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Persistent Organochlorine Pesticide Residues in Tissues and Eggs of White-Backed Vulture, *Gyps bengalensis* from Different Locations in India

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Abstract Organochlorine pesticide residues were determined in tissues of five Indian white-backed vultures and two of their eggs collected from different locations in India. All the samples had varying levels of residues. *p*,*p*′-DDE ranged between 0.002 μg/g in muscle of vulture from Mudumali and 7.30 μg/g in liver of vulture from Delhi. Relatively higher levels of *p*,*p*′-DDT and its metabolites were documented in the bird from Delhi than other places. Dieldrin was 0.003 and 0.015 μg/g while *p*,*p*′-DDE was 2.46 and 3.26 μg/g in egg one and two respectively. Dieldrin appeared to be lower than the threshold level of 0.5 μg/g. *p*,*p*′-DDE exceeded the levels reported to have created toxic effects in eggs of other wild birds. Although varying levels of DDT, HCH, dieldrin, heptachlor epoxide and endosulfan residues were detected in the vulture tissues, they do not appear to be responsible for the present status of population in India.

Keywords Organochlorine pesticide · Indian white-backed vulture · Tissues · Eggs · Threshold levels

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Population of white-backed vulture *Gyps bengalensis* drastically declined up to 92% in its entire known distribution ranges in India during the last 10 years (Prakash et al. 2003). While Cunningham et al. (2003) reported that the most likely cause of vulture decline was due to a novel infectious disease, Pain et al. (2003) said that although there could be more than one factor responsible for such a mysterious situation, the role played by contaminants cannot be entirely ruled out. When the real reason for the vulture mortality across the country and neighbourhood continued to be elusive, Oaks et al. (2004) demonstrated that Diclofenac, an anti-inflammatory drug used for treating cattle, was responsible for the same.

The environmental contaminants especially the organochlorine pesticides (OCPs) are persistent and on many occasions they tend to concentrate in wildlife through the food chain (Guitart et al. 1994) and have profound consequences by way of increased reproductive dysfunction (Custer et al. 2000), increased susceptibility to diseases or other stresses and changes in normal behavior patterns (White et al. 1986). Raptors particularly could be more readily impacted as their numbers and recruitment rates are generally low. Vultures by virtue of their position at the top of the food chain accumulate and concentrate environmental contaminants like pesticides and heavy metals in their tissues and thus serve as sensitive indicators of environmental contamination (Olsen et al. 1993; Guitart et al. 1994).

It is also reported that secondary exposure to insecticide residues through feeding on contaminated carcasses is likely to be the major threat for some vulture species (Ostrowski and Shobrak 2001). Since population decline in many species of birds world over were related to persistent OCPs, efforts were made to find out as to whether any of those pesticides was responsible for the status of vultures in

India. The data on OCPs presented in this paper were collected much before diclofenac was found to be the chemical responsible for the population decline in vultures in India. Since there is no information available in India on the residue levels of OCPs in vultures, it is expected that the data generated in this study will serve as reference values.

Materials and Methods

Five carcasses of the Indian white-backed vulture between 1999 and 2003 (one each from Delhi, Patiala (Punjab), and Mudumalai Wildlife Sanctuary (Tamil Nadu) and two from Ahmedabad (Gujarat)) (Table 1), and two eggs from the vicinity of Bharatpur, Rajasthan during December 1999 were received. Tissues or the whole carcass were air lifted to the laboratory depending on the condition of the birds. Tissues, namely brain, liver, kidney and muscle were stored in deep freezer in clean polythene vials till the time of processing. About 10 g of the tissue was ground with anhydrous sodium sulphate and the mixture was packed in a thimble (Whatman), which was desiccated overnight prior to extraction to remove moisture. The desiccated thimble was extracted with 250 ml of pesticide grade hexane (Merck) in a soxhlet extractor for 6 h. The extract was condensed in a rotary flask evaporator to a specific aliquot (5-ml). The condensed aliquot was placed on a column packed with silica gel (60–120 mesh) and eluted with 250 ml of hexane. The collected elutant was again condensed in a rotary flask evaporator and stored in deep freezer at −20°C till gas chromatography analysis. Extracts with high fat contents were subjected to sulphuric acid digestion. The digested extract was filtered through sodium sulfate and evaporated with rotary flask evaporator to near dryness and residues of OCPs were reconstituted in 2 ml of hexane.

Eggs were cut open along the equator with the help of scalpel and contents were weighed and placed in well cleaned, and hexane-rinsed glass jars. Samples were stored in deep freezer (−20°C) until analysis. A known quantity of egg content was mixed with required amount of anhydrous sodium sulphate and ground with the help of pestle and mortar. Subsequently, celite 545 and deactivated (5% water) aluminum oxide (1:1) were added and mixed thoroughly. The resultant powder was loaded in a clean glass column (30 × 1.5 cm) for extraction and clean up. The column was eluted with 250 ml of hexane. The extract was evaporated with rotary flask evaporator to near dryness and residues of OCPs were reconstituted in 2 ml of hexane. Processed samples were stored in deep freezer (−20°C) until analysis in gas chromatograph.

Samples were injected into a Hewlett Packard 5890 Series II gas chromatograph equipped with Ni^{63} electron

Table 1 Details of white-backed vulture received from different locations in India (1999–2003)

S. No.	Date of collection	Location	Position	Number of vulture
1	10.12.1999	Patiala	30°20.18′ N to 76°23.48′ E	1
2	19.12.2000	Delhi	28°39.00′ N to 77°13.00′ E	1
3	16.02.2003	Ahmedabad	23°03.00′ N to 72°58.00′ E	2
	05.05.2003			
4	22.02.2003	Mudumalai	11°37.00′ N to 76°34.00′ E	1

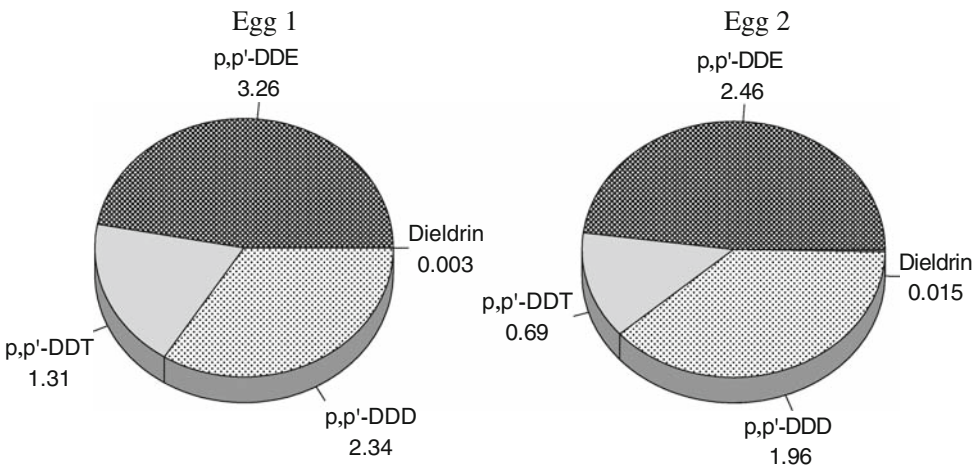

Fig. 1 Organochlorine residues (μg/g, wet weight) in eggs of white-backed vulture received from the vicinity of Bharatpur, Rajasthan (December 1999)

capture detector (ECD). A fused silica capillary column (30 × 0.32 mm × 0.5 μm) DB-608 (5% diphenyl and 95% dimethyl polysiloxane) was used for quantification. Chromatographic conditions were as follow; detector 300°C; injector 250°C; oven temperature was programmed as 180°C – 3 min; 4°C/min – 260°C – 15 min. All the samples were analysed for alpha-hexachlorocyclohexane (α-HCH), β–HCH, δ-HCH, lindane, heptachlor epoxide (HE), dieldrin, *p*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD, α-endosulfan, β-endosulfan and endosulfan sulfate. Spiked and duplicate samples were also analysed. Pesticide residues were analysed and quantified from individually resolved peak areas with the corresponding peak areas of standard (Dr. Ehrenstorfer – Germany). Recoveries averaged from 94% to 103% and the residue levels were not corrected as per the recovery calculation. The detection limits for α-HCH, δ-HCH, lindane, dieldrin, endosulfan sulfate, *p*,*p*′-DDT, heptachlor epoxide (HE) were 0.001 micrograms/gram (μg/g). While the detection limit for *p*,*p*′-DDE was 0.003 μg/g, for α-endosulfan, β-endosulfan, *p*,*p*′-DDD and β–HCH it was 0.005 μg/g. All OCP residues in tissues and eggs are expressed as μg/g wet weight.

Results and Discussion

DDT and its metabolites were the highest in the liver tissues of the bird received from Delhi than the birds from Patiala, Ahmedabad and Mudumalai. The lowest concentrations of *p*,*p*′-DDT, *p*,*p*′-DDE and *p*,*p*′-DDD were below detectable level (BDL) (Patiala-brain, liver), 0.002 μg/g (Mudumalai-muscle) and BDL (Patiala-kidney and Mudumalai liver), respectively. On the whole the vulture received from Delhi had higher concentration of DDT (Table 2). Although the levels of *p*,*p*′-DDT, *p*,*p*′-DDD and *p*,*p*′-DDE detected in the present study were well below the levels reported to be responsible for mortality of birds (Stickel et al. 1970), they were higher than the levels reported in South African white-backed vulture (Wyk et al. 2001). The higher concentration of *p*,*p*′-DDE in the tissues of vulture received from Delhi showed the recent usage of DDT, its persistent and lipophilic nature in the environment.

The most toxic cyclodiene compound, dieldrin was recorded in all the tissues of vulture collected from only Ahmedabad with the maximum of 0.13 μg/g in liver followed by 0.09 μg/g in muscle, while brain recorded the minimum (0.01 μg/g). All these levels are well below the levels expected to create harmful effect to avian population (Heinz and Johnson 1981). However, it may be noted that the Long and Morgan "Effects Range-Low" (ER-L) value (i.e., the contamination level above which adverse biological effects are occasionally observed), is listed as 0.02 μg/kg for dieldrin (Long et al. 1995).

Another cyclodiene pesticide heptachlor epoxide, a metabolite of heptachlor, was detected in the tissues of all the birds except the bird received from Delhi. Maximum levels were recorded in brain (3.05 μg/g) and liver (2.32 μg/g) tissues of vulture received from Ahmedabad.

Table 2 Organochlorine residues (μg/g, wet weight) among various tissues of white-backed vulture received from different locations in India (1999–2003)

Locations	Organ	*p*,*p*′-DDT	*p*,*p*′-DDE	*p*,*p*′-DDD	Dieldrin	∑HCH	∑Endosulfan	Heptachlor epoxide
Ahmedabad	Brain	0.02	0.04	0.09	0.01	1.92	0.08	3.05
	Muscle	0.10	0.13	0.30	0.09	1.73	0.02	0.75
	Liver	0.16	0.28	0.70	0.13	0.67	0.27	2.32
	Kidney	0.04	0.09	0.09	0.04	1.61	0.14	0.55
Patiala	Brain	BDL	0.003	0.02	BDL	0.34	0.01	0.06
	Muscle	0.01	0.32	0.11	BDL	0.22	0.01	0.13
	Liver	BDL	0.003	0.003	BDL	0.01	BDL	0.003
	Kidney	0.004	0.01	BDL	BDL	BDL	BDL	BDL
Mudumalai	Brain	NA	NA	NA	NA	NA	NA	NA
	Muscle	0.003	0.002	0.004	BDL	0.11	0.01	0.03
	Liver	0.05	0.04	BDL	BDL	1.03	0.26	0.39
	Kidney	NA	NA	NA	NA	NA	NA	NA
Delhi	Brain	NA	NA	NA	NA	NA	NA	NA
	Muscle	0.07	4.10	2.55	BDL	BDL	BDL	BDL
	Liver	0.33	7.30	4.00	BDL	BDL	BDL	BDL
	Kidney	NA	NA	NA	NA	NA	NA	NA

NA, not available; BDL, below detectable level

Residues of 0.10–0.73 μg/g have been reported in black-crowned night heron (Heinz et al. 1985) and 0.001–0.042 μg/g in South African vulture (Wyk et al. 2001). The levels recorded in the present study are higher than the LC_{50} values for Japanese Quail, *Coturnix japonica*, 93 ppb and ring-necked pheasant, *Phasianus colchinus*, 224 ppb (Heath et al. 1983). The experimental study by Henny et al. (1983) concluded that residues greater than 1.5 ppm were most definitely associated with decreased reproduction rates in avian species. The birds included in the study had residues of heptachlor epoxide in one or more tissues, and are to be viewed with concern.

Endosulfan (total) was the highest in the liver tissue of the vulture received from Ahmedabad (0.27 μg/g) followed by the vulture from Mudumalai. Among the tissues analyzed, liver and muscle of the vulture from Delhi, liver and kidney of the vulture from Patiala had below detectable levels of endosulfan. Since endosulfan does not remain in the environment longer in contrast to other persistent OCPs, vultures need not reflect the accurate situation. However, it could indicate recent exposure.

Total HCH residues in brain, muscle, kidney and liver of the bird received from Ahmedabad were 1.92, 1.73, 1.61 and 0.67 μg/g, respectively. Bird from Patiala had total HCH between BDL and 0.34 μg/g in the brain. While none of the tissues of the bird received from Delhi had detectable HCH residues, the muscle and liver tissues of the bird from Mudumalai had 0.11 and 1.03 μg/g, respectively. Although the levels are not indicative of any ill effects, they are higher than the levels reported in South African vulture (Wyk et al. 2001).

Of the two eggs examined, one had 3 cm-long embryo and the other had no development. The lipid contents were 6.2% and 17.4%, respectively. While the egg with 3 cm embryo, had 2.46 μg/g of *p,p′*-DDE, the most persistent metabolite, the other egg had 3.26 μg/g. Regarding DDT, all the metabolites exceeded the values which had created toxicity in eggs of many species of wild birds (Nisbet and Reynolds 1984; Hall et al. 1989) (Fig. 1).

Concentration of the most toxic cyclodiene pesticide, dieldrin was 0.003 and 0.015 μg/g in egg one and two, respectively. These levels are less than the values reported in eggs of African white-backed vulture (0.05–0.1 μg/g), and higher than the values reported in eggs of two breeding colonies of Cape griffon vulture (0.002–0.09 μg/g) in South Africa during 1981–1982 (Robertson and Boshoff 1986). Further the recorded levels are lower than the threshold level of 0.5 μg/g (Castillo et al. 1994), and also less than the levels which had impaired reproductive success in pelican (King et al. 1985). Newton et al. (1979) reported 15% eggshell thinning in European kestrel which had 4–5 μg/g of DDE. Clark et al. (1998) reported 50% productivity loss in bald eagle which had 6.3 μg/g of DDE in the eggs. It is also reported that total organochlorine concentration exceeding 0.5 μg/g in eggs of birds will create toxicity and the same is considered as threshold value for the eggs of wild birds (Castillo et al. 1994). The concentration of *p,p′*-DDE recorded in the present study should be viewed with concern owing to its persistence and potentiality to create eggshell thinning, hatching failure and impaired reproduction in avian population. Since there is no published information available in India on the residue levels of OCPs in vultures, it is expected that the data generated in this study will serve as reference values.

Even though many new broad-spectrum pesticides have been developed in recent years, organochlorines continue to be the potential group of chemicals used in control of agricultural pests and vectors of disease, namely malaria in India. India is now both the largest manufacturer and consumer of pesticides in South Asia. Despite the proliferation of different types of pesticides, organochlorines such as HCH and DDT still account for two third of the total consumption in the country for agriculture and public health purposes, respectively (Kumari et al. 2001). In India, no historical data on organochlorine pesticide residues particularly in vulture is available. Although the varying levels of DDT, HCH, dieldrin and endosulfan residues among other organochlorines detected in the tissues in the current investigation are indicative of foodchain accumulation, they do not appear to be responsible for the present status of population in India. Unfortunately, notorious chemicals, namely DDT and dieldrin are still being used in India for specific purposes although banned for agriculture. Hence, regular monitoring of persistent chemicals, especially in breeding colonies of vultures, is recommended.

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