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Levels of Organochlorine Pesticide Residues in Blood Plasma of Various Species of Birds from India

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Abstract Concentrations of organochlorine pesticides (OCPs) were measured in blood plasma of 13 species of birds collected from Ahmedabad, India. Among the various OCPs determined, HCHs and its isomers had higher contribution to the total OCPs. Concentration of $\sum$HCHs varied from 11.4 ng/mL in White ibis *Threskiornis melanocephalus* to 286 ng/mL in Sarus Crane *Grus antigone*, while $\sum$DDT ranged between 19 ng/mL in Black Ibis *Pseudibis papillosa* and 147 ng/mL in Painted Stork *Mycteria leucocephala*. *p,p′*-DDE was accounted for more than 50% of total DDT in many of the samples analysed. However, a *p,p′*-DDT to *p,p′*-DDE ratio higher than one obtained for many species of birds indicates the recent use of DDT in this study region. The concentrations of cyclodiene insecticides, heptachlor epoxide, dieldrin and total endosulfan ranged from 15.8 to 296.2 ng/mL, below detectable level to 15 and 41.1–153.2 ng/mL, respectively. The pattern of total OCP load generally occurred in the following order: granivores < insectivores < omnivores < piscivores < carnivores. Although, the organochlorine residues detected in blood plasma of birds are not indicative of toxicity, the presence of residues in birds over the years (2005–2007) indicates continued exposure to organochlorine compounds. However, continuous monitoring is recommended to facilitate the early identification of risks to the survival of a species.

Keywords HCHs · DDTs · Cyclodiene insecticides · Feeding habit · Avifauna

Organochlorine pesticides (OCPs) are well known environmental contaminants found worldwide and able to concentrate in living organisms, in particular at the top of food chain. Organochlorine pesticides were introduced into the environment in large quantities beginning in the 1940s (Ware 1989). By the early 1960s residues of OCPs were found in tissues and eggs of birds in Europe and North America and dramatic declines in certain bird populations were also noted (Risebrough 1985). OCPs are highly lipid soluble and are thus stored primarily in body fat and very slowly metabolized with half-lives in the order of 1 year in birds. Exposure to *p,p′*-DDE has been associated with reduced reproductive success in bird such as bald eagle *Haliaeetus leucocephalus* and osprey *Pandion haliaetus* (Anderson and Hickey 1972; Wiemeyer et al. 1988). Once widely used in industry and agriculture, most of these compounds were banned from use in developed countries starting in the 1970s mainly because of their detrimental effects on wildlife and concerns regarding human health effects. However, organochlorine contaminants continue to be detected in environmental samples and animal tissues due to their persistence, their ability to bioaccumulate through the food chain, and their continued use in some developing counties for public health purpose (Kunisue et al. 2003). Because of their toxicity and environmental persistence, it is important that OCP contaminants be monitored in wildlife populations. This is especially critical for species that are threatened with extinction, as these compounds may produce detrimental health effects and contribute to population declines in these vulnerable species.

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Table 1 List of birds and number of samples blood samples collected in Ahmedabad, India during 2005–2007

S. no.	Common name	Scientific name	Feeding habit	Year			Total
				2005	2006	2007	
1	Cattle Egret	*Bubulcus Ibis*	Insectivore	2	1	–	3
2	Painted Stork	*Mycteria leucocephala*	Piscivore	1	–	2	3
3	White Ibis	*Threskiornis melanocephalus*	Piscivore	1	–	–	1
4	Black Ibis	*Pseudibis papillosa*	Insectivore	1	–	1	2
5	Pariah Kite	*Milvus migrans govinda*	Omnivore	21	16	14	51
6	Besra Sparrow-hawk	*Accipiter virgatus*	Carnivore	–	–	4	4
7	Indian Peafowl	*Pavo cristatus*	Granivore	–	2	3	5
8	Sarus Crane	*Grus antigone*	Granivore	–	–	4	4
9	Blue Rock Pigeon	*Columba livia*	Granivore	16	9	9	34
10	Little Brown Dove	*Streptopelia senegalensis*	Granivore	1	–	–	1
11	Asian Koel	*Eudynamys scolopacea*	Granivore	–	3	–	3
12	Barn Owl	*Tyto alba*	Carnivore	1	–	–	1
13	House Crow	*Corvus splendens*	Omnivore	2	1	–	3
	Total			46	32	37	115

–, Not available

It was also assumed that the plasma concentrations of organochlorines will be representative of body burden. As blood perfusion rates of lipid storage tissues, such as fat depots, liver and muscle are very high and rates of metabolism are low, persistent organochlorine (OCs) exhibit relatively constant equilibrium between concentrations in blood and in other tissues. As a result, blood sampling is considered as a valid method for measuring body burden of persistent organochlorine contaminants (Rivera-Rodríguez et al. 2007). Studies in humans and various wildlife species have shown consistent relationships between levels of OCs in blood and other tissues (Bernhoft et al. 1997; Bustnes et al. 2001). Many studies have effectively used blood samples in avian contaminant investigations; migrating raptors (Elliott and Shutt 1993), nestling bald eagles (Dykstra et al. 1998; Elliott and Norstrom 1998), peregrine falcons and gyrfalcons nestlings (Jarman et al. 1994) and egyptian vultures (Gomara et al. 2004).

India is now both the largest manufacturer and consumer of pesticides in South Asia. Among the various pesticides used in India, 40% of all the pesticides used belong to organochlorine case of chemicals (FAO 1994) and usage of HCH and DDT continued till recently (Gupta 2004). Studies in India reported the presence of OCPs (Muralidharan 1993; Muralidharan et al. 2008) in a few species of birds. In contrast to the information related to OCPs in tissues and eggs of birds available, there is little information available regarding the levels of contaminants in bird plasma, and currently no long-term monitoring studies are in place for birds in India. Since population decline in many species of birds world over related to persistent OCPs, efforts were made to determine the blood plasma concentration of organochlorine pesticide residues in various species of birds and to evaluate toxicological risk of birds according to food habits. Furthermore, we also aimed to compare these residue levels among differed years. Since there is not much information available on the residue levels of OCPs in plasma of birds in India, it is expected that the data generated in this study will serve as reference values.

Materials and Methods

Between 2005 and 2007, a total of 115 blood samples comprising 13 species of birds were collected from Ahmedabad, India (Table 1, Fig. 1). The samples were collected with the help of Gujarat State Forest Department and Animal Help Foundation (AHF), Ahmedabad on opportunistic basis in all 3 years. It may be noted that people fly kites as an entertainment during a festival called *Uthrayan* which falls in January every year. Each year, thousands of birds are severely injured or killed during *Uttarayan*, the annual kite-flying festival held in several provinces in India. Larger birds such as flamingos, vultures, herons, eagles and cranes are most at risk because they have a broader wingspan; but pigeons, doves, crows and other smaller birds are also in danger. AHF established 33 collection centers across Ahmadabad to aid with the enormous intake of birds during *Uttarayan*. According to AHF almost 70% of injured birds brought to the center during the festival succumb to their wounds. Of those that survive, nearly 90% are left permanently disabled and cannot be released back into their natural habitats. The birds recovered quickly were released after blood collection.

Fig. 1 Map showing the bird's blood collection site at Ahmedabad, India

Blood samples were obtained from healthy birds by jugular venipuncture using a heparinized syringe needle. Samples were kept on wet ice until centrifugation. Blood was transferred from syringe to heparinized capillary tubes and centrifuged at 4,000*g* for 10 min. Plasma was separated and stored in microcentrifuge tubes (1.5 mL). Organochlorine pesticide residues were extracted from the samples by modifying the method described by US EPA (1980) and Bhatnagar et al. (1992). Plasma (0.5–1 mL) was extracted with hexane (6 mL) in a round bottomed tube for 2 h at 50 rpm on a slow speed rotating machine. In case of emulsion formation after extraction, the mixture was centrifuged at 2,000 rpm for 4–5 min to effect sufficient separation and withdrawal of the clear extract. Hexane layer was transferred to a clean test tube and concentrated under a stream flow of nitrogen to near dryness. Then the samples were reconstituted with 1 mL of hexane for analysis in Gas Chromatograph. Blank samples were prepared to identify any contamination throughout the analytical procedure. No background interference was found in the methodology adopted.

The final extracts were analyzed for the following organochlorine pesticides; isomer mixture of hexachlorocyclohexane ($\sum$HCH) consisting α, β, δ and γ-HCH (lindane), DDT metabolites, namely *p,p*′-DDT *p,p*′-DDE and *p,p*′-DDD and the cyclodiene insecticides, heptachlor epoxide, dieldrin, α-endosulfan, β-endosulfan and endosulfan sulfate. An aliquot (1 µL) from the final extract was injected into a Hewlett Packard 5890 series II Gas Chromatography (GC) equipped with a ^{63}Ni electron capture detector and splitless injection port. The GC column employed was DB-608 fused silica capillary column (30 m × 0.32 mm × 0.5 micron thickness; J&W Scientific Inc., Folsom CA) coated with 35% phenyl methyl polysiloxane. The column oven temperature was programmed from 180°C, held for 3 min, then increased to 270°C at 10°C min^{-1} and held for 20 min. Injector and detector temperatures were set at 250 and 280°C, respectively. Nitrogen was used as carrier gas with a column flow rate of 1.5 mL/min. A mixture of organochlorine pesticides provided by Dr. Ehrenstorfer Laboratories, Germany were used as standards. The concentrations of the individual compounds were quantified from the peak area of the sample to that of the corresponding external standard. Recoveries of the compounds from fortified samples (100 ng/mL) ranged from 94 to 103%. Results are not corrected for per cent recovery. Analyses were run in batches of 10 samples plus four Quality Controls (QCs) including one reagent blank, one matrix blank, one QC check sample and one random sample in duplicate. The minimum detection limit for all the compounds analyzed was 1 ng/mL.

Organochlorine residue concentrations were log transformed to satisfy the homogeneity of variance assumption of analysis of variance (ANOVA). For the purpose of analysis, samples below the detection value were given one-half the detection limit before comparison of the means. All statistical calculations were performed using statistical software SPSS student version 11.

Results and Discussion

The mean and range of organochlorine pesticide concentration were presented in Table 2. The highest level of mean total DDT was detected in Painted Stork (147 ng/mL), while lowest was found in Black Ibis (19 ng/mL). The mean ∑DDT calculated from plasma of Indian Peafowl (85.8 ng/mL) was rather similar to that found in Asian Koel (88 ng/mL). Among the various OCPs analyzed *p,p′*-DDE was the most frequently detected metabolite and it contributed more than 50% of total DDT in Black Ibis, House Crow, Sarus Crane and Besra Sparrow-hawk. The mean concentration of *p,p′*-DDE among the birds ranged between 5.3 and 38.6 ng/mL (Table 2). Concentration of *p,p′*-DDE found in the plasma of House Crow, Pariah Kite, Cattle Egret and Blue Rock Pigeon in the present study are in the range of those previously reported in plasma of same species of birds in urban area of Lucknow, India (Kaphalia et al. 1981). However, these levels are far below the concentrations (5 μg/g wet wt.) in eggs which were correlated with toxic effects in different bird species (Hoffman et al. 1998). According to the results obtained by Iseki et al. (2001) the concentration of organochlorine residues in eggs are similar to those found in blood samples. The differences in ∑DDT levels detected among species considered in the present study could probably be related to the characteristics of their habitats (Gomara et al. 2004). The average total burden of DDT in many species of birds revealed that the region was contaminated more with DDT. A, *p,p′*-DDT to *p,p′*-DDE ratio higher than one was obtained for five species of birds (Asian Koel, Blue Rock Pigeon, Indian Peafowl, Painted Stork and Pariah Kite), which could be considered as a possible indicator of recent use of this pesticide.

Relatively high level of ∑HCH was detected in plasma of Sarus Crane (286 ng/mL) an omnivorous bird followed by Besra Sparrow-hawk (166.6 ng/mL) a carnivore. White ibis (11.4 ng/mL) showed generally low concentration of ∑HCH and it was below detectable levels (BDL) in Barn Owl. However, *β*- and *γ*-isomer of HCH accounted for the

Table 2 Organochlorine chemical residues (mean and range, ng/mL) in plasma of birds collected in Ahmedabad, India during 2005–2007

Species	N	*p,p′*-DDE	*p,p′*-DDD	*p,p′*-DDT	∑DDT	∑HCH	HE	Dieldrin	∑Endosulfan
Cattle Egret	3	38.6 (6.5–101)	<DL	<DL	38.6 (6.5–101)	57.7 (23.9–80)	30.2 (8.0–65)	3 (<1–7.9)	64.4 (2.7–143)
Painted Stork	3	22.7 (13.6–40.2)	23.4 (<1–69)	101 (<1–224)	147 (13.6–333)	78.2 (45.6–131)	296.2 (4.5–808)	10.5 (6.2–19)	41.1 (<1–70)
White Ibis	1	27.5	<DL	<DL	27.5	11.4	28.9	<DL	66
Black Ibis	2	10.8 (7.6–14.1)	8.4 (<1–16.3)	<DL	19 (7.6–30.4)	47 (23.7–70.2)	15.8 (11.8–20)	9.9 (5.1–15)	48.3 (42.8–53.7)
Pariah Kite	51	15.3 (<1–137)	8.7 (<1–57.4)	19.2 (<1–227)	42.5 (<1–265)	77.2 (<1–619)	26.2 (<1–156)	9.1 (<1–68.5)	44.4 (<1–290)
Besra Sparrow-hawk	4	35.2 (7.0–115)	1.1 (<1–3.1)	8.6 (<1–23)	44.3 (9.6–115)	166.6 (11.1–541)	21.9 (1 < 1–45)	4.7 (<1–9)	153.2 (45.3–395)
Indian Peafowl	5	37.1 (<1–158)	<DL	48.9 (<1–186)	85.8 (<1–344)	49.3 (<1–127)	35.7 (9.2–96)	15 (<1–53)	60.7 (5.1–223)
Sarus Crane	4	11.9 (5.5–21)	<DL	9.8 (<1–38)	21.4 (8.8–43)	286 (56–944)	53.8 (11.0–92)	5.7 (<1–10)	45.4 (5.5–148)
Blue Rock Pigeon	34	13 (<1–101)	3.3 (<1–66.9)	38.7 (<1–1,015)	54.2 (<1–1,037)	88.3 (<1–422)	28.3 (4.8–159)	7.8 (<1–37.7)	112.8 (4.3–804)
Little Brown Dove	1	5.9	43.2	<1	49.1	73.1	17.9	3.8	58.7
Asian Koel	3	38.3 (<1–102)	<DL	50 (<1–149)	88 (<1–161)	69.6 (<1–154)	24.6 (<1–61)	6.7 (<1–19)	44 (<1–84.3)
Barn Owl	1	5.3	<DL	74	79.3	<DL	28.1	6.3	58.3
House Crow	3	34.2 (4.6–51)	7.6 (4.2–12.2)	3 (<1–8)	44.5 (17.0–63)	73.3 (12.1–192)	53.9 (26.2–78)	4.1 (2.9–6.3)	140 (31.2–267)

HE heptachlor epoxide, *<DL* below detectable limits

Table 3 Comparison of organochlorine residues (ng/mL) in plasma of birds with earlier studies

Region	Species	Year	Tissue	n	p,p'-DDE	Dieldrin	Source
Colorado	Bald Eagle	1977	Plasma	10	140 (30–230)	10 (<10–50)	Henny et al. (1981)
Missouri	Bald Eagle	1978	Plasma	15	60 (10–140)	30 (ND-80)	Henny et al. (1981)
India	House Crow	1980	Plasma	3	35 (20–40)	–	Kaphalia et al. (1981)
India	Pariah Kite	1980	Plasma	3	100 (43–190)	–	Kaphalia et al. (1981)
India	White-backed Vulture	1980	Plasma	3	183 (106–250)	–	Kaphalia et al. (1981)
India	Cattle Egret	1980	Plasma	3	29 (26–30)	–	Kaphalia et al. (1981)
India	Blue Rock Pigeon	1980	Plasma	3	4 (3–10)	–	Kaphalia et al. (1981)
Cousins Island, Great Lake	Caspian terns	1990	Plasma	15	112 (70–180)	10 (7–15)	Mora et al. (1993)
Papoose Island, Great Lake	Caspian terns	1990	Plasma	10	180 (103–320)	15 (10–20)	Mora et al. (1993)
South Greenland	Peregrine falcons	1985–1989	Plasma	5	140 (100–190)	–	Jarman et al. (1994)
West Greenland	Peregrine falcons	1985–1989	Plasma	41	220 (63–720)	–	Jarman et al. (1994)
South Africa	White-backed Vulture	1993–1995	Blood	60	3.44 (1.87–6.64)	8.27 (2.9–16.4)	Van Wyk et al. (2001)
Bear Island, NE Atlantic	Glaucous Gulls	1998	Blood	27	40.8–148.4	–	Bustnes et al. (2001)
Spain	Egyptian vulture	1999–2000	Serum	27	0.5–38	–	Gomara et al. (2004)
India	House Crow	2005–2007	Plasma	3	34.2 (4.6–51)	4.1 (2.9–6.3)	Present study
India	Pariah Kite	2005–2007	Plasma	51	15.3 (<1–137)	9.1 (<1–68.5)	Present study
India	Cattle Egret	2005–2007	Plasma	3	38.6 (6.5–101)	3 (<1–7.9)	Present study
India	Blue Rock Pigeon	2005–2007	Plasma	34	13 (<1–101)	7.8 (<1–37.7)	Present study

major burden of $\sum$HCH. About 84% of blood plasma samples contained residues above the lower quantifiable levels (1 ng/mL) for ΣHCH. HCH also contributed relatively more to the total OCPs. Total HCH and lindane (γ-HCH) levels in the present study are almost twofold higher than the levels reported in Blue Rock Pigeon (48; 16 ng/mL), House Crow (30; 11 ng/mL) and Pariah Kite (60; 28 ng/mL) collected from the urban area of Lucknow, India during 1980 (Kaphalia et al. 1981). Previous studies in Indian biota have also reported high levels of HCH (Tanabe et al. 1998; Senthilkumar et al. 1998, 2001; Muralidharan et al. 2008). There is also substantial use of γ-HCH in India, which might be a contributing factor.

Concentrations of cyclodiene insecticides, namely heptachlor epoxide, dieldrin and total endosulfan ranged from 15.8 to 296.2 ng/mL, BDL to 15 and 41.1–153.2 ng/mL, respectively (Table 2). Dieldrin has been labeled as commonly detected environmental contaminants (Van Wyk et al. 2001), which is detected in 81% of plasma samples analysed. The concentration of dieldrin detected in plasma samples of Pariah Kite was almost equal to the levels reported in plasma of Bald Eagle from Colorado (Henny et al. 1981), whereas, less than the values reported in Caspian Tern *Sterna Caspian* of North Channel of Cousins Island, Georgian Bay, Papoose Island and Northern Lake; Michigan High Island (Mora et al. 1993; Table 3). Heptachlor epoxide was found to be one of the most abundant chlorinated hydrocarbons occurred in 93% of the plasma samples examined. Residue level of heptachlor epoxide in plasma of Besra Sparrow-hawk and Barn Owl are comparable with the levels reported in birds with similar food habits, namely peregrine falcon from South Green land (31; 14–71 ng/mL) and West Greenland (17; 4.5–68 ng/mL; Jarman et al. 1994). The percentage occurrence for the total number of birds included in the study indicate that individuals are being subjected to heptachlor epoxide contamination in one way or the other, although the residual concentrations detected are far too low to pose any imminent danger to the bird populations.

Detectable levels of total endosulfan were found in 75% of the samples tested. Limited information seems to exist regarding residue levels of this metabolite in not only avian species, but in wildlife in general. It was proposed that endosulfan, in contrast to the other organochlorine insecticides, is not very much persistent in the environment and has a half-life in water between 3 and 7 days (Eichelberger and Lichtenberg 1971). It was reported that birds that feed primarily on fish and other aquatic organisms have detectable levels of endosulfan than birds with other food habits (i.e. granivores, insectivores and scavengers; Van Wyk et al. 2001). A study by Aggarwal et al. (2008) revealed that the subchronic concurrent exposure to arsenic and endosulfan at environmentally relevant concentrations significantly altered the functional immunological parameters tested in birds. The movement of endosulfan into the environment, adjacent to the site of application, is of particular concern, especially to fish species (Antonious and Byers 1997). On the contrary, in the present study piscivores had less endosulfan burden. This may be attributed due to the small sample size.

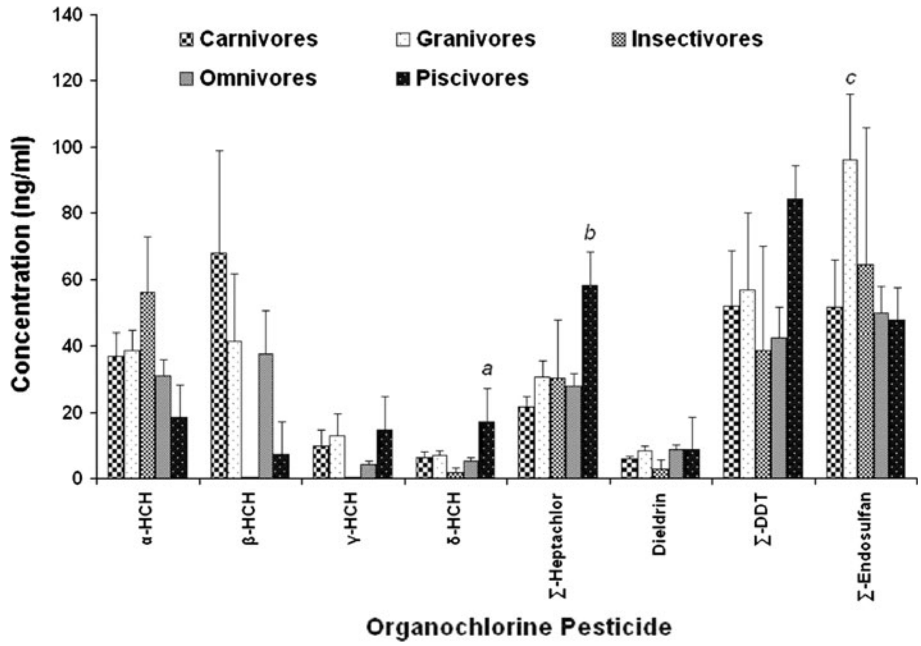

Fig. 2 Organochlorine pesticide residues (mean ± standard error, ng/mL) in plasma of birds collected from Ahmedabad, India grouped according to their feeding habits. $a = p < 0.05$ vs. carnivores, granivores, omnivores and insectivores; $b = p < 0.05$ vs. carnivores, granivores, omnivores and insectivores; $c = p < 0.05$ *vs* omnivores

Table 4 Comparison of organochlorine residues (mean and range, ng/mL) in blood plasma of Pariah Kite and Blue rock pigeon collected between 2005 and 2007 from Ahmedabad, India

Year	2005	2006	2007	ANOVA $p <$ value
Pariah Kite (n)	21	16	14	
∑DDT	20 (<1–69)	36 (<1–172)	83 (<1–265)	NS
∑HCH	47 (<1–201)	85 (<1–290)	114 (<1–619)	<0.05
Heptachlor epoxide	19 (<1–68)	32 (<1–156)	30 (<1–109)	NS
Dieldrin	7 (<1–23)	8 (<1–30)	14 (<1–69)	NS
∑endosulfan	42 (<1–290)	44 (<1–116)	48 (<1–210)	NS
Blue Rock Pigeon (n)	16	9	9	
∑DDT	18 (<1–61)	163 (<1–1,038)	8 (<1–32)	<0.05
∑HCH	68 (<1–269)	141 (14–422)	71 (<1–332)	NS
Heptachlor epoxide	31 (9–159)	30 (<1–86)	21 (5–100)	NS
Dieldrin	6 (<1–15)	9 (<1–38)	8 (<1–22)	NS
∑endosulfan	155 (6–804)	121 (5–672)	29 (4–68)	NS

NS not significant

When birds were grouped according to their food habits, birds belonging to carnivorous category showed higher load of organochlorine residues, followed by piscivores ($p < 0.05$; Fig. 2). Organochlorine residue burden in plasma samples in the present study are concordant with the findings that flesh-eating birds showed higher body burden of total OCs than non-flesh-eating one (Kaphalia et al. 1981). The different accumulation patterns may indicate differences in feeding habits, ecology, movement and metabolic capacity of the species (Senthilkumar et al. 2003). The pattern of total OC load generally occurred in the following order: granivores < insectivores < omnivores < piscivores < carnivores. To know temporal variation in organochlorine residues in plasma, two species, namely Blue Rock Pigeon and Pariah Kite collected in all 3 years were compared (Table 4). Although blood samples were collected between 2005 and 2007, it was not possible to collect from the same species in all 3 years because the sampling was opportunistic. In general, organochlorine pesticide residues in Blue Rock Pigeon showed declining trend from 2006 to 2007, while Pariah Kite showed increasing levels. However, there were no differences over a period of 3 years in many of the organochlorine residues analysed except ΣHCH in Pariah Kite and ΣDDT in Blue Rock Pigeon (Table 4). It is well established that reduction in body fat reserves leads to increased OC concentrations in the tissue and blood (Henriksen et al. 1996, 1998; Van den Brink et al. 1998) and birds in poor body condition generally have higher OC levels than those in good condition. This may suggest that the sources of DDE in the diet of birds have remained constant in India until 2007. Since there is no much study on the residue levels in blood plasma of birds in India, it is very difficult to work out the changing pattern of residue levels over a period of time.

In general the organochlorine residue burden in plasma of birds over the years (between 2005 and 2007) showed no significant decline, probably reflecting the continued exposure to organochlorine compounds. Although, the concentrations detected are not capable of inducing chronic effects, may lead to impairment of reproduction, behavioral, and neurological functions, and suppression of immune function, if the exposure level increases or even remains the same. Further studies on the levels of contaminants and their effects on various species of birds are, however, proposed for continuous monitoring. Ours is the first account of a comprehensive analysis of toxicants present in different species of birds in India. The values reported in this study can serve as guidelines for future research in general as well as control values during the analysis of samples obtained from birds in the event of suspected organochlorine poisoning.

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