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**EXECUTIVE SUMMARY**

There has been an ongoing concern about the presence of different types of contaminants in the environment and their ill effects on wildlife, particularly birds. While substantial information is available on such ill effects on birds elsewhere in the world, very limited data exist in India. A study was intitiated to document the environmental residue levels of certain persistent organic contaminants, chemicals responsible for incidences of mortality of birds and, generate information on the levels of cholinesterase in brain and blood plasma of birds.

The major objectives were to understand tissue specific accumulation of contaminants, namelyorganochlorine pesticies (OCPs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in birds examine the variation in accumulation pattern between sexes and feeding habits, identify normal or reference levels of biomarkers, namely acetylcholinesterase (AchE) and butyrylcholinesterase (BChE) to explain mortality of birds.

Two representative cities, namely Ahmedabad, Gujarat in the west and Coimbatore, Tamil Nadu in the south were selected as study locations. Dead birds collected on opportunistic basis and form specific field visits were sent to the laboratory at SACON immediately. Totally 504 birds comprising 54 species from both the study locations, 202 eggs belonging to 25 species from Coimbatore, 137 blood plasma comprising 16 species form Ahmedabad were collected between 2003 and 2007 for contaminant and biomarker estimations. Depending on the type of sample, a multi residue extraction method was followed. Final qualitative and quantitative analyses were carried out using the standard instruments, namely Gas Chromatograph, High Performance Liquid Chromatograph, Ultracentrifuge and UV-Vis Spectrophotometer using standard operating protocols. Intra laboratory checks improved the quality of the analytical output.

This thesis organized in eight chapters, namely Executive Summary, Introduction, Materials and methods, Organochlorine pesticides residue, Polychlorinated biphenyls, Polycyclic aromatic hydrocarbons, Organochlorine pesticides in eggs, Organochlorine pesticides and PCBs in plasma and Biomarkers. Additional sections at the end of the document include a list of literature cited, publication and annexure.

**Organochlorine pesticide residue in birds**

Varying levels of organochlorine residues were found in all the species studied. HCH and DDT were the most frequently detected at higher levels in various tissues of birds. Among the isomers of HCH, β-HCH was predominat. Similarly, *p,p’*-DDE, the metabolite of *p,p’-*DDT accumulated more in many of the birds studied.

Although HCH residues were high in many species of birds, the levels did not fall within toxic levels, but indicative of exposure. DDT residues were the second highest, among the pesticides analyzed in various tissues of birds. The high DDE concentration in some individuals of Asian Koel *Eudynamys scolopacea*, Common Myna *Acridotheres tristis*, Indian Peafowl *Pavo cristatus*, Pariah Kite *Milvus migrans govinda* and Shikra *Accipiter badius* might be due to incidental exposure. Most of the birds studied showed comparatively less dieldrin burden and were below harmful levels. However, on many birds dieldrin was higher than the “Effects Range-Low” levels, capable of creating biological effects. Heptachlor epoxide detected in Indian Robin *Saxicoloides fulicata*, Shikra and White-headed Babbler *Turdoides affinis* and some individuals of Indian White-backed Vulture *Gyps bengalensis* were above the Food and Drugh Administration (FDA) action levels. AS such levels may pose risk to the referred birds they have to be viewed with concern. Endosulfan levels reported in the present study were less than the lowest dietary toxicity level reported for birds.

Although there was no significant difference observed in accumulation of organochlorine residues among tissues, the differences observed could due to combination of tissue specific retention and olubility in lipids. The carnivorous birds contained the highest concentrations of pesticide residues than the birds with other food habits. The relatively high pesticide residue levels in the predatory birds, namely Shikra and Pariah Kite are of great concern as the results obtained are similar to the general assumption of WHO that predatory birds have higher residues and are more sensitive when compared to other groups of birds.

Significantly, higher levels of OCPS were detected in birds of Ahmedabad than in Coimbatore. This can be explained by the extensive industrial operations and agricultural activities in and around Ahmedabad. Although present levels of organochlorine pesticides in birds from India mostly correspond to their past use, concern on current inputs should persist. In general, in considerable number of cases the measured levels of pesticides were higher than the values reported in similar species in industrialized countries. Although the concentrations found in this study were not exceeding alarming, it allowed revealing the magnitude of contamination among species.

Among the various groups of birds studied, carnivorous brids appeared to be good indicators of contaminants. Almost all the group of brids explained the pattern of organochlorine pesticide load over the period. Residues of cyclodiene insecticides, mostly dieldrin showed declining trend among the years. It could be due to complete ban and/or restricted usage. The current study proposes that specis, namely Pariah Kite and Blue Rock Pigeon Columba livia could be used in understanding temporal trends in contamination, as well as pattern of local or regional differences. Although some information on organochlorine pesticide residues in birds among different geographical areas is available, this information is scattered among trophic groups and chemical classes in India.

Polychlorinated Biphenyls (PCBs) and Polycyclic Aromatic Hydrocardons (PAHs) in birds

A total of 330 individuals comprising 13 species of birds received were analyzed for PCB and PAH contamination. There was no significant variation observed in contamination among tissues and species, except a few individuals. Although the birds collected from Ahmedabad were victims to kite flying in this region, the concentration documented may affect embryo development. In many cases PCB and PAH levels exceeded the levels associated with potential avian health effects. Although such levels are not likely to show impact on the demographic performances of the birds. May cause decreased reproduction or survival in some years, in combination with other non-anthropogenic stressors such as food scarcity. Incidentally, none of the tissues tested was free from PCB and PAH and their congeners and metabolites respectively.

Although experimental studies elsewhere have shown the effects of polycyclic aromatic hydrocarbons (PAHs) on bird behavior, field assessments are invariably confounded by ecological differences between contaminated and uncontaminated sites. Presence of PAH residues in birds of Ahmedabad city shows continuous input through industrial and other petrochemical industries to the environment. Comparatively lesser loads of PCBs and PAHs in the birds of Coimbatore is due to less oil based industries surrounding the city. This study recommends a continuous monitoring programme on PCBs and PAHs in select of birds across the country.

Organochlorine pesticide residues in eggs of birds

Two hundred twenty two eggs of 25 species of birds collected from seven districts in Tamil Nadu were analysed for organochlorine residues. Concentrations of OCPs found in the eggs were compared with available information in India and abroad. The levels of DDE, HCH and heptachlor epoxide in eggs of several species of brids were higher than the levels which are reported to have caused reproductive impairment in many species of birds elsewhere.

Although, OC residues in eggs of some species are lower than the levels documented elsewhere, it is of concern as even low levels of contamination if continued, can pose serious ill effects. Levels of *p,p’*-DDE in the eggs of Grey Partride *Francolinus pondicerainus* and Purple Sunbird *Nectarinia asatica*, heptachlor epoxide in the eggs of Plain Prinia *Prinia inornata*, Grey Partridge, House Sparrow *Passer domesticus*, Spotted Munia *Lonchura punctulata* and Red-whiskere Bulbul *Pycnonotus jocosus* were above the levels which were associate dwith impaired reproduction. All the species in the present study recorded less than 1µg/g of dieldrin, which is not expected to create any ill effects to the bird populations. Although use of HCH in agricultural fields has been banned, ɣ-HCH is still used on many crops including paddy and pules. Varying levels of HCH and its isomers are recorded in many species of birds, but their effects on reproductive success are not clear.

Declining population of House Sparrow is being reported all over India and several other countries around the world. Since levels of *p,p’-*DDE and heptachlor epoxide in the eggs studied are above the threshold levels, the present situation should be viewed with concern. *p,p’*-DDE concentration in the eggs of many species of birds were negatively correlated with eggshell thickness, but not significant. Levels of other pollutants were relatively low, and appear to have less significant influence on the reproduction.

High frequency of detection of HCH isomers and total endosulfan in all study sites indicates the extensive application of these pesticides in agriculture fields. Additive and possible synergetic effects may also occur where many different agricultural pesticides are used. Agro-ecosystem in Tamil Nadu appears to be a contaminated environment with respect to HCH and endosulfan. Results of this stud could serve as a basis for monitoring persistent OCPs on temporal and spatial scale in India using birds’ eggs as non-invasive tool.

OCPs and PCBs in plasma of birds

Information on the residue levels of OCPs and PCBs in plasma samples of 16 species of birds collected from Ahmedabad have been compiled. Relatively high levels of total HCH were detected in plasma of Sarus Crane *Grus antigone* (286 ng/g) an omnivorous bird followed by Besra Sparrow-hawk *Accipiter virgatus* (166.6 ng/g) a carnivore. White Ibis *Threskiornis melanocephalus* (11.4 ng/g) showed generally low concentration of HCH. Highest level of total DDT (147 ng/g) was detected in Painted Stork *Mycteria leucocephala*, while the lowest was in Black Ibis (19ng/g). Among various OCPs analyzed, *p,p’*-DDE was detected most frequently which accounted for more than 50% of total DDT in many of the speices anlysed. The concentrations of cyclodiene insecticides, heptachlor epoxide, dieldrin and total endosulfan ranged from 12.9 to 296.2 ng/g, BDL to 10.5 and 26.2 to 153.2 ng/g respectively. The PCB levels were high in carnivores and piscivores, while was low in granivores. The temporal variation in organochlorines were not significant.

The detected organochlorine residue levels are relatively higher in comparison with other studies carried out elsewhere. Although, the concentrations, detected are not capable of inducing chronic effects, may led to impairment of reproduction, behavioural and neurological functions, and suppression of immune function, if the exposure levels increases or even remains the same. DDE concentration was less than those of PCBs and threshold concentrations reported to cause significant eggshell thinning in birds. However, *p,p’-*DDT to *p,p’*-DDE ratio higher than that for many species of birds which indicates the recent use of DDT in this study sites. The presence of organochlorine residues in birds over the years (2005-2007) indicates continued exposure to organochlorine compounds. Attention should be paid, in particular, to breeding areas of different geographical regions so as to ultimately facilitate the early identification of increased risks to the survival of any particular species.

The documentation and monitoring of pesticide residues in a region may be facilitated by establishing certain organisms as basic bioindicators. Based on the present study Blue Rock Pigeon and Pariah Kite could be considered as excellent choice as bioindicators of chlorinated hydrocarbon contamination. This study is the first accound of a comprehensive analysis of toxicants in different species of bird in India. The values reported in this study can serve as guidenlines 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.

Biomarkers in birds

The highest levels of brain AChE activity was recorded in Asian Openbill *Anastomus oscitans* (5.52 *µ*mles/min/g) followed by House Crow *Corvus splendens* (4.06 *µ*moles/min/g) and Painted Stork (3.01*µ*moles/min/g), while lowest levels of AChE activity was in Bank Myna *Acridotheres ginginianus*, White Ibis and Barn Owl Tyto alba 0.51, 0.65 and 0.93 *µ*moles/min/g respectively. The mean brain AChE and BChE values among years were not varying in any of the species studied (ANOVA, *P* > 0.05).

Plasma AChE activity was significantly different among species. The highest AChE activity was observed in Blue Rock Pigeon (1.09 *µ*moles/min/ml) followed by Painted Stork (1.06 *µ*moles/min/ml) and the lowest in Besra Sparrow-hawk (0.1*µ*moles/min/ml). The highest mean plasma BChE activity was recorded in Blue Rock Pigeon (1.01 *µ*moles/min/ml) and the lowest in Painted Stork.

The estimated normal whole brain ChE activities presented in this study serve as reference values with the recommendation that the database initially be confirmed and expanded rather than used exclusively in lieu of concurrent controls. Present study encourages the use of presented values as emergency substitutes in diagnosis of lethal anticholinesterase poisoning when concurrent controls cannot be obtained. This study is the first report of AChE and BChE activity in birds in India and constitutes a starting point for future studies that evaluate impact of pesticides in birds. This study further recommends continuous monitoring of AChE and BChE activity in brain and plasma tissues of various species of wild bird and creation of database.

**Conclusions and Recommendations**

The study has documented levels of OCPs, PCBs, and PAHs in tissues of 504 birds comprising 54 species. OCPs residues in 202 eggs belonging to 25 species of birds and biomarkers (AChE and BChE) in brain and plasma of 21 species of birds also have been documented. About 12% of birds studied exceeded in organochlorine, PCB and PAH levels reported to have caused risk to birds, or to wildlife. Overall the OCPs, PCBs and PAHs in this study ranged between below detectable limits and levels that could pose threat to birds or to animals those consume them. The highest concentrations of most of the contaminants were found in those species which are relatively higher in the food chain, namely vultures, kites, and crows. Birds received from Ahmedabad, Gujarat had higher levels of contaminants than birds of Coimbatore, Tamil Nadu.

Different species may vary in their sensitivity to the same compound so that a direct relationship between the effects observed in one species may not manifest itself in another, even when exposed to the same concentration of a chemical. Additionally, among the majority of the birds included in the present study, Pariah Kite and Blue Rock Pigeon appeared to be consider as biosentinal or indicator species for continuous monitoring of contaminants in the environment.

In the present study, as majority of the birds received form Ahmedabad had died due to injuries caused by kite flying, the contaminant load recorded from this area could serve as a good data bank or reference point except a few cases. This protocol can be expanded to include additional species for monitoring the Indian environment. The data collected in this study therefore, could be used to determine if thresholds have been reached for contaminants those are known to cause reproductive impairment or developmental deformities; and determine changes in contaminant load over the years within and between states.

Routine monitoring should be based upon standardized protocols for selection of species collection sites, storage and transport of samples, post-mortem techniques and reporting procedures. Plasma and specimen banks will be required to house samples for future reference. Site specific and species specific monitoring programmes should be initiated based on the needs. Appropriate control sites, away from human activities, will be essential for reliable interpretation of monitoring results. Data should be reported regularly and coordinated by responsible teams for intended use.

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