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Organochlorine Pesticide Residues in Eggs and Tissues of House Sparrow, *Passer domesticus*, from Tamil Nadu, India

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Abstract This study provides information on the current status of contamination by organochlorine pesticides (OCPs) in eggs and tissues of House Sparrow, *Passer domesticus*, in Tamil Nadu, India. The mean concentration of total hexachlorocyclohexane ($\sum$HCH) and total dichloro-diphenyl-trichloroethane ($\sum$DDT) in eggs ranged from 0.01 to 1.81 μg/g and 0.02 to 1.29 μg/g, respectively. Concentration of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (*p,p′*-DDE) ranged from below detectable limit (BDL) to 0.64 μg/g, representing more than 60% of the $\sum$DDTs. About 28% of samples had *p,p′*-DDE levels above the critical concentration associated with reproductive impairment. However, the mean concentrations of cyclodiene insecticides were less than 0.5 μg/g. Although OCPs levels detected in tissues are not indicative of toxicity, continuous monitoring is recommended.

Keywords OCPs · House sparrow · Eggs · Eggshell thickness · Tissues

Organochlorine pesticides (OCPs) are persistent, and on many occasions, they tend to concentrate in wildlife through the food chain (Henriques et al. 1997) and have profound consequences by way of increased reproductive dysfunction (Custer et al. 2000). Several investigators have reported that OCPs are associated with eggshell thinning, inhibition of egg laying, a decrease in hatching success (Castillo et al. 1994) and population decline (Lundholm 1997). A large number of studies on humans and wildlife demonstrated that contamination by organochlorine compounds have become a generalized problem (Colborn 1995). About 25,000 mt of chlorinated pesticides were used annually in India and DDT accounted for 40% of the total in the past (Mathur 1993) and usage of HCH and DDT continued till recently (Gupta 2004). This has raised toxicological concerns for both wildlife and mankind, based on historical and ongoing trends in the use of OCPs.

The House Sparrow, *Passer domesticus,* is one of most common birds in India become of rare bird now days. Infrequent sitting and absence of population in many parts of world have been reported (Vijayan 2003). The Royal Society for Protection of Birds (RSPB), UK recently added the House Sparrow to its red list (for rapidly declining bird population, which poses global conservation concern). This avian species can still be reported at over two-thirds of the world's land surface. But reports are pouring in from all over India and around the world of rapid decline in population of House Sparrow (Vijayan 2003). Studies in India reported the presence of OCPs in various species of birds (Dhananjayan 2009; Dhananjayan et al. 2011). Although many studies have reported varying levels of OCPs on different species of birds, study on contamination status on House Sparrow is scare in literature. 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 were responsible for the status of sparrows in India.

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Materials and Methods

Between 2001 and 2006 a total of 32 unhatched eggs of House Sparrow (7-Coimbatore 11°00′N 77°00′E, 6–Namakkal 11°13′N 78°13′E, 9-Kothakiri, 11°24′N 76°44′E, 4-Mettur 11°52′N 77°50′E, 6-Anaikatty forests, Coimbatore 11°05′N 76°50′E) were collected during nest monitoring visits in the study areas as soon as it became evident that they were not going to hatch. The general features of House Sparrow are given in Table 1. Dead House Sparrow (n = 10) and chick (n = 4) were also collected from Anaikatty forests, Coimbatore during nest monitoring. Efforts were made not to disturb any active nests. All the samples were collected in opportunists' manner with help of field biologists and research fellows. The collected eggs and dead birds were wrapped in aluminum foil transported to the laboratory and refrigerated at 3–4°C until they were opened for chemical analysis.

All the eggs were cleaned with detergent in warm water and air dried prior to processing. Each egg was weighed using top loading electronic balance, Metler AE420. Length and diameter of egg was measured with 0.1 mm precision (Science ware Dial Caliper). Egg samples were cut open along the equator with the help of scalpel and the contents were weighed and placed in well cleaned, and hexane rinsed glass jars. Until analysis samples were stored in deep freezer at −20°C. Eggshells were cleaned thoroughly and allowed to dry in room temperature for 4 weeks prior to measuring thickness. Eggshell thickness was measured to the nearest 0.01 mm with a digital micrometer (Mitutoyo, IP65, No.395–271). Known quantity of egg contents were dried with anhydrous sodium sulphate (1:4) and ground with the help of pestle and mortar. Subsequently Celite 545 (1:1) and deactivated (5% water) aluminum oxide were added and mixed thoroughly. The resultant powder was loaded in a clean glass column (30 × 1.5 cm) for extraction and clean up and eluted with 250 mL of 1:3 ratios of dichloromethane and hexane. Extracts with high fat contents were subjected to sulphuric acid treatment. The extract was evaporated to a volume of about 10–15 mL and transferred to a 100 mL separating funnel. Then 5 mL of sulphuric acid was added drop-by-drop, mixed and washed with 20–30 mL of aqueous sodium bicarbonate solution (1%). This cleaning procedure was repeated until the extract became colorless with a pH of 7. The extract was filtered through sodium sulphate and evaporated with rotary flask evaporator to almost dryness and residues of OCP were reconstituted in 2 mL of hexane. Processed samples were stored in deep freezer at −20°C until gas chromatography (GC) analysis.

Table 1 General features of House Sparrow

Parameters	Description
Size (cm)	14–17
Wingspan (cm)	19–25
Weight (g)	26–32
Bill	Thick bill
Legs	Short legs
Chest	Unstreaked
Food	Primarily seed eaters but also eat insects especially during the breading season
Egg clutch size	1–8
Egg description	Light white to greenish white or blue white, usually spelled with grey or brown
Incubation period	10–14 days

Tissues, namely brain, liver, muscle (2–8 g), and chicks were 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 aliquot was placed on a glass 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 to near dryness and residues of OCPs were reconstituted in 2 mL of hexane.

Samples (1 μL) were injected into a Hewlett Packard 5890 Series II gas chromatograph equipped with Ni^{63} electron capture detector (ECD). A fused silica capillary column (30 × 0.32 mm × 0.5 μm) DB-608 (5% diphenyl and 95% dimethyl polysiloxane) stationary phase (J & W Scientific Co., USA) was used for quantification. Chromatographic conditions for analysis 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, heptachlor epoxide (HE), aldrin, dieldrin, *p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, α-endosulfan, β-endosulfan, endosulfan sulfate, endrin and endrin aldehyde. Spiked and duplicate samples were also analysed. OCPs were analysed and quantified from individually resolved peak areas with the corresponding peak areas of standard (Pesticide composition, Dr. Ehrenstorfer, Germany). The recovery of pesticides in fortified samples (n = 3) averaged between 94% and 105%. The residue levels were not corrected as per the recovery calculation. 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 to check for cross-contamination. The minimum detection limit for all the compounds analysed was

0.001 μg/g. The residue levels in tissues and eggs are expressed as μg/g wet weight.

Concentrations of OCPs were tested for homogeneity of variance and log transformed to approximate a normal distribution of the data. Before transformation, a value one-half the lower limit of detection was assigned to samples with undetectable concentrations of contaminants if detectable quantities were found in at least half of the samples. Differences in concentrations of OCPs in eggs of various places were evaluated by one-way analysis of variance (ANOVA). When significant differences were observed among the species, the Bonferroni multiple comparisons test was applied to determine which means were significantly different. All data were analysed using statistical software, Statistical Packages for the Social Sciences (SPSS) student version 11.

Results and Discussion

Eggshell parameters (egg weight, egg length, egg width, eggshell weight, eggshell thickness and egg content weight) measured in samples collected from various locations are presented in Table 2. Organochlorine pesticide residues were detected in all the samples of eggs and tissues of House Sparrow tested. Mean and range of pesticide residues are presented in Table 3. The fat content among eggs was ranged from 7.8% to 13.2%. HCH residues were detected in 80% of samples analyzed. Residues of heptachlor epoxide, *p,p′*-DDT, dieldrin, endosulfan and *p,p′*-DDD were detected in 73%, 63%, 60%, 47% and 46% of the samples analyzed, respectively. Aldrin, endrin, endrin aldehyde were not detected in any of the sample analyzed.

The mean concentration for ∑HCH, ∑DDT, heptachlor epoxide, dieldrin, and ∑endosulfan in eggs ranging from 0.01 to 1.81 μg/g, 0.02 to1.29 μg/g, below detection limit (BDL) to 0.23 μg/g, BDL to 0.2 μg/g and BDL to 0.4 μg/g, respectively. There was no significant difference found in organochlorine residues in eggs collected from various places ($p > 0.05$) except *p,p′*-DDE ($p < 0.05$). Eggs collected from Namakkal and Mettur had detected above 0.5 μg/g of *p,p′*-DDE (Fig. 1). *p,p′*-DDE, the most stable metabolite of DDT ranging from below detection limit (BDL) to 0.64 μg/g. Of the various tissue of House Sparrow analysed for OCPs, liver had the maximum load of residues (Table 4) although, the levels were not significantly different (ANOVA, $p > 0.05$). The mean concentration for ∑HCH, ∑DDT and heptachlor epoxide in tissues ranging from 0.04 to 0.23 μg/g, BDL to 0.45 μg/g and 0.004 to 0.34 μg/g, respectively. However, the cyclodiene insecticides dieldrin and endosulfan were not detected in any of the tissues analysed.

The most predominant occurrence of isomers of HCH in eggs and tissues reflects its stable nature than the other isomers. Lindane has not received much attention as far its effects on birds are concerned. Although several studies have been carried out, investigations pertaining to the levels of lindane are a few (King et al. 1978; Becker et al. 1993). However, varying levels of ∑HCH and its metabolites in eggs and tissues of House Sparrow included in the present study indicate the regular use of this chemical for agriculture. Although use of HCH in agricultural field has been restricted, it is still used on many crops including paddy and pulse (Mukherjee and Gopal 1996). The levels reported in the present study are higher than the levels reported in colonial water birds of Keoladeo National Park in Bharatpur, India (Muralidharan et al. 1992). It is also reported that the HCH and lindane (gamma HCH) used as heptachlor substitutes for seed treatment, are safe to wild birds (Goutner et al. 1997). Although varying levels of HCH and its isomers are recorded in many species of birds, their effects on reproductive success are not clear.

The high frequency of occurrence of *p,p′*-DDE confirms the presence of DDT and its metabolites in the environment. This could be largely due to the chemical persistence and also its use in malaria control. Many studies have associated the high concentration of DDT (between 0.5 and 6 μg/g) specifically *p,p′*-DDE in bird's eggs with impaired reproduction, eggshell thickness and decline of bird species (Castillo et al. 1994; Mateo et al. 2000). The concentrations recorded in the present study are lower than the DDE concentration (5.6 μg/g), which was responsible for 10.5% eggshell thinning in the eggs of Merlin in Norway. Although possible effects of DDE on populations of many species of bird are unknown in Indian context, widespread occurrences of OCPs on various species of birds have been

Table 2 Eggshell parameters (mean ± SD) of House Sparrow collected from various locations in Tamil Nadu, India

Eggshell parameters	Coimbatore (n = 7)	Namakkal (n = 6)	Kothagiri (n = 9)	Mettur (n = 4)	Anaikatty (n = 6)
Egg weight (g)	0.93 ± 0.04	0.88 ± 0.11	1.18 ± 0.21	0.93 ± 0.02	0.66 ± 0.10
Egg length (cm)	1.53 ± 0.07	1.50 ± 0.09	1.63 ± 0.07	1.53 ± 0.06	1.87 ± 0.06
Egg width(cm)	1.17 ± 0.23	1.30 ± 0.19	1.50 ± 0.14	1.17 ± 0.18	1.23 ± 0.23
Eggshell weight (g)	0.21 ± 0.16	0.19 ± 0.06	0.19 ± 0.04	0.21 ± 0.15	0.16 ± 0.09
Eggshell thickness (mm)	0.06 ± 0.03	0.08 ± 0.03	0.09 ± 0.02	0.06 ± 0.01	0.08 ± 0.02
Egg content wt (g)	0.72 ± 0.01	0.69 ± 0.08	0.99 ± 0.19	0.72 ± 0.01	0.58 ± 0.08

Table 3 Mean (range) organochlorine pesticide residues (μg/g, wet wt.) in eggs of House Sparrow collected from different places in Tamil Nadu, India

Organochlorine pesticide	Coimbatore (n = 7)	Namakkal (n = 6)	Kothagiri (n = 9)	Mettur (n = 4)	Anaikatty (n = 6)
Fat content (%)	10.2–12.1	9.1–11.4	8.2–13.1	10.6–13.2	7.8–11.9
α-HCH	<DL	<DL	0.07 (<DL-0.14)	<DL	<DL
β-HCH	0.5 (0.04–1.23)	0.37 (0.02–1.89)	0.28 (<DL-1.75)	<DL	0.01 (<DL-0.02)
γ-HCH	1.1 (0.08–2.03)	<DL	0.79 (0.05–2.63)	0.89 (0.03–2.46)	<DL
δ-HCH	0.2 (<DL-0.39)	<DL	<DL	0.46 (<DL-0.66)	<DL
∑HCH	1.81 (0.19–3.65)	0.37 (0.02–1.89)	1.14 (0.12–4.52)	1.35 (0.55–3.12)	0.01 (0.01–0.02)
p,p′-DDT	0.1 (0.02–0.22)	0.27 (0.01–1.30)	0.06 (<DL-0.11)	0.03 (0.01–0.05)	0.02 (<DL-0.03)
p,p′-DDD	<DL	0.38 (0.01–0.75)	0.01 (<DL-0.03)	<DL	<DL
p,p′-DDE*	0.11 (0.04–0.16)	0.64 (0.03–1.48)	0.07 (0.02–0.29)	0.59 (0.48–0.70)	<DL
∑DDT	0.2 (0.09–0.36)	1.29 (0.05–3.53)	0.14 (0.04–0.43)	0.62 (0.52–0.75)	0.02 (0.01–0.03)
Heptachlor epoxide	<DL	0.19 (0.02–0.66)	0.07 (0.02–0.19)	0.23 (0.01–0.44)	<DL
Dieldrin	0.2 (0.05–0.35)	0.13 (0.01–0.36)	<DL	<DL	<DL
∑Endosulfan	0.4 (< DL -0.44)	0.33 (< DL-1.73)	<DL	<DL	<DL
∑OCPs	2.6 (0.33–5.01)	2.31 (0.10–8.17)	1.35 (0.18–5.15)	2.2 (1.08–4.31)	0.03 (0.02–0.08)

<DL = values below detection limits

* Significantly different among locations (ANOVA, $p < 0.05$)

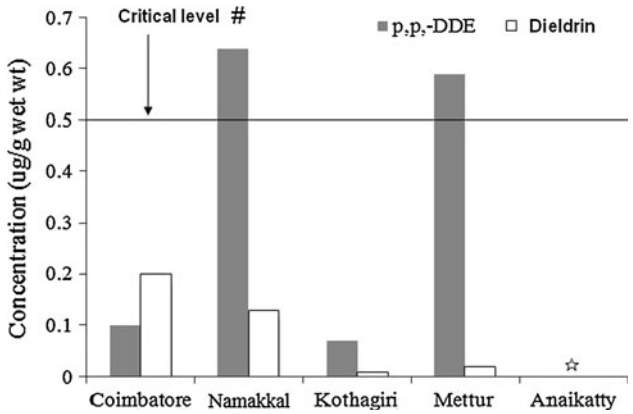

Fig. 1 Concentration of *p,p′*-DDE and dieldrin in eggs of House Sparrow collected from different places in Tamil Nadu, India. *Star*- Values below detection limit. *Number sign*- Proposed by Castillo et al. (1994)

reported (Tanabe et al. 1998; Dhananjayan 2009; Dhananjayan et al. 2011). Castillo et al. (1994) reported that the *p,p'*-DDE concentration exceeding 0.5 μg/g created impact of many species of wildbird and proposed threshold levels. The metabolites of *p,p′*-DDT detected in tissues of House Sparrow of the present study were below the levels (brain; >30 ppm) reported to be responsible for the mortality of birds (Stickel et al. 1970). Considering the present status of House Sparrow the above threshold levels of *p,p′*-DDE in eggs should be viewed with concern.

Residues of heptachlor epoxide detected in the eggs and tissues of the present study species are lesser than the levels reported by Muralidharan et al. (1992) in the eggs of birds in Keoladeo National Park, Bharatpur, India. However, this study does not show any direct evidence that heptachlor epoxide residues was related to reduced eggshell thickness and poisoning of birds. Although, the concentration detected is less than the reported levels in Canada Goose (>10 μg/g, Blus et al. 1984) and American Kestrel (>1.5 μg/g, Henny et al. 1983), the maximum residues in the eggs of House Sparrow in this study were above the levels which were associated with impaired reproductive success. The levels of dieldrin are very low when compared with levels reported in eggs of the select species of colonial water birds at Keoladeo National Park, Bharatpur, India (Muralidharan et al. 1992). All the samples tested in the present study recorded less than 1 μg/g of dieldrin, which is not expected to create ill effects to the bird populations (King et al. 1978).

In general the levels OCPs measured in tissues and eggs of House Sparrow reflect the widespread occurrence of these pesticides in the environment. Although, the measured levels in tissues of House Sparrow were lower than those found in field birds that reported poor hatching success, about 28% of eggs had detected above the critical concentration reported for wildbirds. The chemical substances considered in this study are toxic, persistent, and they biomagnify in lipid-rich tissues throughout the food-chain. Furthermore, they can negatively influence the birds' health, survival and reproduction. In order to estimate the potential risk for birds, the threshold levels known and mentioned in the literature must considered carefully, although threshold levels vary among chemicals compounds, bird species, and other environmental factors. Hence, continuous monitoring is recommended along with

Table 4 Mean (range) organochlorine pesticide residues (μg/g, wet wt.) among various tissues of House Sparrow in Tamil Nadu, India

Organochlorine pesticide	Chick (n = 4)	Muscle (n = 10)	Brain (n = 10)	Liver (n = 10)	ANOVA
α-HCH	0.15 (<DL-0.34)	<DL	<DL	0.02 (<DL-0.03)	–
β-HCH	<DL	0.01 (<DL-0.03)	0.13 (0.02–0.51)	0.06 (<DL-0.25)	NS
γ-HCH	<DL	0.006 (<DL-0.02)	<DL	0.05 (0.01–0.09)	–
δ-HCH	0.08 (<DL-0.38)	0.02 (<DL-0.11)	0.04 (<DL-0.15)	<DL	NS
∑HCH	0.23 (0.02–0.72)	0.04 (0.01–0.27)	0.17 (0.02–0.69)	0.13 (0.01–0.34)	NS
p,p′-DDE	0.06 (0.003–0.35)	0.06 (< DL-0.40)	<DL	0.42 (0.03–1.01)	NS
p,p′-DDD	<DL	<DL	<DL	0.03 (0.006–0.26)	–
p,p′-DDT	<DL	<DL	<DL	<DL	–
∑DDT	0.06 (0.003–0.35)	0.06 (<DL-0.40)	<DL	0.45 (0.04–1.26)	NS
Heptachlor epoxide	0.19 (<DL-0.37)	0.004 (<DL-0.08)	0.34 (0.05–0.82)	0.25 (0.01–0.46)	NS
dieldrin	<DL	<DL	<DL	<DL	–
∑endosulfan	<DL	<DL	<DL	<DL	–
∑OCPs	0.48 (0.023–1.44)	0.10 (0.01–0.75)	0.51 (0.07–1.51)	0.85 (0.07–2.09)	NS

<DL = values below detection limits, – = not tested for ANOVA, *NS* not significant ($p > 0.05$)

ecological aspect with higher sample size. This study is the first report of OCP contamination in eggs and tissues of House Sparrow of southern region of India and can serve as guidelines for future studies that evaluate organochlorine poisoning.

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