5)	<LOQ
PCBs[c]	40.0 (11.0–58.0)	12.0 (8.0–16.0)	43.0 (6.0–95.0)	34.0 (20.5–56.5)	53.0 (23.0–83.0)	84.5 (6.0–123.0)

LOQ Limit of quantification

[a] sum *o,p′*- DDE and *p-p′* DDE

[b] sum *o,p′*- DDT and *p-p′* DDT

[c] sum PCB28, PCB 52, PCB 118, PCB 138, PCB 153, PCB 180

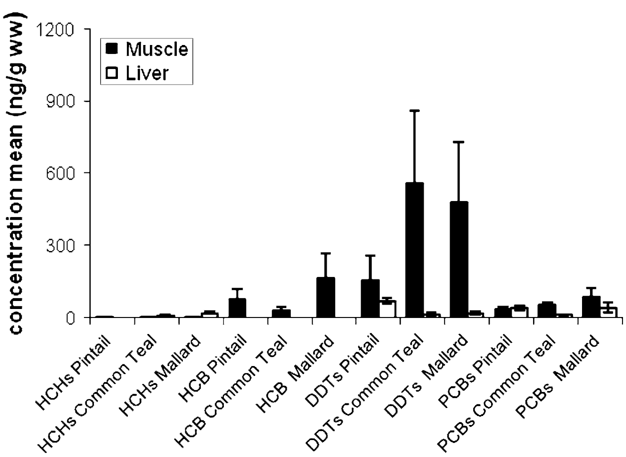

Fig. 2 Levels of organochlorine compounds in muscle, and liver of three avian species [(Pintail (*Anas acuta*), Common Teal (*Anas creaca*), and Mallard (*Anas platyhynchos*)] collected in Fereydoon-kenar Wildlife Refuge in Mazandaran province of Iran during winter of 2008

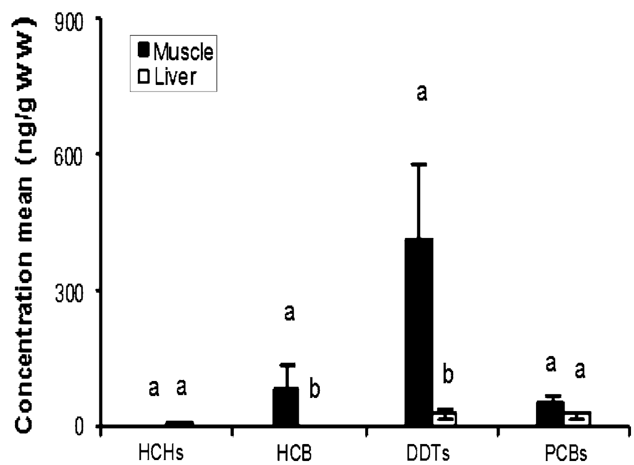

Fig. 3 Overall comparison of organochlorine compounds in muscle, and liver of three avian species [(Pintail (*Anas acuta*), Common Teal (*Anas creaca*), and Mallard (*Anas platyhynchos*)] collected in Fereydoon-kenar Wildlife Refuge in Mazandaran province of Iran during winter of 2008. *Different letters* indicate statistically significant difference at $p < 0.05$

explain differences in toxicant levels and in these birds by their migratory habits. Mallards we collected during the winter in Fereydoon-kenar Wildlife Refuge can be migratory or resident. But Common Teal and Pintail are migratory and they only winter in this area. In migratory birds, accumulation of organochlorinated compounds depends not only on the degree of pollution in the area of collection, but pollution in stopover sites and breeding grounds. We know that many birds that winter in Iran have their breeding ground in Russia, where high levels of DDTs have been reported in Ringed Seal from the Russian Arctic; suggesting that DDTs are widely distributed in this region (Nakata et al. 1998). To add another level of complication to this picture, we know that over-wintering areas can also vary from year to year and also between individual birds, making it extremely difficult to explain the underlying reasons for different levels of toxicant in birds (Jasper et al. 2006).

Presence of toxicants and their levels in food and food safety has been the subject of much debate and controversy. Various national agencies and international food safety organizations have set levels for safety of food but the issue is still being debated worldwide. For DDT in meat of mammals, WHO and the American FDA have set the level of 5 mg/kg as limit values for foodstuffs (WHO/FAO 2002). The European Maximum Residue Limits for Pesticides Residues in Food Commodities which is a similar value for presence of pesticides in meat is significantly lower, at 1 mg/kg (EU 2002). U.S. Food and Drug

Administration has set the tolerances for PCB residues in red meat at 3 mg/kg, while the German Umweltbundesamt (Federal Environmental Agency, (FEA) has marked a significantly lower levels of PCB (15 ng/kg) in red meats. Levels of DDT and PCB we found in this study are lower than USDA and WHO limits but higher than the German Umweltbundesamt. However since individuals consuming wild caught meat are usually exposed to a broad spectrum of toxicants, many investigator argue that the approach of setting a limit such as the one insisted upon by US and other agencies, may be inadequate as the joint toxic action of the pollutants is not taken into account (Verslycke et al. 2003; Steevens and Benson 2001) and mixture toxicity studies have indicated that toxicants may have additive effects on living organisms (Das et al. 2008; Prato and Biandolino 2007). Therefore we conclude that consuming duck meat from this region may be hazardous to health.

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