# LEVELS OF ORGANIC CONTAMINANTS AND SELECT BIOMARKERS IN THE BIRDS OF GUJARAT AND TAMIL NADU, INDIA

Thesis submitted to the
**BHARATHIAR UNIVERSITY**
Coimbatore



for the award of the Degree of Doctor of Philosophy in
**ENVIRONMENTAL SCIENCE**

by
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***Division of Ecotoxicology***

**Sálim Ali Centre for Ornithology and Natural History**
**(An Autonomous Centre of the Ministry of Environment & Forests, Govt. of India)**

**Anaikatty, Coimbatore – 641 108**

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# CERTIFICATE

This is to certify that the thesis, entitled **"LEVELS OF ORGANIC CONTAMINANTS AND SELECT BIOMARKERS IN THE BIRDS OF GUJARAT AND TAMIL NADU, INDIA"** submitted to the Bharathiar University, in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy in **ENVIRONMENTAL SCIENCE** is a record of original research work done by **Mr. V. DHANANJAYAN** during the period **AUGUST 2004 to AUGUST 2008** in the Department of **ECOTOXICOLOGY** at **SÁLIM ALI CENTRE FOR ORNITHOLOGY AND NATURAL HISTORY, ANAIKATTY, COIMBATORE – 641 108** under my supervision and guidance, and the thesis has not formed the basis for the award of any Degree / Diploma / Associateship /Fellowship or any other similar title of any candidate of any University.

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# DECLARATION

I, **V. DHANANJAYAN** hereby declare that the thesis, entitled **"LEVELS OF ORGANIC CONTAMINANTS AND SELECT BIOMARKERS IN THE BIRDS OF GUJARAT AND TAMIL NADU, INDIA"** submitted to the Bharathiar University, in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy in **ENVIRONMENTAL SCIENCE** is a record of original research work done by me during **AUGUST 2004 to AUGUST 2008** under the supervision and guidance of **Dr. S. MURALIDHARAN**, Division of **ECOTOXICOLOGY** at **SÁLIM ALI CENTRE FOR ORNITHOLOGY AND NATURAL HISTORY, ANAIKATTY, COIMBATORE – 641 108** and it has not formed the basis for the award of any Degree / Diploma / Associateship / Fellowship or any other similar title of any candidate of any University.

Signature of the Candidate

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## CONTENTS

## 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 initiated 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, namely organochlorine pesticides (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 from 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 from 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 is 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 residues 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, $\beta$-HCH was predominant. 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 Drug 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 solubility 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 birds appeared to be good indicators of contaminants. Almost all the group of birds 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 species, 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 Hydrocarbons (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 species 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 birds 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 Partridge *Francolinus pondicerianus* and Purple Sunbird *Nectarinia asiatica* , heptachlor epoxide in the eggs of Plain Prinia *Prinia inornata,* Grey Partridge, House Sparrow *Passer domesticus,* Spotted Munia *Lonchura punctulata* and Red-whiskered Bulbul *Pycnonotus jocosus* were above the levels which were associated with impaired reproduction. All the species in the present study recorded less than 1 µg/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, γ-HCH is still used on many crops including paddy and pulses. 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 the 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 study 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 (19 ng/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 species analysed. 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, behavioral 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 account of a comprehensive analysis of toxicants 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.

**Biomarkers in birds**

The highest levels of brain AChE activity was recorded in Asian Openbill *Anastomus oscitans* (5.52 μmoles/min/g) followed by House Crow *Corvus splendens* (4.06 μmoles/min/g) and Painted Stork (3.01 μmoles/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 μmoles/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 μmoles/min/ml) followed by Painted Stork (1.06 μmoles/min/ml) and the lowest in Besra Sparrow-hawk (0.1 μmoles/min/ml). The highest mean plasma BChE activity was recorded in Blue Rock Pigeon (1.01 μmoles/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 birds, 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 from 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.

# Chapter 1
# INTRODUCTION

## 1.1. General Introduction

Enormous quantities of man made chemicals are used in modern agriculture for producing food commodities as efficiently as possible to meet the ever increasing human food demands. Despite obvious benefits, the indiscriminate use of such chemicals has resulted in over intoxication of animals and accumulation of residues in feeds and foods causing environmental and ecological impacts (Moore, 1980). Rachel Carson through her book, Silent Spring (Carson, 1962) might be regarded as the first to bring attention to the sensitive nature of birds to the deleterious affects of toxic contaminants.

The persistence of pesticide residues in soil, water, air, plant, and animal raised concern about their long-term accumulation and effects. Residues of contaminants in foods, eggs and tissues have continued to be used to evaluate reproductive failures and population declaines in many wild vertebrates. Birds are a visible and important part of the world we share and they appear to be the major targets of environmental contaminants because they occupy a wide range of tropic levels in different food chains and are thereby exposed to different concentration of pollutants through their food (Ankley *et al.*, 1993).

The pesticide, dichloro-diphenyl-trichloroethane (DDT) and its breakdown product DDE gained notoriety about 40 years back in North America. Ratcliffe (1967) investigating the Peregrine Falcon *Falco peregrinus* and Sparrow Hawk *Accipiter brachyurus* in Britain, was the first to record a correlation between the magnitude of decrease in eggshell weights, decrease in breeding populations and exposure of populations of these species to persistent organic pesticides. Health *et al.*, (1969) showed experimentally that DDE impaired the reproductive performances.

In general, birds those eat other birds, or fish, have higher residues than those that eat seeds and other plant products. It is also reported that a synchronous, rapid, and widespread decline in weight and thickness of shells of eggs laid by many species of wild birds and fish-eating birds occurred in the late 1940's and had persisted in the United States (Stickel, 1973). Later it was proved responsible for eggshell thinning that led to population crashes and reproductive impairments in many species of birds in North America (King *et al.*, 1978; Fleming *et al.*, 1984) and in United Kingdom.

The effects of organic pollutants on wildlife, waterfowl and fish-eating birds have been extensively studied in many countries (Stickel *et al.*, 1969; Fleming *et al.*, 1984; Ambrose *et al.*, 1988; Custer *et al.*, 1990; Kuiken *et al.*, 1999; Balseiro *et al.*, 2005). Birds have been widely used as bioindicators, for environmental pollution, particularly for persistent organochlorines, which have been considered as potential endocrine-disrupting chemicals in wildlife (Minh *et al.*, 2002).

The US Environmental Protection Agency tests chemicals for avian reproduction hazards and reports shell thickness measurements as part of the investigation process. Therefore, measuring eggshell quality remains a valuable tool for testing or monitoring a variety of contemporary environmental chemicals. Studies also support one of the basic assumptions of the sample egg technique, that the chemical residues in one egg in a clutch accurately reflects residues in the remaining eggs of the clutch (Custer *et al.*, 1990).

Programme for monitoring pesticide residues were recommended by individuals and official committee (US President's Science Advisory Committee). An international convention aiming to restrict persistent organic pollutants (POPs) was formally adopted in May 2001, and global actions to reduce and eliminate release of the pollutants have been recommended. The organochlorines (OCs) known as the 'dirty dozen' have already had a strong impact on wildlife and human beings (Choi *et al.*, 2001).

Replacement of OC insecticides with shorter-lived chemicals such as organophosphates (OP), carbamates (CB) and other compounds alleviated many problems with persistence and bioaccumulation of lipid-soluble OCs (Blus, 1982; Wiemeyer *et al.,* 1984). However, further studies reveal that even newer compounds, particularly those which do not stay longer in the environment lead to acute or sub-acute toxicity (Grue *et al.,* 1983; Henny *et al.,* 1985) and reduction in the food base (Rands, 1985; Potts, 1986). It was also demonstrated that some OP compounds could temporary affects the eggshell quality and reduce eggshell thickness in Northern Bobwhite *Colinus virginianus* (Driver *et al.,* 1991). Organophosphorous compounds also alter bird behaviour; important among them were reduction in singing (Grue and Shipley, 1981), decreased vigilance, disturbance of nesting (Grue *et al.,* 1982) and feeding behaviour (Adams, 1977).

In addition to agricultural pesticides, Polychlorinated Biphenyls (PCBs) and Polycyclic Aromatic Hydrocarbons (PAHs) which are basically industrial chemicals composed of many isomers and congeners also highly differ in toxicity and persistence. PCBs and PAHs being highly lipophilic, they accumulate in lipid fractions of cell and tissues of living biota including human beings and birds (Falandysz *et al.,* 2003).

In recent years use of blood as a non-destructive technique has become increasingly common in assessing both exposure and effect of environmental toxicants in avian wildlife. Reports on the residue levels of organochlorine pesticides and PCBs were available in plasma of Peregrine Falcons and Gyrfalcons (Jarman *et al.,* 1994) and Egyptian Vulture (Gomara *et al.,* 2004). However, there is little information available in India on the residue levels of contaminants in birds.

In recent years, the concept of "Biomarker" has received considerable attention among ecotoxicologists as a new and powerful tool for detecting and documenting exposure and effects of environmental contaminants. A biomarker is a xenobiotically induced variation in cellular or biochemical components or process, structure, or functions that is measurable in a biological system or samples (National Research Council, 1989). Inhibition of brain acetylcholinesterase (AChE) activity and plasma butyrylcholinesterase (BChE) activity has been the most widely used biomarker to diagnose the OP poisoning in a wide variety of organisms (Franson *et al.,* 1983; Hill and Murray, 1987; Fairbrother, 1990; Day and Scott, 1990).

Cholinesterase is essential for the normal functioning of the central and peripheral nervous system (Hart, 1993) and is responsible for the transmission of signals from the terminals of motor nerves to the muscles as well as the synaptic transmission between nerve cells (Schmidt Nielson, 1990). Plasma and brain cholinesterase activity were measured in 27 species of birds (Westlake *et al.,* 1983). Many species of birds have plasma butyrylcholinesterase (BChE) activity less than plasma acetylcholinesterase (AChE) values. Interestingly, there has been no further additions to this till date. Recently a few studies have documented the relative amounts of AChE and BChE in avian serum (Busby, 1991; Rainwater *et al.,* 1995). However, for diagnostic interpretation, normal cholinesterase activity is very essential. Although, such definite information on the impact of environmental contaminants on many species of birds is available across the globe, the information on birds in India is very limited.

Following the success of DDT in World War II, several organochlorine pesticides were discovered for the control of vector born diseases of crops. The use of pesticides in agriculture in India commenced around 1948-49 (Gupta, 1986) and it increased manifold with the green revolution. Today India is the second largest manufacturer of pesticides in Asia and third largest consumer in the world. There has been steady growth in the production of technical grade pesticides in India, from 5,000 mt in 1958 to 1,02,240 mt in 1998 (Saiyed *et al.,* 1999). Estimated usage of pesticides in India increased from 2350 mt in 1955-56 to around 1 lakh mt in 2003 and it is decreased to 41,350 mt during 2005 (www.ncipm.org.in/ConsumptionGroupwise.htm.).

Although DDT has now been banned for use in agriculture, it is still available for public health care programmes particularly malaria eradication, while BHC use is restricted. The annual production of gamma HCH in India increased from 40 mt in 1996-97 to 250 mt in 1997-98. It was also reported that residues of HCH persist in the environment, concentrate in the crops and continue to be detected in the food chain (Nigam and Siddiqui, 2001). There is now overwhelming evidence that most of the pesticides used in agriculture and vector control operations do pose potential health hazards to humanity.

In India, the first tragic report of pesticide poisoning was from Kerala in 1958 when about 100 people died after consuming wheat flour contaminated with parathion. Therefore, the reports of the special committee on harmful effects of pesticides focused further attention on the problem. Subsequently, Insecticide Act (1968) and the Insecticide Rules (1971) were enacted in the country to ensure safe use of these chemicals for the benefits of the society. Indian Council for Medical Research's (ICMR) National Institute of Occupational Health (NIOH), Ahmedabad, India conducted studies to evaluate toxicological implications amongst workers in unorganized and organized sectors of the pesticide industry, people involved in malaria control programmes, pest control agency workers warehouse workers and other user groups (Kashyap and Bhatnagar, 1996). Several studies confirm that a large variety of food and feeds are contaminated with persistent organochlorine group of chemicals, such as DDT, HCH, chlordane and PCBs. Similarly bioaccumulation of these chemicals in milk, butter, meat and even human fat has been observed.

Vijayan *et al.,* (2004) documented residues of organochlorine pesticides in 66 species of fishes from inlandwetlands in 14 states in the country. Indian Council of Agricultural Research (ICAR) initiated a programme to monitor residue levels of persistent pesticides in various agricultural produces involving several agricultural universities across the country during 2006. Later additional components including fishes were added to monitor the OC residues and other problem chemicals (Chandrasekaran, 2006). As has been referred earlier in this section, although there are some information available on the residue levels and impacts on birds in the country, effective long-term monitoring studies are lacking.

In India, declining population of piscivorous birds breeding at regular sites and infrequent sightings or total absence of many species of birds, in the agricultural fields in various places is being reported. During the last two decades, impacts of pesticides on many species of birds have been abundantly obvious.

Pesticide levels in the eggs of eight species of fish-eating birds in Keoladeo National Park (Muralidharan *et al.,* 1992), accumulation of organochlorine in birds from South India (Tanabe *et al.,* 1998; Muralidharan and Murugavel, 2001), and isomeric accumulation pattern and toxic assessment of polychlorinated biphenyls in resident, wintering and migrant birds and bat collected from South India (Senthilkumar *et al.,* 1999) give credence to the concern of ornithologists. Between 1987 and 1990, 18 Sarus Cranes and more than 50 Collared Doves had fallen victim to pesticides in Keoladeo National Park, Bharatpur (Muralidharan, 1993). Even after the ban on aldrin, death of 15 Sarus Cranes due to monocrotophos poisoning was reported (Pain *et al.,* 2004). Mass mortality of Indian Peafowl happened in Madhya Pradesh and some parts of western India during the year 1999 (Muralidharan, 2002).

In India subsequent to the apprehensions expressed by ornithologists about the declining population trend in many species of common birds, Sálim Ali Centre for Ornithology and Natural History (SACON), initiated a programme to document the residue levels of pesticides in birds in the year 1999. Till today information on 1050 birds comprising 105 species of birds received from limited locations in the country has been gathered. While some information is published (Vijayan and Muralidharan, 1999; Muralidharan and Murugavel, 2001; Muralidharan, 2002; Muralidharan *et al.,* 2004; 2008), the rest is being compiled.

## 1.2. Study Design

- There are hundreds of chemical compounds to potentially monitor in the environment, particularly birds. However, this study narrowed the scope to those pollutants those are persistent, known to bioaccumulate and identified as chemicals of concern.

## 1.3. Major Objectives

- Document the residue levels of persistent organic contaminants, namely Organochlorine Pesticides, Polychlorinated Biphenyls and Polycyclic Aromatic Hydrocarbons in the birds of Ahmedabad, Gujarat and Coimbatore, Tamil Nadu, India.

- Understand tissue specific accumulation pattern of the referred contaminants in birds.

- Examine the variation in accumulation pattern between sex and also among feeding habits.

- Identify the normal or reference levels of biomarkers, namely Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) to explain mortality of birds.

## 1.4. Organization of thesis

The findings of this study are presented and discussed in eight different chapters.

The general introduction and significance of the study, overall outlook and study design, objectives and structure of the thesis are given in *Chapter 1*. The detailed methodology including study area, sample collection, processing, analysis using various instrumentation techniques and quality control measures are given in *Chapter 2*. Organochlorine pesticide residues in tissues of various species of birds collected between 2003 and 2007 in Ahmedabad and Coimbatore are discussed in *Chapter 3*. Polychlorinated biphenyls (PCBs) and Polycyclic Aromatic Hydrocarbons (PAHs) in tissues of various species of birds collected between 2005 and 2007 in Ahmedabad are treated separately in *Chapter 4*. Organochlorine pesticide residues in eggs of birds collected between 2001 and 2006 in and around Coimbatore are described in *Chapter 5*. The varying levels of organochlorine residues (OCPs and PCBs) in plasma of birds collected (2005-2007) from Ahmedabad, Gujarat are given *Chapter 6*. The normal and reference values of biomarkers, namely Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) in brain and plasma of birds of Ahmedabad are discussed in *Chapter 7*. Major findings of the study and conclusion are given as an executive summary.

# MATERIALS AND METHODS

## 2.1. Introduction

India is the seventh largest country in the world and also ranks second in population. Surrounded by Bangladesh, Burma, Bhutan, China, Nepal and Pakistan, India covers an area of 32,87,590 sq km. The geographical coordinates extends between $20^{\circ}00'$ N and $77^{\circ}00'$ E with the total length of the coastline is over 7,000 kms. Agriculture and allied activities constitute the single largest contributor to the Gross Domestic Product (GDP) and provide the means of livelihood to about two-thirds of the work force in the country. The total food production in India is likely to double in the next ten years and there is an opportunity for large investments in food and food processing. Climate of India varied from tropical monsoon in South to temperate in north (http://geography.about.com/library/cia/blcindia.htm).

India is now the second largest manufacturer of pesticides in Asia after China and it ranks twelfth globally. In India 76% of the pesticide used are insecticides, as against 44% globally (Mathur *et al.,* 1992; Saiyed *et al.,* 1999). Major demand of the pesticide in the country is met from the indigenous production for control of disease, insect pests and weeds. The annual production capacity of pesticides in the country is around 1,39,000 mt with more than 125 technical grade/manufacturing units and over 800 formulation units. At present, the consumption of chemical pesticides is the highest in Andhra Pradesh (33%), followed by Punjab (14%), Karnataka (11%), Tamil Nadu (9%), Maharashtra (7%), Haryana (6%), Gujarat (5%), Uttar Pradesh (5%) and the remaining states account for 9.5% of the total consumption (Singhal, 1999).

## 2.2. Study Area

This current study was designed as part of a major project titled monitoring and surveillance of environmental contaminations in Indian Avifauna at the Ecotoxicology Division, Sálim Ali Centre for Ornithology and Natural History, Coimbatore, India. While the objective of the project was to study the impact of pesticides on birds across the country, the present study concentrates only on two major cities, namely Ahmedabad, Gujarat and Coimbatore, Tamil Nadu. $10^{\circ}55'^{\circ}$

### *2.2.1. Ahmedabad, Gujarat*

Ahmedabad is situated between $23^{\circ}03'$ N and $72^{\circ}40'$ E having 11 taluks, covering an area of 8707 square Km in the state of Gujarat. Gujarat is situated in the west coast of India. The state is bounded by the Arabian Sea on the west, Pakistan and Rajasthan in the north and north-east respectively, Madhya Pradesh in the south-east and Maharashtra in the south. The state is divided into 25 administrative districts (as of 2006).

**Climate and Temperature:** State has diverse climatic conditions. The winters are mild, pleasant, and dry with average daytime temperatures around 83°F (29°C) and nights around 53°F (12°C) with 100 per cent sunny days and clear nights. The summers are extremely hot and dry with daytime temperatures around 105°F (41°C) and at night no lower than 85°F (29°C).

**Agriculture:** Gujarat is the main producer of tobacco, cotton and groundnuts in the country. Other major food crops grown in and around Ahmedabad are rice, wheat, jowar, bajra, maize, tur, and gram. Gujarat has sound agricultural economy; the total crop area accounts to more than one-half of the total land area. Animal husbandry and dairy play a vital role in the rural economy of Gujarat.

**Industrial profile**: Ahmedabad one of India's most industrialized cities in Gujarat houses a variety of industries, the principal ones being general and electrical engineering and the manufacture of textiles, vegetable oils, chemicals, soda ash, and cement. New industries include the production of fertilizers and petrochemicals. In general, Gujarat produces about 90% of India's required amount of Soda Ash and gives the country about 66% of its national requirement of salt. Gujarat contributes inputs to industries like textiles, oil and soap.

### *2.2.2. Coimbatore, Tamil Nadu*

Coimbatore is an important industrial town in Tamil Nadu, South of India. It is located between 10°55'and 11°10'N, and 77°50' and 76°50'E at an approximate altitude of 470 m. The population of Coimbatore as per the 2001 census of India is 0.9 million. Though it is an industrial town, agriculture is practiced in vast areas in the district. The present study was focused on birds collected from both Coimbatore and surrounding area. Tamil Nadu state is situated between 8°5' and 13°35' of northern latitude and 76°15' and 80°20' of eastern longitude with an area of 1,30,058 square kilometers which constitutes about 4% of the land area of the country. The state is divided into 30 districts. It is bounded on the north by Karnataka and Andhra Pradesh on the east by Bay of Bengal, on the South by the Indian Ocean and on the West by Kerala State.

**Agricultural Profile:** The agricultural sector's growth rate in Tamil Nadu was among the highest in India during 80's and in early 90's (Profile of Tamil Nadu). Rice and various pulses are grown extensively here. The major cash crops grown in the state are sugarcane, tobacco, chilly, and cotton, giving rich scope for the growth and development of the sugar, alcohol-based and textile industries. The diverse agro-ecological conditions also make this land ideal for growing fruits and vegetables.

**Industrial Profile:** Traditionally, Coimbatore is one of the well developed cities in terms of industrial development. Agro-based Industries: Ideal climatic conditions for the growth of fruits and vegetables have given rise to a vibrant agro-based food industry in the state.

### 2.3. Sample collection and transportation

Details of the samples collected from the study locations are given in the respective chapters. Since all the birds were collected on opportunistic basis and during sporadic field visits to suspected places based on the information collected from field biologists, local people and media. Dead birds collected were sent to SACON laboratory over gel packs by courier without delay. Flyers were circulated among people as to what they should do on locating a bird dead.

**Dead Birds:** A total of 510 individuals (1445 tissues) comprising 73 species belonging to 33 families were collected from Ahmedabad, Gujarat and in and around Coimbatore, Tamil Nadu (Figure 2.1; Table 2.1; Plates 2.1, 2.2).

**Blood Plasma:** About 137 blood plasma samples were collected from Ahmedabad, Gujarat for contaminants analysis (Plate 2.2).

**Eggs:** Totally 202 eggs belonging to 25 species of birds were collected/received only from seven districts in Tamil Nadu for organochlorine pesticide residues analysis (Plate 2.3).

Dead birds were collected from the study sites with the help of field biologists, forest officials, farmers and brought to the laboratory at SACON, Coimbatore. In the events of any mass mortality of birds exclusive visits were made to collect the samples.

### 2.4. Sample processing

All the birds received at the laboratory were subjected to postmortem examination and tissues, namely brain, muscle, liver and gut contents were dissected out and preserved at -20°C for possible contaminant analyses.

Methodologies were designed for residue analyses, depending on the type of samples and evidences collected from the field. The biomarkers, namely acetylcholinesterase and butyrylcholinesterase levels were also estimated in plasma and brain samples of birds.

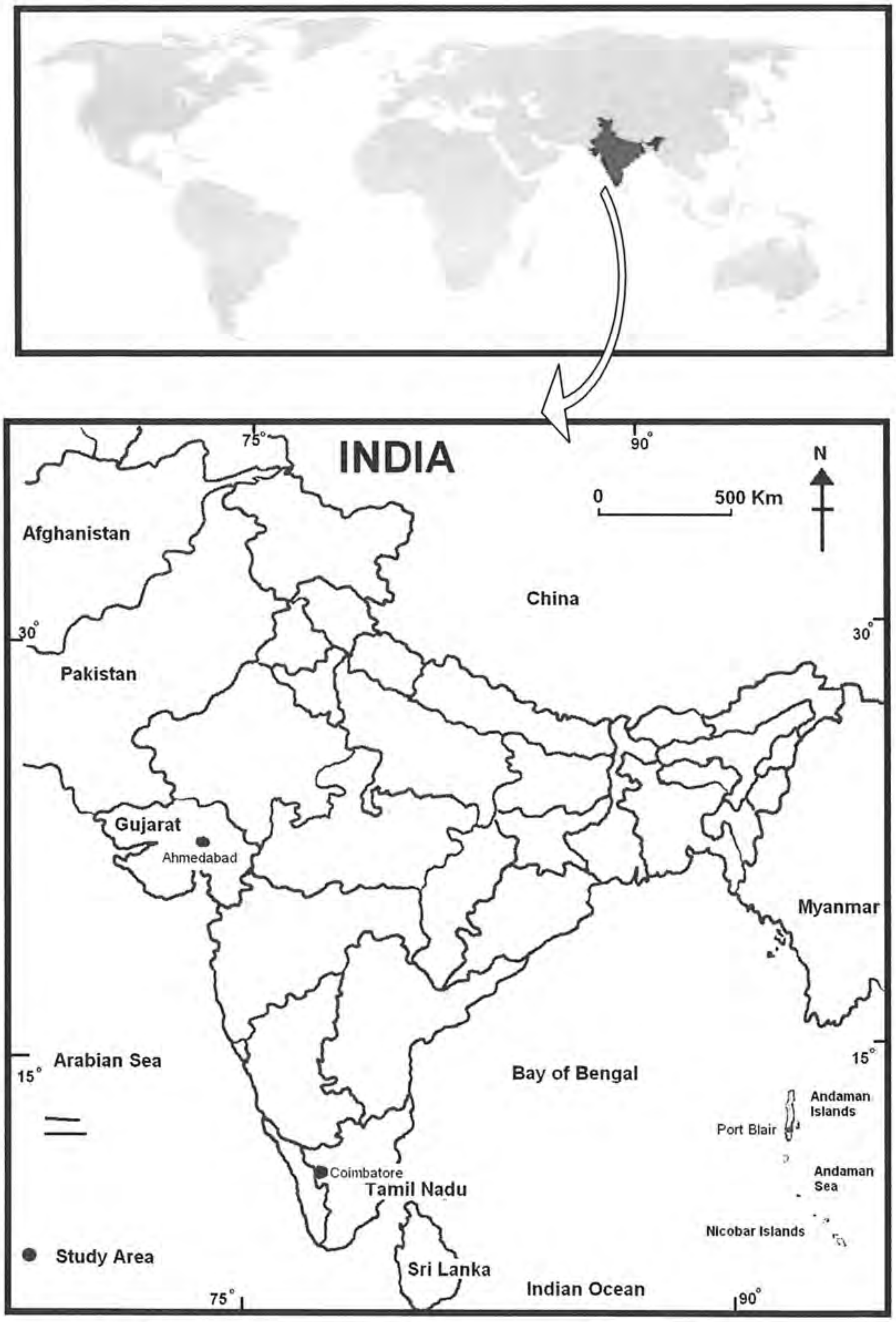



**Figure 2.1.** Map showing the dead bird collection sites at Ahmedabad, Gujarat and Coimbatore, Tamil Nadu.

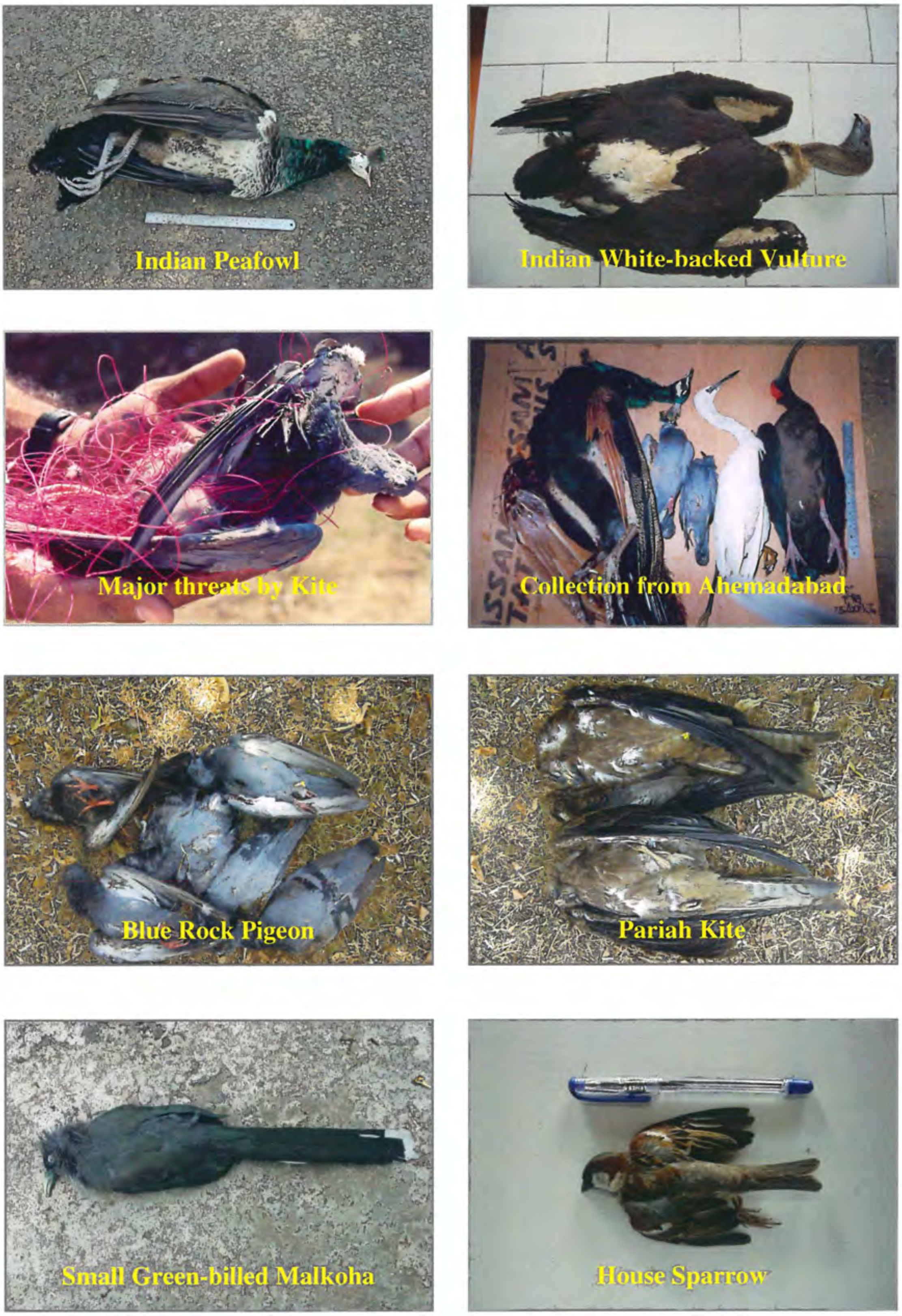



**Plate 2.1.** Dead birds collected from various places in Ahmedabad and Coimbatore.


**Plate 2.2.** Postmortem, sample processing and analysis of samples in the laboratory at SACON


**Plate 2.3.** Eggs of select species of birds collected from various places in Tamil Nadu, India

**Table 2.1.** Details of samples and parameters analyzed

| Type of samples | Contaminants | Species | Birds | No. of tissue samples analysed |
|---|---|---|---|---|
| Tissues (Brain, Liver, Muscle, Fat and Gut content) | Organochlorine pesticides (OCPs) | 54 | 504 | 1534 |
| Tissues (Brain, Liver, Muscle | Polychlorinated Biphenyls (PCBs) and Polycyclic Aromatic Hydrocarbons (PAHs) | 13 | 330 | 1016 |
| Eggs | Organochlorine pesticides (OCPs) | 25 | 202 | 202 |
| Blood Plasma | OCPs and PCBs | 16 | 137 | 137 |
| Brain & Plasma | Biomarkers (Acetylcholinesterase Butyrylcholinesterase) | 31 | 114+ 149 | 263 |

By and large a multi residue extraction with suitable solvent and clean up with suitable adsorbent, namely florisil or/and silica gel with specific mesh sizes was followed. Final qualitative and quantitative analyses were carried out using the appropriate instruments. The standard operating protocol (SOP) was followed for all the laboratory work and the detailed processing procedures are given in the respective chapters.

**2.5. Sample analysis**

The following instruments were used for quantitative analyses of the components.

**a) Gas Chromatograph (GC),** Hewlett Packard (HP), 5890 Series II equipped with two detectors namely a) Electron Capture Detector (ECD) and Nitrogen Phosphorous Detectors (NPD) were used for quantification of pesticides and PCB residues.

**b) High Performance Liquid Chromatograph (HPLC):** Agilent 1100 Series, equipped with binary pump along with the Diode Array Detector (DAD) and Florescence Detectors (FLD) was employed for estimation of PAHs in the tissues of birds.

**c) Ultracentrifuge:** Beckman (Coulter- Optima LE- 80 K) was used for plasma separation and brain sample preparation for UV-Vis spectrophotometer analysis.

**d) UV-Vis Spectrophotometer** PerkinElmer Lambda 35 was employed for quantifying the enzyme activity in brain and plasma samples of birds.

Other ancillary instruments used for laboratory analysis were **Rotary Evoporator** (BUCHI), **Sonicator, Rotospin** and **Vortex shaker**.

### 2.6. Quality control

Quality control measures were adopted to ensure accuracy and precision of analytical results. This study was formulated involving a few established residue testing laboratories for this purpose for inter- and intra laboratory checks. This helped to identify weak methodology and upgrade overall quality of laboratory performance. Intra laboratory check, with the objectives in parallel with the inter laboratory check improved the quality of the analytical output in the analyses within the laboratory.

Chapter 3

# ORGANOCHLORINE PESTICIDE RESIDUES IN BIRDS OF AHMEDABAD AND COIMBATORE

## 3.1. Introduction

Among the innumerable chemicals introduced into the environment, pesticides have been posing serious problems. Among the different groups of pesticides, organochlorines have got slow decomposition rate and long life thereby they remain in the system and exert toxic effects on all living biota including man and birds. Some are very persistent and become widely distributed by air and water (White and Stickel, 1975). Presently, use of many organochlorine compounds has been banned or severely restricted all over the world.

Organochlorine pesticides and certain other persistent toxic chemicals are widely dispersed in the environment and are commonly found in tissues and eggs of wild birds from the Arctic to Antarctic. The organochlorines due to their persistent nature remain stored in the body fat and largely disturb calcium metabolism and thereby reduce eggshell thinning over a period of time eventually leading to population decline (Tanabe *et al.,* 1998). Some organochlorine chemicals are highly toxic and can cause mortality directly, whereas others reduce reproductive success or cause subtle effects on the ecosystem (Ohlendorf, 1981).

Birds appear to be the major targets of pesticide pollution as they occupy a wide range of tropic levels in different food chains and thereby get exposed through their food (Ankley *et al.,* 1993). Birds have been widely used as bioindicators, for environmental pollution, particularly persistent organochlorines, which have been considered as potential endocrine-disrupting chemicals in wildlife (Minh *et al.,* 2002).

Birds, fish and other wild animals are placed in intimate contact with the toxic materials or may ingest them with their food (De Witt *et al.,* 1960). The effects of pesticide on wildlife, especially raptors, waterfowl and fish-eating birds have been extensively studied around the world

(Stickel *et al.,* 1969; Fleming *et al.,* 1984). Additional information is also available on common resident birds like Partridges (Herrera *et al.,* 2000), Passerine birds (Frick *et al.,* 1998), and Kingfisher (Minh *et al.,* 2002). Although, food abundance and quality greatly impact reproductive effort and success in many predatory birds (Korpinaki and Norrdahl, 1991; Rohner, 1996), there has been increasing concern about the potential deleterious effects of organochlorine and other pesticides on the population of many species of birds (Gard *et al.,* 1995; Block *et al.,* 1995). Chlorinated pesticides have shown to cause estrogen-dependent reproductive effects in several avian species (Fry, 1995). However, for the clarification of the toxic effects of OCs, basic information such as the distribution of OCs among organ (or tissue) is still insufficient (Takazawa *et al.,* 2004).

Declining population of birds breeding at regular sites and infrequent sightings or total absence of insectivorous birds, such as drongos and bee-eaters in the agricultural fields in various parts of India are being reported. Declining breeding population of Sarus Crane *Grus antigone,* (Walkinshaw, 1973; Vijayan, 1991; Muralidharan, 1993) in Keoladeo National Park, Bharatpur, higher accumulation of organochlorine in resident and migratory birds from south India (Tanabe *et al.,* 1998), fast disappearing common bird 'House Sparrow' in India (Vijayan, 2003) and accumulation of various OCPs in tissues of Vultures (Muralidharan *et al.,* 2008) give credence to the concern of Indian ornithologists. In the case of Sarus Crane in Bharatpur, it was inferred that the decline in population was mainly due to application of aldrin in the agricultural fields around the Park (Muralidharan, 1993). Even after the ban on aldrin, death of 15 Sarus Cranes due to monocrotophos poisoning was reported (Pain *et al.,* 2004). Unfortunately mortality of Sarus Crane has become rather frequent. Although such definite information are not available for many species of birds in India, study conducted elsewhere give definite proof for the deleterious effects of pesticides on avifauna and the ecosystem.

There is a sequential rise in the production and consumption of pesticides in the India during the last three decades. Currently, there are about 165 pesticides registered for use in India. Although we keep guessing that pesticide could be the reason for the decreasing population trend in many insectivorous and piscivorous birds, nothing has been proved till date (Muralidharan, 2002) except a very few studies on the problems of pesticide contamination in Indian wildlife and birds especially resident birds (Muralidharan, 1993; Tanabe *et al.*, 1998; Senthilkumar *et al.*, 1999; Muralidharan *et al.*, 2008). Not much of research has been carried out to bring out the exact picture of pesticide contamination in a specific region. In this background the current study is an attempt to add on to the existing database.

## 3.2. Background Information

### *3.2.1. Impact of pesticides on birds around the world*

Studies in Europe and North America have linked the decline in populations of several species of birds to chlorinated hydrocarbons, exposure (Moore, 1980). In Spain, Red-legged Partridge *Alectoris rufa* population has declined by around 30% (Delibes, 1992). Bioaccumulated DDE has been linked to eggshell thinning and reduced reproductive success in a variety of bird species (King *et al.*, 1991; Fasola *et al.*, 1998). Long range transport of these contaminants into different geographical areas has been recognized and chlorinated hydrocarbon residues are widely dispersed throughout the ecosystems.

The pesticides don't kill the individual birds often but do affect their bodies so that they lay eggs with very thin shell. Often these thin-shelled eggs break or the birds are unable to reproduce and pesticides also affect birds physiological function. In most cases, pyrethroids are less toxic to mammals but they produced harmful effect on birds. Natural conservation committee and Royal Society for Protection of Birds suggested that indirect effect of pesticides was a major cause of decline in birds (Tabassum *et al.*, 2003).

Many species of animals including birds possess biochemical defense systems which can detoxify some of these foreign substances. However, the organochlorine residues are relatively toxic to animals, and birds, particularly raptors which have relatively inefficient mechanism for eliminating these lipophilic contaminants (Ewins, 1994). Depletion of body fat during times of hunger could be expected to mobilize chlorinated hydrocarbons, providing additional stress when the birds are poorly equipped to cope with it (Te Luttik and Snoo, 2004).

Fish-easting birds appear to be vulnerable to contaminant effects because of biological magnification of contaminants in the aquatic chain. Measurable levels of organochlorines were found in aquatic birds from highly polluted areas in North America and in some remote areas (Bourne and Bogan, 1972; Ohlendorf *et al.,* 1974). Besides fish-eating birds, plankton and invertebrate feeding birds also accumulate pesticides through their respective food chains.

The concentrations of residues in the brain provide the most rigorous criteria for diagnosis of death due to these chemicals, and levels are generally similar across a wide range of species of birds and mammals. Residues in liver are closely correlated with recent dose, either from direct intake or from mobilization from storage, and so reflect hazardous exposure. Residues in the whole carcass show the storage reserve, and so indicate the potential for adverse effects from lethal mobilization or from the continuous slow mobilization that occurs during the normal processes of metabolism and excretion (Stickel, 1973).

Among birds with similar food habits, smaller individuals are likely to ingest larger amounts of pesticide residues. Age and body size of the species are also important in influencing the accumulation of pesticide residues. However, food habits and the food-chain concentration mechanism are more important in determining the total body burden of pesticide residues. The studies which began in 1960 on the White Pelican *Pelecanus erythrorhynchos* colonies at Lower Klamath and Clear lake Nation Wildlife Refuges (NWRS) when a die off of fish eating birds occurred concluded that run-off containing organochlorine pesticides from surrounding agricultural areas caused the moralities (Keith, 1966). Fulvous Whistling-Ducks *Dendrocygna bicolor* nesting in rice fields along the gulf coast of southeastern Texas suffered a major population decline in the late 1960s. The decline was attributed to mortality from dieldrin or aldrin (Flickinger and King, 1972). In 1974 the US EPA cancelled the use of aldrin as a treatment for rice seed.

The pesticide DDT and its breakdown product DDE gained notoriety about 40 years back in North America. Ratcliffe (1967) investigating the Peregrine Falcon *Falco peregrinus* and Sparrow Hawk *Accipiter brachyurus* in Britain, was the first to note a correlation between the magnitude of decrease in eggshell weights, decreases in breeding populations and exposure of populations of these species to persistent organic pesticides. Health *et al.*, (1969) showed experimentally that DDE impaired the reproductive performances. Later it was proved responsible for eggshell thinning that led to population crashes and reproductive impairments in many species of birds in North America (King *et al.*, 1978; Fleming *et al.*, 1984; Ohlendrof *et al.*, 1988).

DDT has been shown to have an estrogen-like activity and possible carcinogenic activity in humans (UNEP, 2002). DDT may be metabolized easily to DDE and DDD in the environment and its derivatives are more stable and persistent than the parent molecule. Concentration of DDE, total DDT and dieldrin in fat, uropygial gland and brain of Florida Turkey Vultures *Cathartes aura* and Black Vultures *Coragyps atratus* were low with DDE not exceeding 1 mg/kg in brain and 12 mg/kg in fat (Johnston, 1978). DDE concentration amounted to 50 and 12 mg/kg in muscles of two wild California condors that died in 1974 and 1976 (Wiemeyer *et al.*, 1983) respectively.

Combined DDT and DDE residues of 30 mg/kg represent the practical separation point between dead birds and survivors in laboratory studies (Stickel *et al.*, 1966, 1970). DDT and its metabolites show a consistent biomagnification in wild birds, presumably through the food-chain concentration mechanism. Flesh-eating birds had shown higher body burdens of DDT than non-flesh-eating ones. Relatively high levels of *p,p*-DDE were observed in different body organs of Cattle Egret *Bubulcus ibis*, compared with those present in other birds examined. More than 70% of total DDT was present as *p,p-* DDE in most body organs of pigeons.

Eight years after DDT was banned, Black-crowned Night-Herons *Nycticorax nycticorax* nesting in Washingtion, Oregon and Nevada were showing DDE related reproductive problems (Henny *et al.,* 1984). In an evaluation of residues trend at Oregon and Nevada, Black-crowned Night-Heron colonies showed DDE ranging from 0.31-15.6 mg/kg in 40 eggs. In White-faced Ibis *Plegadis chihi*, DDE was detected at levels as high as 20 mg/kg (Henny *et al.,* 1985). Of eleven female Tree Swallows *Tachycineta bicolor* collected from Denver, Colorado, five had carcass levels of DDE below 5 mg/kg. About 1,150 individuals of Passeriformes and other small birds were collected in Western U.S. for organochlorine residue analysis. Concentrations of HCH, DDE, heptachlor epoxide, dieldrin and toxaphene were more than 0.05 mg/kg of which DDE alone accounted for 72% of total organochlorine concentration (DeWeese *et al.,* 1986).

Nineteen of 38 White Pelicans *Pelecanus Onocrotalus* analysed from Klamath basin refuges, were suspected to have died from endrin or possible additive effects of endrin + dieldrin poisoning (Sileo *et al.,* 1977). The endrin residues exceeding 0.8 mg/kg level generally considered as lethal (Stickel *et al.,* 1979). Havera and Duzan (1986) reported that 5 of 57 birds of prey from Illinois, whose brains were analysed, had dieldrin levels that approached or exceeded the diagnostic lethal level of 4 to 5 mg/kg.

Heptachlor has been extensively used for seed treatment, which is reported to enter the raptor food chain through ingestion of heptachlor treated seeds by their prey. A nesting American Kestrel *Falco sparverius* in Columbia basin of Oregon is reported to have died in its nest with 28 mg/kg heptachlor epoxide in brain (Henny *et al.,* 1983). Heptachlor epoxide has been detected in eggs of raptors from Columbia basin of Oregon, which ranged as high as 4.75 mg/kg (Henny *et al.,* 1984). Residues of heptachlor epoxide in brain of moffitti found dead in 1978-79 equalled or exceeded the lethal hazard zone of 8-9 mg/kg in experimental passerine birds (Blus *et al.,* 1984). High heptachlor epoxide residues varying between 1.5 and 9.3 mg/kg were found in Teal and Pintail carcasses in Playa Lakes, Texas (Flickinger, 1987).

It is fact that problem to birds due to persistent chemicals with the introduction of fast degrading chemicals such as OP, carbamates and pyrethroids came down considerably, world over, specifically in the western countries. Several instance of poisoning in birds due to OP and carbamates have been reported in North America (Stickel *et al.*, 1969; Fleming *et al.*, 1984; Ambrose *et al.*, 1988; Custer *et al.*, 1990) and Europe (Moore, 1980; Dewit *et al.*, 2002).

### *3.2.2. Impact of pesticides on birds in India*

In India, unfortunately evaluation of the impact of pesticides on the ecosystem with special reference to birds has not been carried out systematically. However, limited studies carried out by various authors are mainly confined to very small number of individuals to a particular place and periods. Species of birds considered for the studies were largely opportunistic rather than organized collection. The results of such scattered studies in the Indian environment are briefly discussed below:

HCH and DDT residues of the internal body organs, depot fat and blood plasma of a few species of Indian wild birds from Lucknow, were estimated by Kaphalia *et al.*, (1981). High levels of DDT were detected in deport fat of Crow, Kite and Vulture (50.8, 67.0 and 95.3 mg/kg respectively). Total HCH detected in depot fat of Crow was 29.7 mg/kg. Comparatively lesser levels were found in vulture, kite and cattle egret. A single specimen of pigeon ovary examined contained 1.21 mg/kg total HCH and 0.31 mg/kg lindane. They further reported that within the same food habit group, smaller individuals are likely to ingest larger amounts of pesticide residues. HCH level in liver, lung and kidney were generally high in Pigeon and Crows and in the breast muscles and spleen of Vulture. Total HCH and $\gamma$ - HCH (lindane) levels were high in breast muscle, liver, heart and lung tissues of Pigeon, Crow and Vulture were compared with the respective tissues of Chicken, Cattle Egret and Kite.

A study by Misra (1989) in Mahala water reservoir with reference to its avifauna reported relatively higher levels of total organochlorine pesticide residues in brain tissues of Flamingo and Red-wattled Lapwing *Vanellus indicus* brain, than other tissues. Flamingo contained 7.39 $\mu g/g$ beta HCH and 7.46 $\mu g/g$ *p,p'*-DDE in brain while Red-wattled Lapwing had 3.45 $\mu g/g$ alfa HCH, 5.82 $\mu g/g$ gamma HCH and 3.66 $\mu g/g$ aldrin. Large-pied Wagtail *Motacilla maderaspatensis* had 5.42 $\mu g/g$ *p,p'*-DDE in the brains which are many times higher than the concentrations in other species of birds studied. Total DDT residues were 2.0, 5.42 and 6.34 $\mu g/g$ in brain of Black-winged Stilt *Himantopus himantopus*, Large-pied Wagtail and Flamingo respectively.

Muralidharan *et al.,* (1992) conducted a study on organochlorine residues in the eggs of selected colonial waterbirds breeding at Keoladeo National Park, Bharatpur. The study recorded higher concentration of dieldrin in eggs of Large Cormorant (1.54 mg/kg), Indian Shag (2.94 mg/kg), Darter (1.52 mg/kg), Grey Heron (5.95 mg/kg), Cattle Egret (2.52 mg/kg), Painted Stork (5.78 mg/kg) and Spoonbill (1.3 mg/kg). As the concentration in all the eggs was higher than one mg/kg, it was suggested that the concentration would have been associated with reproductive impairment (Stickel, 1973).

The breeding population of Sarus Crane in Keoladeo National Park in Rajasthan has come down to six pairs as against 27 pairs in 1973 (Walkinshaw, 1973). As diagnostic and circumstantial evidence prove the cause of dead of 18 Sarus Crane between 1987-1988 and 1989-1990 to be aldrin poisoning (Muralidharan, 1993), the reason for the decline in the breeding population become obvious. Brain tissue of Sarus Cranes, Collared Doves and Blue Rock Pigeons collected from Keoladeo National Park, Bharatpur showed an average of 19.33, 15.19 and 20.42 mg/kg of dieldrin respectively. Dieldrin in other tissues ranged from 0.78 mg/kg to 92.26 mg/kg in, Sarus Cranes, 3.44 mg/kg to 66.17 mg/kg in Collared Doves and 16.92 mg/kg to 20.99 mg/kg in Blue Rock Pigeons. Very high residues of aldrin in the gut

(89.75 mg/kg) and dieldrin at much higher quantities in the brain than the lethal level (4-5 mg/kg) clearly indicated that dieldrin after being metabolized from aldrin was responsible for the death of many Sarus Cranes and other granivores in Bharatpur (Muralidharan, 1993).

Himalayan Grey-headed fishing Eagle *Ichthyophaja nana* an endangered species has been breeding unsuccessfully for the last several years in Corbet National Park at Uttrakand. Detection of high levels of DDT, HCH, dieldrin, endosulfan and heptachlor in the unhatched egg (Rishad, 1995) makes the reason all the most strong.

Senthilkumar *et al.,* (1999, 2001) measured levels of pesticides, dioxins and Polychlorinated Biphenyls (PCBs) in a few species of birds. Tanabe *et al.* (1998) refered that generally the organochlorine contamination levels in individual species of birds vary with feeding habits and the residue pattern of organochlorine in most species of resident birds analyzed followed the order of HCHs > DDTs > PCBs. They also reported that the concentrations of HCHs in resident birds of South India were in the range of 14 – 8,800 ng/g followed by DDTs ranging from 0.3 to 3, 600 ng/g. Global comparison of organochlorine concentrations indicated that resident birds in India had the highest residues of HCHs and moderate to high residues of DDTs.

Organochlorine residues analyzed in the tissue of six species of birds collected from Nilgiri District, Tamil Nadu showed high levels of endosulfan residues in the tissues of Large Cormorant with values of 10.9, 126.8, 112.88, 217.2 and 233 mg/kg in brain liver, kidney, muscle and gut content respectively (Muralidharan and Murugavel, 2001). Death of 58 aquatic birds comprising six species had been recently reported in Okhla Bird Sanctuary, Uttar Pradesh (Gopi Sundar, 2006) due to suspected poisoning but the reason was not confirmed.

Muralidharan *et al.,* (2008) reported the levels of persistent organochlorine pesticide residues in tissues of Indian White-backed Vulture from different locations in India. The levels of DDT, HCH, dieldrin and endosulfan residues among other organochlorine detected in the tissues and eggs, none of them was indicative of either food chain buildup or poisoning leading to population decline. Organochlorine residues in the avifauna of Tamil Nadu (Southeast coast of India) (Sethuraman and Subramanian, 2003), agricultural chemicals on the Indian avifauna and varying concentration of organochlorine pesticide residues in various tissues of birds (Muralidharan, 2004; Muralidharan and Dhananjayan, 2007), and the information on the declining trend of House Sparrow (Vijayan, 2003) was reported all over India. Although, reductions in population of many species of birds have been reported in India, no study attempted to figure out if pesticides were responsible. However, the limited information and circumstantial evidences could point towards pesticides. Systematic studies on the impact of agricultural chemicals on any species of bird are absent.

## 3.3. Objectives

- To document the organochlorine pesticide (OCP) residues among various species of birds from Ahmedabad, Gujarat and Coimbatore, Tamil Nadu.
- To identify the inter specific and temporal variation in OCP levels among various groups of birds.
- To generate baseline information on pesticide residues among the birds.

## 3.4. Materials and Methods

*Study Sites:* Details about the study area are given in chapter 2.

### *3.4.1. Pesticide consumption in Tamil Nadu*

Tamil Nadu is one among the four major states which utilize about 48.8% of the total pesticide consumption in India. In recent years, consumption of pesticides in Tamil Nadu has seen a downward trend; 75,000 mt in 1991-1992 to around 37,959 mt in 2006-2007. This is due to popularization of Integrated Pest Management approach and other techniques (Pesticide Monitoring Unit 2008). Although the consumption of pesticides reduced across the country, Tamil Nadu seems to be stable in the consumption pattern. The consumption was 3324 mt in 2003-2004 and declined during 2003-2004.

### *3.4.2. Pesticide consumption in Gujarat*

Paddy cultivation is very high in Gujarat occupying about 5% of the gross cropped area (7.5 to 8.0 lakhs ha). About 80% of the area is confined to south and central Gujarat, comprising the districts of Kheda, Surat, Valsad, Panchmahal, Ahmedabad and Vadodara with an average production of 2.5tons/ha of transplanted paddy (Korat and Mehta, 1996). Gujarat stood fourth in the quantity of pesticide use (1.48 kg/ha) (NCAER, 1967) with the total consumption rate of 2900 mt during 2004-2005 (NCIPM database) in the state is Rich farmers with large land holdings are of the notion that heavy use of pesticides can prevent pest attacks and increase crop yield.

### *3.4.3. Sample collection and transportation*

Dead birds were collected from Ahmedabad, Gujarat and in and around Coimbatore, Tamil Nadu between 2003 and 2007 are given in the tables (Table 3.1 and Table 3.2). Field biologists, forest officials, keen bird watches and farmers were requested to send the samples whenever they come across any dead bird. In the events of any mass mortality of birds or spillage of contaminants due to accidents which caused mortality of birds, exclusive visits wee made to study the same on opportunistic sampling basis. Based on the circumstantial evidence and symptoms, dead birds collected were double-bagged and preserved, and sent to laboratory at SACON by air or courier without delay for contaminant analysis.

### *3.4.4. Sample processing*

All the birds were subjected to post-mortem examination and organ, namely liver, brain and muscle were dissected out using acid/acetone washed dissection tools and sterile scalpel. Gut contents were considered, if available.

**Table 3.1.** Birds collected dead from Ahmedabad and Coimbatore for organochlorine residues analysis (2003 -2005).

| S.No. | Species | Scientific Name | Food habits | Ahmedabad | Coimbatore | Total |
|---|---|---|---|---|---|---|
| 1 | Little Cormorant | *Phalacrocorax niger* | Piscivore | NA | 8 | 8 |
| 2 | Grey Heron | *Ardea cinerea* | Piscivore | NA | 1 | 1 |
| 3 | Indian Pond-Heron | *Ardeola grayii* | Insectivor | NA | 12 | 12 |
| 4 | Cattle Egret | *Bubulcus ibis* | Insectivore | 4 | 5 | 9 |
| 5 | Median Egret | *Mesophoyx intermedia* | Carnivore | NA | 1 | 1 |
| 6 | Little Egret | *Egretta garzetta* | Carnivore | 4 | 6 | 10 |
| 7 | Black-crowned Night-Heron | *Nycticorax nycticorax* | Carnivore | NA | 1 | 1 |
| 8 | Painted Stork | *Mycteria leucocephala* | Piscivore | 1 | NA | 1 |
| 9 | Asian Openbill | *Anastomus oscitans* | Piscivore | 1 | NA | 1 |
| 10 | White Ibis | *Threskiornis melanocephalus* | Carnivore | 1 | NA | 1 |
| 11 | Black Ibis | *Pseudibis papillosa* | Carnivore | 3 | NA | 3 |
| 12 | Bar-headed Goose | *Anser indicus* | Herbivore | NA | 1 | 1 |
| 13 | Pariah Kite | *Milvus migrans govinda* | Omnivore | 62 | 3 | 65 |
| 14 | Shikra | *Accipiter badius* | Carnivore | 5 | 5 | 10 |
| 15 | Besra Sparrow-hawk | *Accipiter virgatus* | Carnivore | 5 | 3 | 8 |
| 16 | Indian White-backed Vulture | *Gyps bengalensis* | Carnivore | 13 | NA | 13 |
| 17 | Common Kestrel | *Falco tinnunculus* | Insectivore | 1 | NA | 1 |
| 18 | Grey Partridge | *Francolinus pondicerianus* | Granivore | NA | 11 | 11 |
| 19 | Common Quail | *Coturnix coturnix* | Omnivore | NA | 6 | 6 |
| 20 | Indian Peafowl | *Pavo cristatus* | Omnivore | 5 | 13 | 18 |
| 21 | Sarus Crane | *Grus antigone* | Omnivore | 1 | NA | 1 |
| 22 | Common Coot | *Fulica atra* | Herbivore | NA | 3 | 3 |
| 23 | Blue Rock Pigeon | *Columba livia* | Granivore | 70 | 7 | 77 |
| 24 | Nilgiri Wood Pigeon | *Columba elphinstonii* | Granivore | NA | 1 | 1 |
| 25 | Indian Ring Dove | *Streptopelia decaocto* | Granivore | 8 | NA | 8 |
| 26 | Spotted Dove | *Streptopelia chinensis* | Granivore | NA | 8 | 8 |
| 27 | Little-brown Dove | *Streptopelia senegalensis* | Granivore | NA | 1 | 1 |

**Table 3.1** *Continued...*

| | | | | | | |
|---|---|---|---|---|---|---|
| 28 | Rose-ringed Parakeet | *Psittacula krameri* | Frugivore | 3 | 4 | 7 |
| 29 | Asian Koel | *Eudynamys scolopacea* | Frugivore | 7 | 15 | 22 |
| 30 | Small Green-billed Malkoha | *Phaenicophaeus viridirostris* | Insectivore | NA | 16 | 16 |
| 31 | Crow Pheasant | *Centropus sinensis* | Carnivore | NA | 11 | 11 |
| 32 | Barn Owl | *Tyto alba* | Carnivore | 5 | 1 | 6 |
| 33 | Collared Scops Owl | *Otus bakkamoena* | Carnivore | NA | 3 | 3 |
| 34 | Spotted Owlet | *Athene brama* | Insectivore | 3 | 3 | 6 |
| 35 | Long-tailed Nightjar | *Caprimulgus macrurus* | Insectivore | NA | 1 | 1 |
| 36 | Common Indian Nightjar | *Caprimulgus asiaticus* | Insectivore | NA | 3 | 3 |
| 37 | White-breasted Kingfisher | *Halcyon smyrnensis* | Piscivore | NA | 3 | 3 |
| 38 | Indian Roller | *Coracias benghalensis* | Insectivore | 3 | 1 | 4 |
| 39 | Indian Pitta | *Pitta brachyura* | Insectivore | NA | 5 | 5 |
| 40 | White-bellied Drongo | *Dicrurus caerulescens* | Carnivore | NA | 1 | 1 |
| 41 | Common Myna | *Acridotheres tristis* | Omnivore | 6 | 12 | 18 |
| 42 | House Crow | *Corvus splendens* | Omnivore | 6 | 20 | 26 |
| 43 | Jungle Crow | *Corvus macrorhynchos* | Omnivore | NA | 11 | 11 |
| 44 | Red-whiskered Bulbul | *Pycnonotus jocosus* | Frugivore | NA | 6 | 6 |
| 45 | Red-vented Bulbul | *Pycnonotus cafer* | Frugivore | NA | 10 | 10 |
| 46 | White-browed Bulbul | *Pycnonotus luteolus* | Frugivore | NA | 3 | 3 |
| 47 | Blyth's Reed Warbler | *Acrocephalus dumetorum* | Insectivore | NA | 3 | 3 |
| 48 | White-headed Babbler | *Turdoides affinis* | Insectivore | NA | 27 | 27 |
| 49 | Magpie Robin | *Copsychus saularis* | Insectivore | NA | 4 | 4 |
| 50 | Indian Robin | *Saxicoloides fulicata* | Insectivore | NA | 6 | 6 |
| 51 | Nilgiri Pipit | *Anthus nilghiriensis* | Insectivore | NA | 1 | 1 |
| 52 | House Sparrow | *Passer domesticus* | Granivore | NA | 13 | 13 |
| 53 | Baya Weaver | *Ploceus philippinus* | Granivore | NA | 2 | 2 |
| 54 | Spotted Munia | *Lonchura punctulata* | Granivore | NA | 6 | 6 |
| | **Total** | | | **220** | **284** | **504** |

NA- Not Available

**Table 3.2.** Number of species, individuals and tissues included for organochlorine pesticide residue analysis between 2003 and 2007.

| Year | Ahmedabad | Tamil Nadu | Total |
|---|---|---|---|
| 2003 | 22 | 46 | 68 |
| 2004 | 18 | 40 | 58 |
| 2005 | 58 | 64 | 122 |
| 2006 | 62 | 61 | 123 |
| 2007 | 60 | 73 | 133 |
| Birds (individuals) | 220 | 284 | 504 |
| Species* | 22 | 46 | 54* |
| Tissues # | 682 | 852 | 1534 |

* Cumulative number, # = brain, liver, muscle, gut content and fat.

About 10 g of the sample was ground with anhydrous sodium sulphate (Qualigents) and the mixture was packed in a chromatograph grade thimble (Whatman No.1) and desiccated overnight prior to extraction to remove moisture. The desiccated thimble was loaded in a soxhlet extractor and extracted with 1:1 ratio of 250 ml of pesticide grade hexane and dichloromethane (Merck) for 6 hrs. The extract was condensed in a rotary flask evaporator to a specific aliquot (about 5 ml) and placed on a glass column packed with silica gel (60-120 mesh) and eluted with hexane and dichloromethane in two fractions. The first fraction was eluted with 100 ml hexane for PCB residues (dicussed in chapter 4) and second fraction with 3:1 ration of hexane and dichloromethane for organochlorine pesticides. The eluate was condensed in a rotary flask evaporator and stored in deep freezer at -20°C untill analysis in Gas Chromatograph (El Nemr *et al.*, 2003).

Extracts with high fat contents were subjected to sulphuric acid digestion. The digested extracts were evaporated to a volume of about 10-15 ml and transferred to a 50-100 ml separating funnel. Then 5 ml of sulphuric acid was added drop by drop and washed with 20-30 ml of aqueous sodium bicarbonate solution (1%). This procedure was repeated until the extract became colorless with a pH of 7. The extract was filtered, and the filtrate was evaporated with rotary flask evaporator to almost dryness and residues of organochlorines were reconstituted with 1 ml of hexane and stored for residue analysis.

Gut contents of the birds were homogenized in 40 ml vials. 20 ml of Acetone: Water: Phosphoric acid (98:1:1 v/v/v) was added to each sample after homogenization. The samples were shaken for 30 minutes and centrifuged for 10 minutes at 1200 rpm. A 10 ml aliquot of each samples was transferred to a 15 ml of water: phosphoric acid (99:1: v/v) and 2 ml of hexane and the samples were shaken by hand for 1 minute and then centrifuged for 5 minutes at approximately 1200 rpm. The supernatant was diluted with hexane and the samples were stored at – 20°C untill analysis in gas chromatography.

### 3.4.5. Chemical analysis

Quantification of organochlorine pesticide residues were done with Hewlett-Packard 5890 Series II Gas Chromatograph equipped with $Ni^{63}$ Electron Capture Detector (ECD) and DB-608 fused silica capillary column (30 m x 0.32 mm x 0.5 $\mu$m) coated with 35% phenyl methyl polysiloxane. Chromatographic conditions were as follow: detector 300°C; injector 250°C; oven temperature was programmed as 180°C -3min; 4°C /min-260°C -15 min. All the samples were analysed for alpha-hexachlorocyclohexane ($\alpha$-HCH), $\beta$-HCH, $\delta$-HCH, lindane ($\gamma$-HCH), heptachlor epoxide (HE), dieldrin, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, $\alpha$-endosulfan, $\beta$-endosulfan and endosulfan sulfate. An equivalent mixture provided by Dr. Ehrenstorfer Laboratories (Germany) was used as standard. The concentrations of the individual compounds were quantified from the peak area of the samples to that of the corresponding external standard.

Recoveries of the compounds from fortified samples (50 ng/g) ranged from 91 to 102% and the results were not corrected for per cent recovery and the results were expressed in wet weight basis. 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 analysed was 1 ng/g.

and one random sample in duplicate. The minimum detection limit for all the compounds analysed was 1 ng/g.

### 3.4.6. Statistical analysis

Prior to comparison of the means, a value one-half the limit of detection was assigned to samples with below detectable concentrations of contaminants if detectable quantities were found in at least half of the samples as followed by Anthony *et al.,* (1999). Ranges were compiled from minimum and maximum values for levels detected in individuals for each toxicant included in the study. Due to skewed distributions, all the contaminant concentrations were $\log_{10}$-transformed to satisfy the homogeneity of variance assumption, of analysis of variance (Hernandez *et al.,* 1993) and tested using general linear model (GLM) procedure. One, two or three way analysis of variance (ANOVA) was carried out in order to avoid confounding affects of factors. When there were significant differences, the Tukey's multiple comparison procedure was used to determine significance among means (Custer and Custer, 1995). Student *t*-test was used to compare the residue levels between male and female of each species. The probability level determining significance was $P < 0.05$ for all statistical tests. Pearson's correlation coefficient was used to determine the relation among residues. All these calculations were performed using statistical software, SPSS student version 10.

## 3.5. Results

Among the various organochlorines tested, isomers of HCH and metabolites of DDT dominated the total OCPs followed by heptachlor epoxide. Over all $\beta$-HCH accounted for 44% of total HCH and $\gamma$-, $\alpha$- and $\delta$-HCH contributed 29%, 17% and 10% respectively. Of the various metabolites of *p,p'*-DDT, the *p,p'*-DDE dominated (63%) the total DDT indicating its higher persistency in tissues of birds.

### *3.5.1. Variation among tissues and between sexes*

For multiple comparison of residue data among birds, all the 54 species were grouped into six categories, namely frugivores, granivores, carnivores, insectivores, omnivores and piscivores based on their feeding habits. The variation in organochlorine pesticide residues among the tissues were not statistically significant ($P = 0.08$). However, the maximum accumulation of organochlorine residues was detected in fat followed by liver and minimum in gut content (Figure 3.1 a, b).

Sex differences were not significantly varied among birds ($t$-test, $P > 0.05$). Hence, birds were grouped on the basis of food habits to know the difference in OCPs between sexes (Figure 3.2). However, the sex differences were not statistically significant among the birds with various food habits (Two way ANOVA, $P = 0.784$) except frugivorous birds. The mean total OCPs were lower in females than males in frugivorous and Carnivorous birds, while males of all other categories showed higher concentration (Figure 3.2). Several reports suggest comparatively greater concentrations in females than males (Lemmetyinen *et al.,* 1982; Elliott and Shutt, 1993). The reduction of organochlorine residues in females suggests excretion through egg laying.

### *3.5.2. Variation in OCP residues among species*

Organochlorine pesticide residues determined for all the species of birds collected between 2003 and 2007 are tabulated (Table 3.3). The maximum load of organochlorine pesticides was recorded in carnivorous birds. The minimum contamination load was observed in granivorous birds. Significant variation in HCH residues were observed among various species of birds studied ($P < 0.05$). Comparatively higher load of total HCH (> 2000 ng/g) was recorded in Nilgiri Wood Pigeon, Blyth's Reed Warbler, Indian Robin, Collared Scops Owl and White-headed Babbler (Figure 3.3).

**Table 3.3.** Organochlorine pesticide residues (mean ng/g, wet wt) in various species of birds collected from Ahmedabad and Coimbatore between 2003 and 2007 (Range in parentheses).

| Species | N | $\alpha$-HCH | $\beta$-HCH | $\gamma$-HCH | $\delta$-HCH | DDE | DDD | DDT | HE | Dieldrin | Σ-endosulfan |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Little Cormorant | 8 | 75.8 (<DL-1527) | 207 (<DL-2123) | 108 (<DL-1120) | 68.1 (<DL-788) | 12.9 (<DL-147) | 5.07 (<DL-59.3) | 13.7 (<DL-102) | 165 (<DL-2400) | 8.24 (<DL-95) | 96.1 (<DL-845) |
| Grey Heron | 1 | 36.1 | 126 | 123 | 72.9 | 17.5 | 53.3 | 341 | 106 | 1220 | 287 |
| Indian Pond-Heron | 12 | 41.6 (<DL-457) | 306 (<DL-2769) | 296 (<DL-3808) | 134 (<DL-969) | 20.7 (<DL-150) | 15.2 (<DL-171) | 24.3 (<DL-201) | 211 (<DL-2264) | 44.9 (<DL-390) | 85.8 (<DL-441) |
| Cattle Egret | 9 | 252 (<DL-4826) | 211 (<DL-3075) | 39.9 (<DL-571) | 11.5 (<DL-109) | 130 (<DL-2391) | 9.3 (<DL-106) | 157 (<DL-3260) | 185 (<DL-3357) | 16.5 (<DL-100) | 198 (<DL-1751) |
| Median Egret | 1 | 83.4 | 279 | 218 | 61.1 | 4.31 | 77.7 | 182 | 241 | 492 | 300 |
| Little Egret | 10 | 67.3 (<DL-528) | 500 (<DL-5021) | 284 (<DL-2476) | 55.1 (<DL-463) | 50.2 (<DL-340) | 7.81 (<DL-46.3) | 34.9 (<DL-178) | 177 (<DL-1615) | 27.9 (<DL-317) | 69.7 (<DL-571) |
| Black-crowned Night-Heron | 1 | <DL | <DL | 10.3 | <DL | <DL | 14.7 | <DL | <DL | 33.3 | 86.2 |
| Painted Stork | 1 | 1.05 | 2.11 | <DL | <DL | 4.65 | 1.99 | <DL | 9.75 | 5.01 | 1.08 |
| Asian Openbill | 1 | 6.03 | <DL | <DL | <DL | 61.1 | <DL | 4.75 | 1.34 | <DL | 1.64 |
| White Ibis | 1 | 1.33 | 5.33 | 5.5 | 9.01 | 4.83 | <DL | <DL | 8.33 | 20.7 | 3.01 |
| Black Ibis | 3 | 11.1 (<DL-49.2) | 83.4 (1-313) | 4.82 (<DL-15.6) | 17.8 (1.2-39.4) | 241 (<DL-855) | 5.83 (1.95-22.3) | 30.2 (<DL-138) | 25.2 (3.48-88.6) | 9.34 (<DL-25.8) | 203 (3.5-600) |

**Table 3.3.** *Continued...*

| Species | N | $\alpha$-HCH | $\beta$-HCH | $\gamma$-HCH | $\delta$-HCH | DDE | DDD | DDT | HE | Dieldrin | $\Sigma$-endosulfan |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bar-headed Goose | 1 | <DL | <DL | 1.75 | 1.98 | <DL | <DL | 1.75 | <DL | <DL | <DL |
| Pariah Kite | 65 | 162 (<DL-5183) | 102 (<DL-3079) | 68.3 (<DL-5316) | 12.3 (<DL-384) | 233 (<DL-6252) | 69.2 (<DL-1868) | 181 (<DL-8806) | 112 (<DL-2707) | 65.9 (<DL-1662) | 305 (<DL-4958) |
| Shikra | 10 | 265 (<DL-1560) | 1115 (<DL-6269) | 958 (<DL-5661) | 314 (<DL-1301) | 4667 (<DL-7114) | 23.3 (<DL-139) | 52.1 (<DL-534) | 834 (<DL-3646) | 295 (<DL-4550) | 101 (<DL-330) |
| Besra Sparrow-hawk | 8 | 134 (<DL-545) | 670 (<DL-2743) | 60.7 (<DL-247) | 142 (<DL-549) | 110 (<DL-410) | 2.31 (<DL-6.54) | 7.51 (<DL-30.1) | 193 (<DL-890) | 32.3 (<DL-146) | 276 (<DL-1324) |
| Indian White-backed Vulture | 13 | 54.6 (<DL-461) | 284 (<DL-3503) | 299 (<DL-3163) | 62.8 (<DL-381) | 126 (<DL-1760) | 75.9 (<DL-1373) | 45.8 (<DL-437) | 166 (<DL-1206) | 34.3 (<DL-769) | 149 (<DL-2009) |
| Common Kestrel | 1 | 1.01 | <DL | 1.36 | 5.01 | 2.31 | 3.71 | <DL | 12.02 | <DL | 3.28 |
| Grey Partridge | 11 | 14.9 (<DL-264) | 17.1 (<DL-107) | 15.6 (<DL-134) | 8.53 (<DL-54.4) | 7.61 (<DL-107) | 67.6 (<DL-465) | 13.6 (<DL-92.5) | 66.4 (<DL-926) | 10.5 (<DL-97.1) | 34.2 (<DL-263) |
| Common Quail | 6 | 540 (3.4-2731) | 762 (<DL-6741) | 143 (3.84-775) | 139 (<DL-1146) | 93.8 (<DL-701) | <DL | 16.9 (<DL-180) | 234 (<DL-1889) | 17.4 (<DL-151) | 116 (<DL-734) |
| Indian Peafowl | 18 | 148 (<DL-1248) | 378 (<DL-8310) | 208 (<DL-4139) | 29.3 (<DL-487) | 205 (<DL-4932) | 12.8 (<DL-126) | 62.8 (<DL-1028) | 106 (<DL-2637) | 67.2 (<DL-1624) | 332 (<DL-4707) |
| Sarus Crane | 1 | <DL | 16.8 | <DL | <DL | <DL | <DL | <DL | 19.3 | 2.75 | 77.8 |

**Table 3.3.** *Continued...*

| Species | N | $\alpha$-HCH | $\beta$-HCH | $\gamma$-HCH | $\delta$-HCH | DDE | DDD | DDT | HE | Dieldrin | Σ-endosulfan |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Common Coot | 3 | 3.25 (<DL-17) | 2.51 (<DL-12) | 10.8 (<DL-43) | 2.17 (<DL-10) | 51.8 (<DL-287) | 3.17 (<DL-14.4) | 1.08 (<DL-2.07) | 32.9 (<DL-149) | 7.33 (<DL-24.6) | 429 (<DL-2488) |
| Blue Rock Pigeon | 79 | 73.5 (<DL-3059) | 72.4 (<DL-2764) | 41.5 (<DL-2999) | 14.7 (<DL-456) | 69.8 (<DL-384) | 18.1 (<DL-197) | 141 (<DL-1254) | 67.4 (<DL-294) | 25.6 (<DL-169) | 185 (<DL-456) |
| Nilgiri Wood Pigeon | 1 | 813 | 1872 | 2597 | 724 | 81.1 | 4.75 | 56.3 | 1729 | 63.4 | 722 |
| Indian Ring Dove | 8 | 276 (<DL-2272) | 253 (<DL-1393) | 31.3 (<DL-191) | 30.1 (<DL-127) | 156 (<DL-1323) | 60.7 (<DL-486) | 84.3 (<DL-622) | 25.6 (<DL-60.2) | 12.8 (<DL-67) | 33.4 (<DL-120) |
| Spotted Dove | 8 | 29.8 (<DL-114) | 919 (<DL-3653) | 1066 (<DL-4224) | 128 (<DL-506) | 121 (<DL-423) | <DL | 3.75 (<DL-12.5) | 458 (<DL-1801) | 6.63 (<DL-25.4) | 76.8 (<DL-303) |
| Little-brown Dove | 1 | 2.01 | 1.5 | 10.4 | <DL | <DL | <DL | <DL | 14.5 | 3.4 | 5.24 |
| Rose-ringed Parakeet | 7 | 173 (2.09-643) | 46.9 (<DL-115) | 298 (10.1-1010) | 115 (<DL-447) | 112 (2.25-416) | 1.5 (<DL-3.23) | 1.63 (<DL-5.49) | 14.9 (<DL-33) | 6.13 (<DL-14.8) | 7.88 (<DL-14.2) |
| Asian Koel | 22 | 165 (<DL-3417) | 568 (<DL-7232) | 217 (<DL-3792) | 101 (<DL-2006) | 291 (<DL-7267) | 24.1 (<DL-551) | 56.9 (<DL-907) | 254 (<DL-5297) | 25.3 (<DL-433) | 156 (<DL-3062) |
| Small Green-billed Malkoha | 16 | 355 (<DL-2353) | 631 (<DL-5724) | 604 (<DL-3491) | 85.8 (<DL-783) | 93.5 (<DL-1367) | 15.5 (<DL-182) | 351 (<DL-3249) | 131 (<DL-952) | 33.8 (<DL-190) | 177 (<DL-734) |
| Crow Pheasant | 11 | 40.2 (<DL-283) | 100 (<DL-672) | 184 (<DL-2126) | 11.2 (<DL-55) | 28.8 (<DL-211) | 5.34 (<DL-35.2) | 123 (<DL-2128) | 58.6 (<DL-409) | 21.9 (<DL-141) | 225 (<DL-1727) |
| Barn Owl | 6 | 411 (<DL-2757) | 337 (1.96-2315) | 67.1 (<DL-336) | 45.3 (<DL-225) | 23.7 (<DL-94.2) | 9.91 (<DL-32) | 115 (<DL-314) | 60.2 (4.05-156) | 134 (45-276) | 165 (<DL-997) |

**Table 3.3.** *Continued...*

| Species | N | $\alpha$-HCH | $\beta$-HCH | $\gamma$-HCH | $\delta$-HCH | DDE | DDD | DDT | HE | Dieldrin | $\Sigma$-endosulfan |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Collared Scops Owl | 3 | 961 (<DL-2533) | 1932 (<DL-3082) | 269 (<DL-1074) | 233 (<DL-375) | 151 (<DL-453) | 78.8 (<DL-202) | 34.9 (<DL-138) | 458 (<DL-767) | <DL | 142 (<DL-428) |
| Spotted Owlet | 6 | 506 (3.23-2102) | 236 (6.98-784) | 11.8 (<DL-57.3) | 8.06 (<DL-36.7) | 8.31 (<DL-19.5) | 5.63 (<DL-23.7) | 38.6 (<DL-232) | 70.8 (<DL-237) | 1.88 (<DL-11.1) | 64.6 (4-353) |
| Long-tailed Nightjar | 1 | <DL | <DL | 17.5 | 8.87 | 2579 | 43.5 | 36.2 | 33.8 | 23.3 | 76.1 |
| Common Indian NightJar | 3 | 327 (<DL-964) | 520 (<DL-2478) | 46.8 (<DL-321) | 932 (<DL-3448) | 92.6 (<DL-391) | 26.1 (<DL-117) | 29.1 (<DL-201) | 182 (<DL-683) | 12.9 (<DL-81) | 59.1 (<DL-317) |
| White-breasted Kingfisher | 3 | 25.5 (<DL-80.2) | 190 (<DL-1323) | 176 (<DL-1081) | 54.7 (<DL-214) | 98.4 (<DL-574) | 67.2 (<DL-230) | 146 (<DL-623) | 149 (<DL-781) | 29.4 (<DL-115) | 379 (<DL-1199) |
| Indian Roller | 3 | 4.88 (<DL-18.9) | 99.4 (<DL-248) | 15.8 (<DL-53.8) | 49.9 (<DL-163) | 7.38 (<DL-15.5) | 9.63 (<DL-37.4) | <DL | 57.6 (<DL-174) | 53.4 (<DL-169) | 35.1 (<DL-106) |
| Indian Pitta | 5 | 31.7 (<DL-330) | 174 (<DL-2377) | 571 (<DL-7504) | 140 (<DL-1363) | 35.6 (<DL-330) | 23.4 (<DL-191) | 80.7 (<DL-576) | 67.5 (<DL-715) | 28 (<DL-141) | 205 (<DL-2432) |
| White-bellied Drongo | 1 | 2.17 | 25.2 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL |
| Common Myna | 18 | 114 (<DL-1328) | 864 (<DL-18952) | 391 (<DL-6956) | 88.1 (<DL-1163) | 170 (<DL-3310) | 24.3 (<DL-508) | 67.2 (<DL-1317) | 273 (<DL-3626) | 40.3 (<DL-1070) | 175 (<DL-2030) |
| House Crow | 27 | 26.7 (<DL-369) | 144 (<DL-2813) | 84.9 (<DL-2317) | 56.7 (<DL-1249) | 79 (<DL-925) | 55.5 (<DL-1249) | 29.2 (<DL-194) | 98.5 (<DL-2089) | 45.2 (<DL-1321) | 125 (<DL-2505) |
| Jungle Crow | 11 | 30.1 (<DL-392) | 55.9 (<DL-474) | 34.8 (<DL-249) | 72 (<DL-843) | 19.9 (<DL-137) | 11.1 (<DL-85.3) | 119 (<DL-650) | 49.1 (<DL-422) | 15.2 (<DL-130) | 56 (<DL-485) |

**Table 3.3.** *Continued...*

| Species | N | $\alpha$-HCH | $\beta$-HCH | $\gamma$-HCH | $\delta$-HCH | DDE | DDD | DDT | HE | Dieldrin | $\sum$-endosulfan |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Red-whiskered Bulbul | 6 | 982 (<DL-3328) | 377 (<DL-1301) | 434 (<DL-1479) | 43.8 (<DL-120) | 125 (<DL-478) | 8.92 (<DL-21.4) | 369 (<DL-1153) | 222 (<DL-697) | 14.3 (<DL-48.6) | 164 (<DL-584) |
| Red-vented Bulbul | 10 | 107 (<DL-673) | 817 (<DL-7648) | 654 (<DL-4905) | 136 (<DL-986) | 20.1 (<DL-124) | 3.19 (<DL-42.2) | 50.1 (<DL-713) | 462 (<DL-3836) | 8.95 (<DL-59.9) | 75.7 (<DL-575) |
| White-browed Bulbul | 3 | 50.3 (<DL-104) | 94 (<DL-301) | 43 (<DL-107) | 39 (<DL-144) | 48.5 (<DL-190) | 27 (<DL-68.8) | 48.8 (<DL-181) | 32.4 (<DL-98) | 35.6 (<DL-93.5) | 256 (1.96-417) |
| Blyth's reed Warbler | 3 | 248 (<DL-590) | 2783 (24-10227) | 685 (<DL-2839) | 316 (<DL-848) | 35 (<DL-110) | 12.8 (<DL-62.2) | 71.5 (<DL-204) | 1563 (1-4911) | 36.1 (<DL-90.4) | 712 (<DL-3072) |
| White-headed Babbler | 27 | 445 (<DL-2332) | 1170 (<DL-5801) | 844 (<DL-5015) | 282 (<DL-1587) | 488 (<DL-1215) | 38 (<DL-675) | 229 (<DL-2609) | 678 (<DL-4412) | 71.7 (<DL-1459) | 316 (<DL-1440) |
| Magpie Robin | 4 | 112 (2-400) | 177 (<DL-669) | 1.38 (<DL-4.24). | 108 (<DL-292) | 14.3 (<DL-31.7) | 4.9 (<DL-81.4) | 70.9 (<DL-227) | 73 (<DL-200) | 51 (<DL-178) | 81.5 (<DL-182) |
| Indian Robin | 6 | 268 (<DL-935) | 1324 (<DL-6450) | 1403 (<DL-5470) | 430 (<DL-2021) | 89 (<DL-624) | 4.8 (<DL-25.2) | 139 (<DL-990) | 1504 (<DL-5520) | <DL | 85 (<DL-226) |
| Nilgiri Pipit | 1 | 68.7 | 644 | <DL | <DL | <DL | <DL | <DL | <DL | <DL | <DL |
| House Sparrow | 13 | 26.6 (<DL-316) | 67.9 (<DL-429) | 49.6 (<DL-678) | 23.9 (<DL-160) | 39.5 (<DL-323) | 1.05 (<DL-3.95) | <DL | 29.5 (<DL-168) | 28.6 (<DL-367) | 29.9 (<DL-337) |
| Baya Weaver | 2 | 25.8 (<DL-51.6) | 4.75 (<DL-9.5) | 137 (3.99-270) | 17.8 (<DL-35.6) | 8.01 (7.98-8.04) | 56 (7-105) | 101 (92-109) | 19.5 (16-23) | 7.25 (<DL-14.5) | 9.75 (<DL-19.5) |
| Spotted Munia | 6 | 27.2 (<DL-68.1) | 185 (<DL-362) | 43.1 (<DL-111) | 54.3 (<DL-173) | 25.8 (<DL-137) | 16.1 (<DL-125) | <DL | 97.8 (<DL-462) | 20.3 (<DL-157) | 35 (<DL-276) |

HE= heptachlor epoxide; $\sum$-endosulfan= $\alpha$- endosulfan + $\beta$-endosulfan + endosulfan sulfate; < DL- below detection limits (1 ng/g).

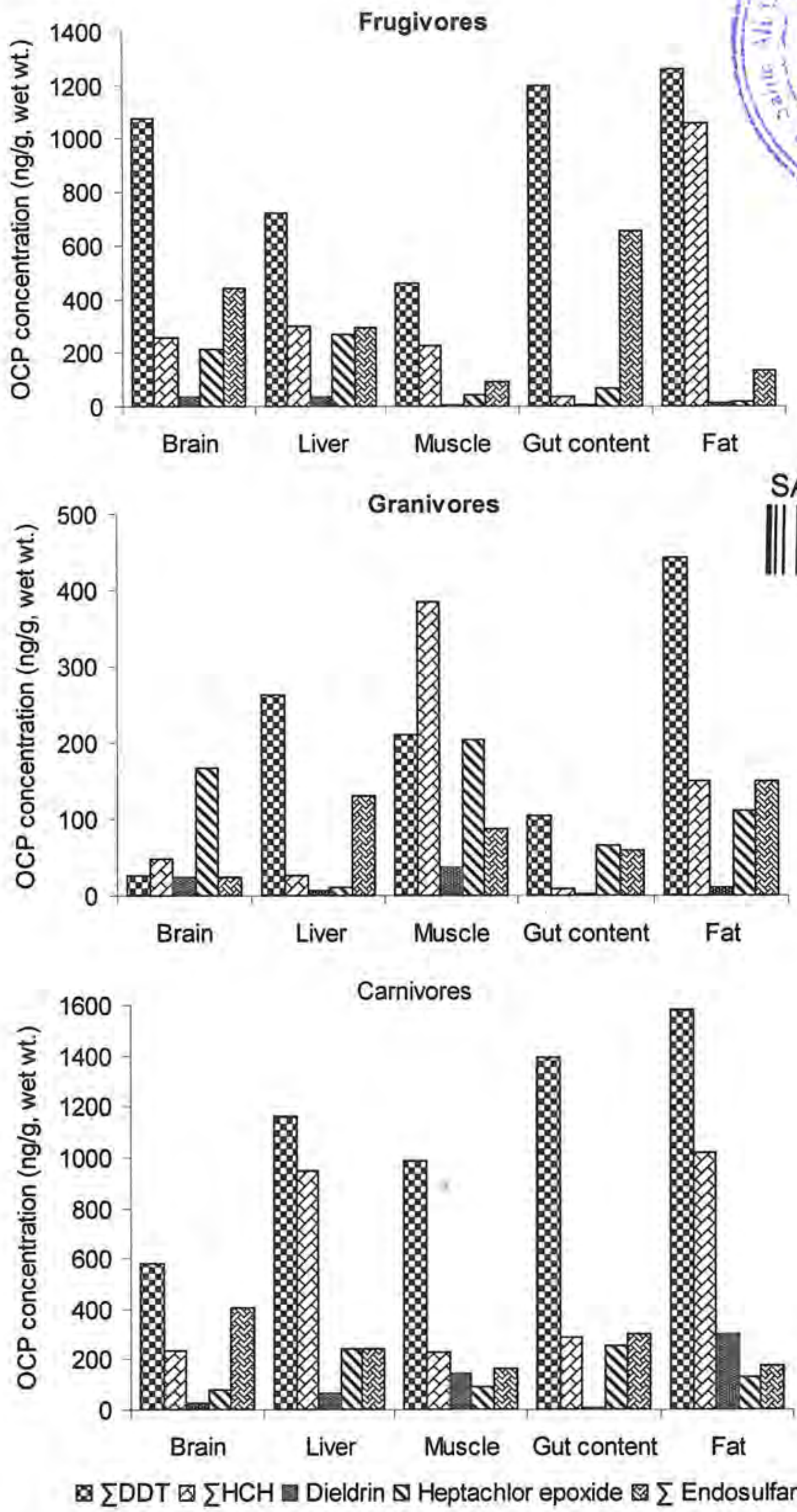



**Figure 3.1a.** Total organochlorine residues among various tissues of birds with different food habits (B- Brain, L- Liver, M-Muscle, G-Gut content, F-Fat).

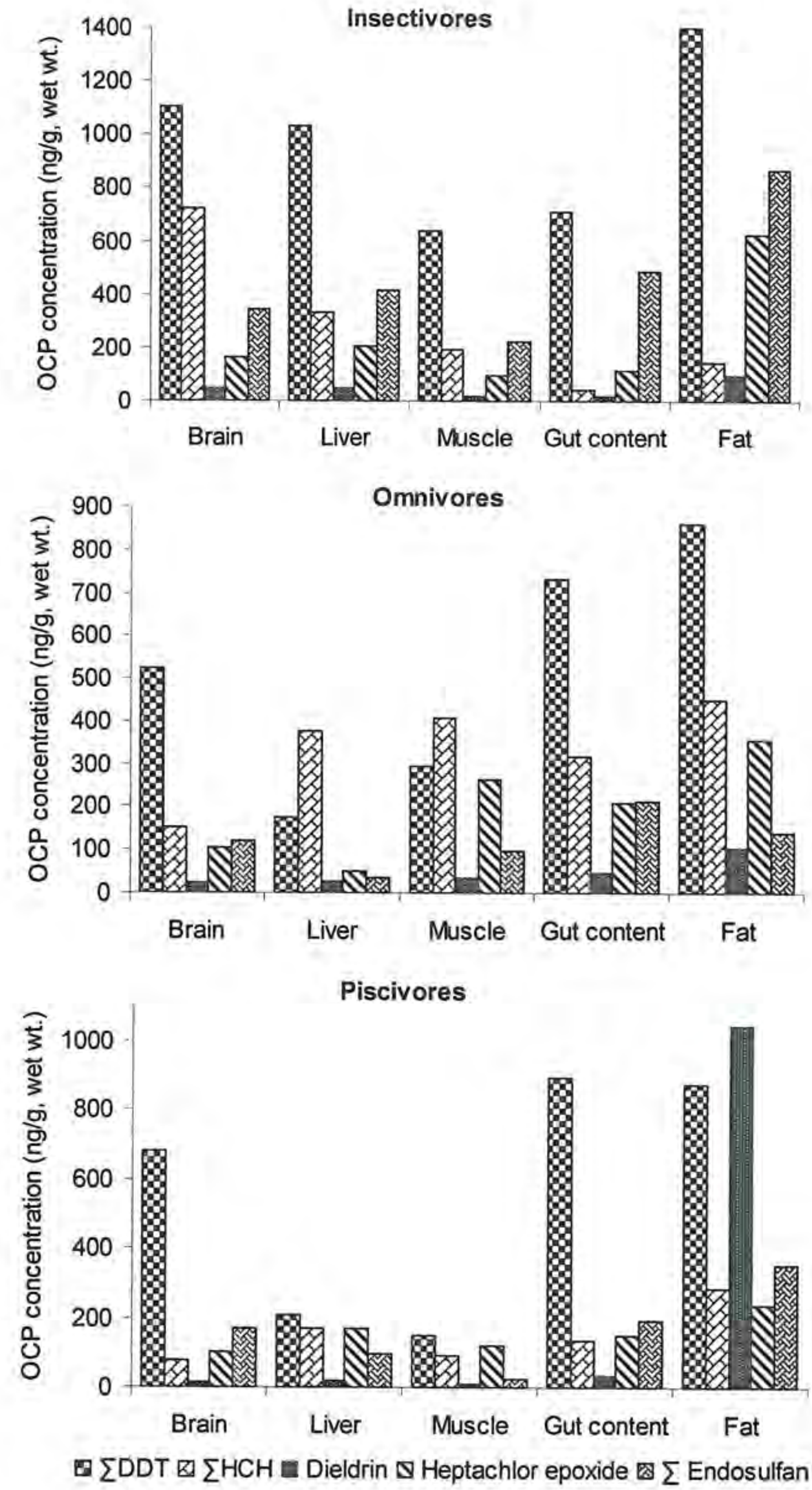



**Figure 3.1b.** Total organochlorine residues among various tissues of birds with different food habits (B- Brain, L- Liver, M-Muscle, G-Gut content, F-Fat).

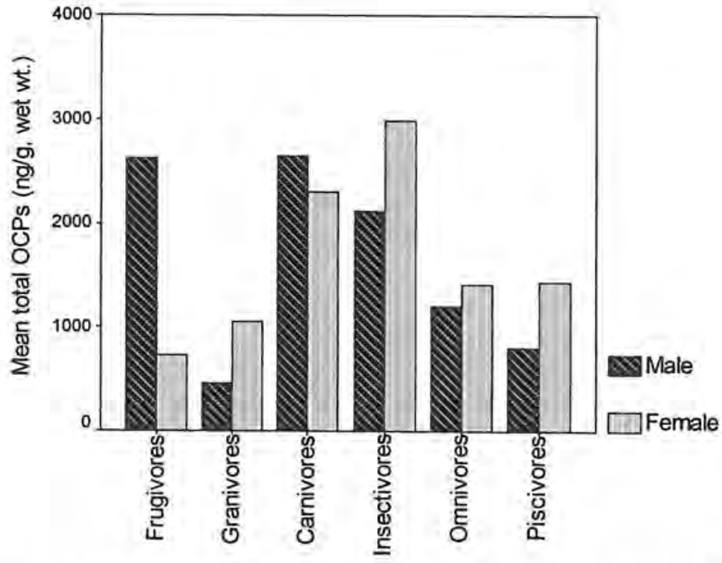



**Figure 3.2.** Variation in total organochlorine pesticide residues between male and female birds (birds were grouped based on their food habits).

The maximum concentration of total DDT was recorded in Shikra (4742 ng/g) followed by Long-tailed Nightjar (2658 ng/g), White-headed Babbler (755 ng/g) and Red-whiskered Bulbul (503 ng/g) (Figure 3.3). Of the various DDT metabolites, *p,p'*-DDE was present at the highest concentration. *p,p'*-DDE ranged from below detectable levels to 7267 ng/g among various species tested. *p,p'*-DDE concentration range was above 1000 ng/g in Cattle Egret, Pariah Kite, Shikra, Indian White-backed Vulture, Indian Peafowl, Indian Ring Dove, Asian Koel, Small Green-billed Molkoha and White-headed Babbler (Table 3.3).

Among the cyclodiene groups of pesticides tested, heptachlor epoxide showed higher accumulation followed by total endosulfan and dieldrin. As recorded for HCH and *p,p'*-DDE, cyclodiene insecticide residues were higher in many species of birds, namely Nilgiri Wood Pigeon, Blyth's Reed Warbler, Indian Robin, Grey Heron and White-headed Babbler (Table 3.3, Figure 3.4). Dieldrin concentration was above 1000 ng/g in some individuals of Pariah Kite, Indian Peafowl, Common Myna, House Crow and White-headed Babbler, whereas one individual of Shikra showed 4550 ng/g of dieldrin.

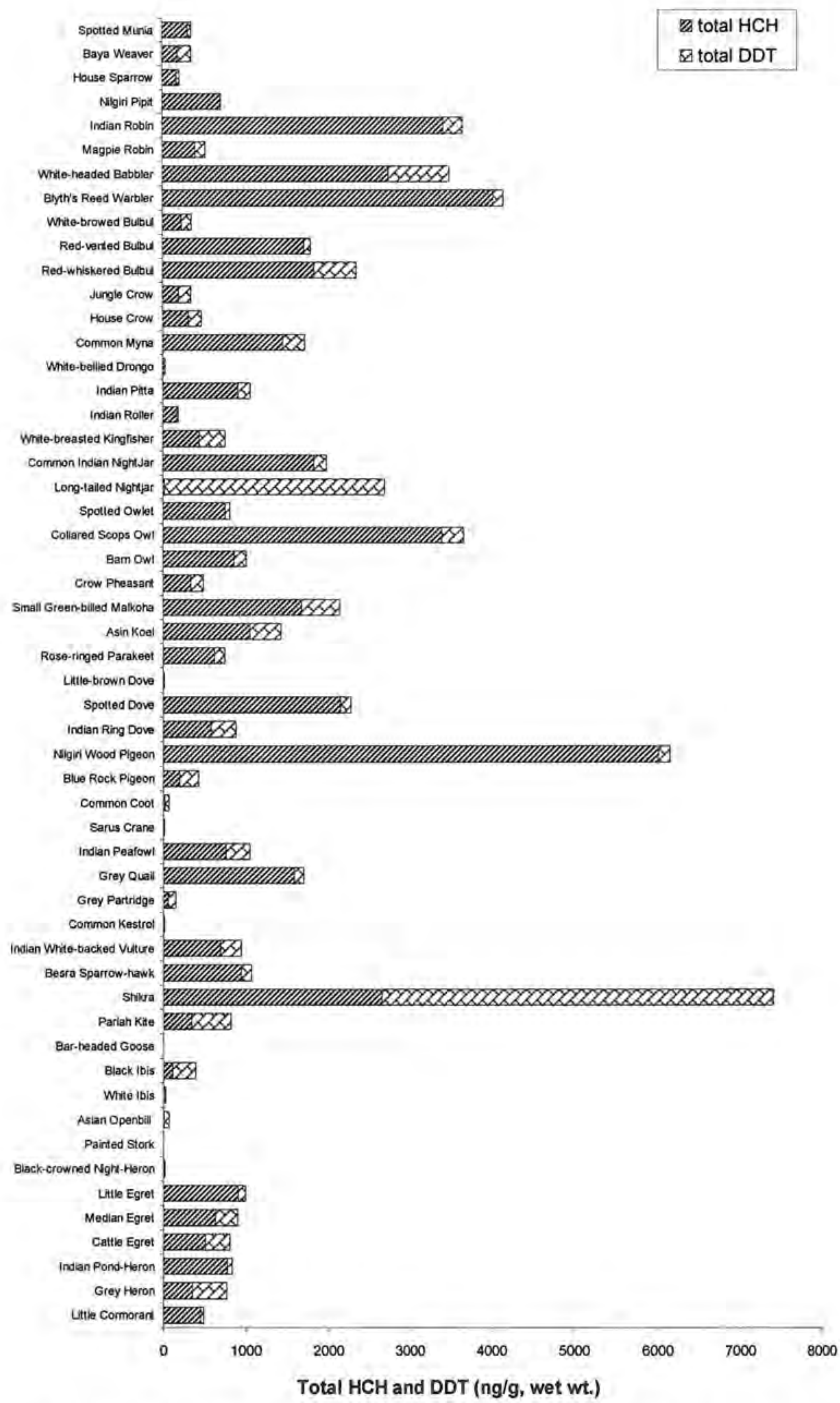



**Figure 3.3.** Total HCH and DDT residues (mean, ng/g, wet wt.) in various species of birds collected from Ahmedabad and Coimbatore, India.

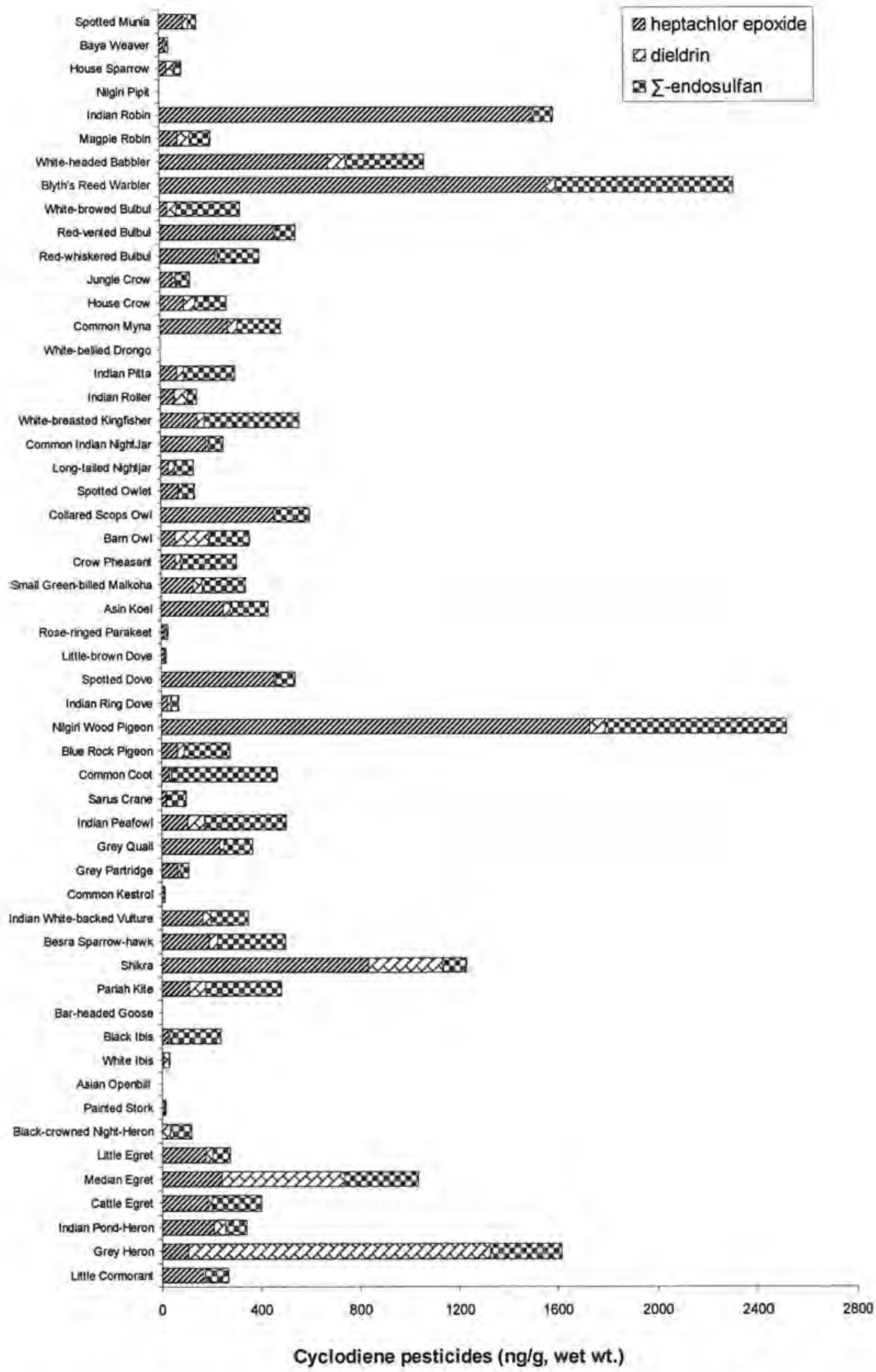



**Figure 3.4.** Cyclodiene pesticide residues (mean, ng/g, wet wt.) in various species of birds collected from Ahmedabad and Coimbatore, India.

**Table 3.4** Variation in organochlorine pesticide residues among birds with different food habits (One way ANOVA).

| Organochlorine pesticides | df | F | $P$-Value |
|---|---|---|---|
| $\alpha$-HCH | 5 | 14.67 | 0.05 |
| $\beta$-HCH | 5 | 15.62 | 0.05 |
| $\gamma$_HCH | 5 | 23.57 | 0.05 |
| $\delta$_HCH | 5 | 18.31 | 0.05 |
| *p.p'*-DDE | 5 | 8.94 | 0.05 |
| *p.p'*-DDD | 5 | 10.09 | 0.05 |
| *p.p'*-DDT | 5 | 8.06 | 0.05 |
| Heptachlor epoxide | 5 | 13.24 | 0.05 |
| dieldrin | 5 | 6.95 | 0.05 |
| $\sum$-endosulfan | 5 | 6.29 | 0.05 |
| $\sum$-Organochlorine pesticide | 5 | 14.45 | 0.05 |

Significant differences were observed for all the organochlorine pesticides among the various species of birds (Table 3.4). Organochlorines pesticide residues were detected in appreciable concentrations in many of the bird studied. However, *p,p'*-DDE and isomers of HCH were detected in most or all individuals. Cyclodiene insecticides were found at levels near the analytical detection limit. Total DDT and HCH levels high in many individuals were of concern.

### *3.5.3. Spatial and temporal variation in organochlorine residues*

The comparison of organochlorine pesticide residues among birds with different food habits collected from Ahmedabad and Coimbatore were presented in figures (Figure 3.5 and 3.6).

General Leaner Model (GLM) including all log-transformed residue data in dependent variable and place, year and birds with different food habits as factors revealed high differences due to interactions of places and years ($P < 0.05$). The interaction effects of food habits and place were not significant ($P > 0.05$) (Table 3.5). Significantly ($P < 0.05$) higher load of pesticide residues were accumulated on carnivorous birds followed by insectivores and piscivores.

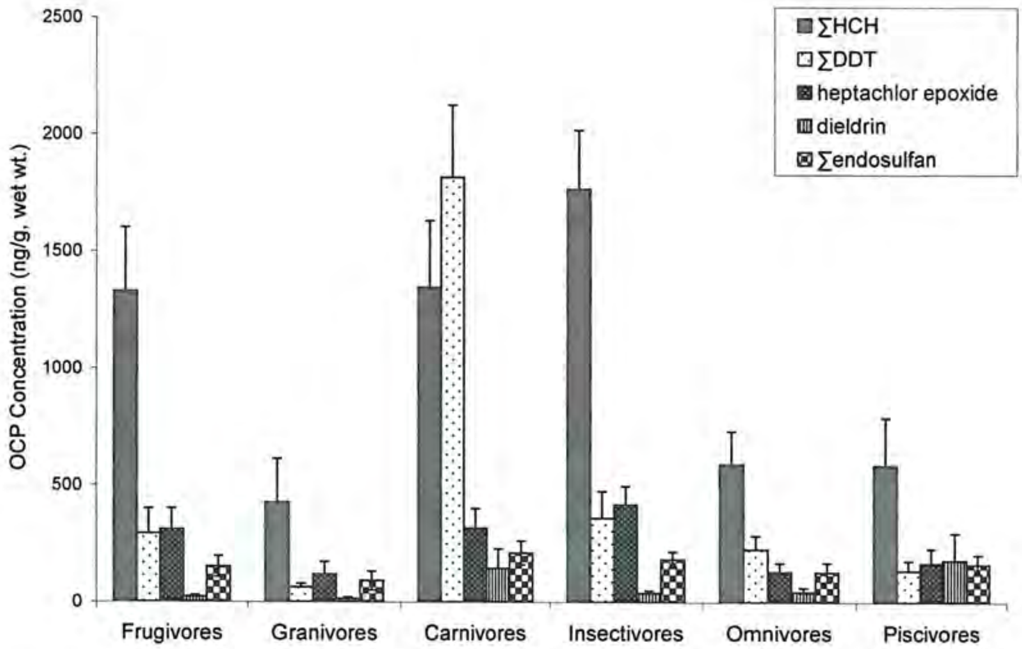



**Figure 3.5.** Organochlorine pesticide residues among birds with different food habits collected in Ahmedabad between 2003 and 2007.

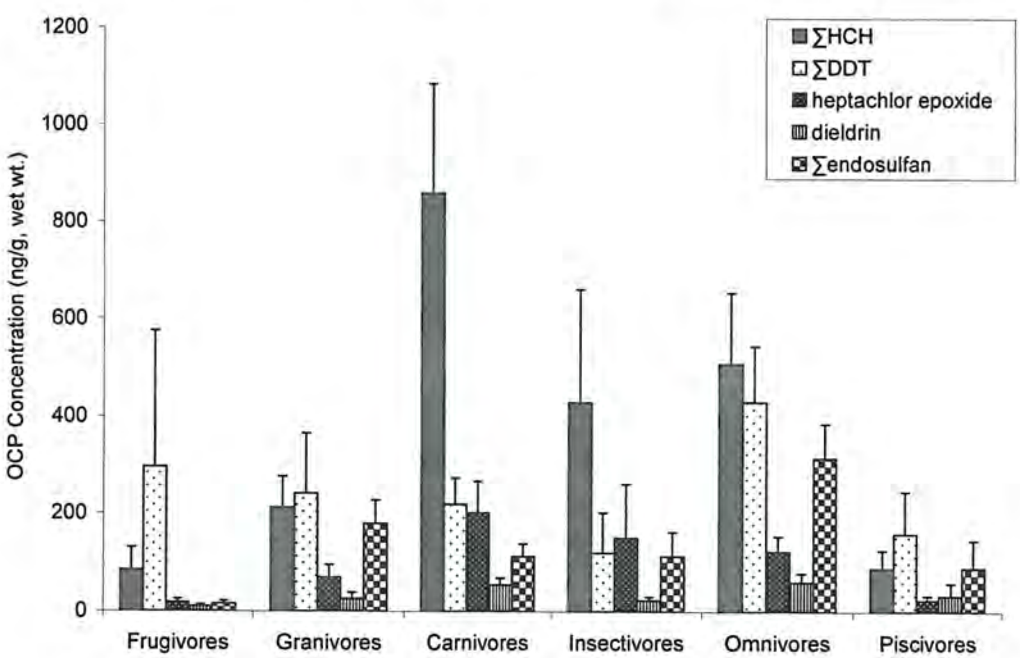



**Figure 3.6.** Organochlorine pesticide residues among birds with different food habits collected in Coimbatore between 2003 and 2007.

**Table 3.5.** Three way ANOVA interaction between places, among years and food habits.

| Source | df | F | P values |
|---|---|---|---|
| Place | 1 | 18.648 | < 0.05 |
| Year | 4 | 29.596 | < 0.05 |
| Food habits | 5 | 5.567 | < 0.05 |
| Place * Year | 3 | 3.024 | < 0.05 |
| Place * Food habits | 5 | 0.172 | 0.972 |
| Year * Food habits | 19 | 1.435 | 0.101 |
| State * Year * Food habits | 4 | 0.113 | 0.978 |

The total organochlorine pesticides in birds of various categories were in the order of carnivores > insectivores > omnivores > piscivores > frugivores > granivores.

All the residues tested showed the higher load of organochlorine pesticide accumulation in birds of Ahmedabad than those in Coimbatore. The maximum load of HCH and DDT were recorded in carnivorous birds from Ahmedabad than that of Coimbatore, while the minimum was in granivorous birds. The maximum total HCH was recorded in insectivorous (1766 ± 251 ng/g) birds followed by carnivorous (1348 ± 282 ng/g) species of birds from Ahmedabad (Figure 3.5). While the minimum was in piscivores (88 ± 37 ng/g) followed by frugivorous (84 ± 46 ng/g) birds of Coimbatore (Figure 3.6). The DDT was the highest in carnivorous birds, while it was the lowest in granivorous birds of Ahmedabad. There was no variation in cyclodiene insecticide among the various groups of birds analysed between two cities (Figure 3.5 and 3.6). However, seven species of birds, namely Nilgiri Wood Pigeon, Blyth's Reed Warbler, Indian Robin, Shikra, White-headed Babbler, Collared Scops Owl and Red-vended Bulbul had more than 300 ng/g of heptachlor epoxide (Figure 3.4). OCPs levels were significantly different between places and among years.

Hence, all the data were considered together to identify the temporal changes in OCPs among birds with different food habits studied. There was a significant difference in total OCPs observed among years (ANOVA, $P < 0.05$). The maximum load of OCPs was detected during 2003 and the minimum in 2004, while the residue levels were almost equal during 2005-2007 (Figure 3.7). HCH levels were high in 2003 and decreased in 2004. Further the levels continuously increased till 2007 among various groups of birds tested. DDT level was constantly maintained during 2005-2007. However, the residues of cyclodiene insecticides showed a declining trend in all groups of birds.

#### *3.5.4. Correlations among chemicals*

The Pearson's correlation coefficient was determined for each pair of organochlorine pesticide residues (Table 3.6). Most of the residue vs residue correlations were at least moderately strong. The positive correlation was generally strong between heptachlor epoxide and isomers of HCH.

## 3.6. Discussion

### *3.6.1. Possible impact of pesticides on birds*

#### *3.6.1.1. HCH and its isomers*

HCH is the most predominantly detected contaminant among the birds. The frequency of HCH isomers, namely alpha, beta, gamma and delta was high in the tissues of all the species of birds studied. There is not much of information available on HCH poisoning in wild birds in India and elsewhere. However, some data generated in the laboratory give some basic information about HCH and its toxicity. The levels recorded in the present study are lower than the $LC_{50}$ values reported from the dietary toxicity study for Bobwhite 882 mg/kg, Japanese Quail 425 mg/kg, Ring-necked Pheasant 561 mg/kg and Mallard 1500 mg/kg (Hill *et al.,* 1975). The HCH concentration reported in the tissue of birds included in the present study do not fall within toxic levels, but indicative of exposure.

**Table 3.6.** Pearson's correlation matrix among organochlorine residues in birds received during 2003 and 2007.

| | *β*-HCH | *γ*-HCH | *δ*-HCH | *p,p'*-DDE | *p,p'*-DDD | *p,p'*-DDT | Heptachlor epoxide | Dieldrin | ∑-endosulfan |
|---|---|---|---|---|---|---|---|---|---|
| **Ahmedabad** | | | | | | | | | |
| *α*-HCH | 0.763 | **0.695** | **0.677** | 0.495 | 0.230 | 0.470 | **0.695** | 0.279 | 0.538 |
| *β*-HCH | | 0.590 | **0.718** | 0.452 | 0.241 | 0.465 | **0.740** | 0.315 | 0.588 |
| *γ*-HCH | | | **0.678** | 0.462 | 0.183 | 0.507 | **0.728** | 0.367 | 0.591 |
| *δ*-HCH | | | | 0.469 | 0.256 | 0.497 | **0.761** | 0.333 | 0.558 |
| DDE | | | | | 0.363 | 0.435 | 0.538 | 0.274 | 0.478 |
| DDD | | | | | | 0.408 | 0.324 | 0.392 | 0.447 |
| DDT | | | | | | | 0.501 | 0.381 | 0.557 |
| Heptachlor epoxide | | | | | | | | 0.432 | **0.716** |
| Dieldrin | | | | | | | | | 0.493 |
| **Coimbatore** | | | | | | | | | |
| *α*-HCH | 0.579 | 0.587 | 0.515 | 0.523 | 0.500 | 0.557 | **0.691** | 0.430 | 0.451 |
| *β*-HCH | | 0.419 | 0.528 | 0.437 | 0.387 | 0.458 | 0.569 | 0.409 | 0.456 |
| *γ*-HCH | | | **0.610** | 0.414 | 0.509 | 0.369 | 0.548 | 0.458 | 0.292 |
| *δ*-HCH | | | | 0.355 | 0.406 | 0.359 | 0.536 | 0.468 | 0.304 |
| DDE | | | | | **0.658** | **0.604** | 0.497 | 0.396 | 0.542 |
| DDD | | | | | | 0.593 | 0.498 | 0.471 | 0.465 |
| DDT | | | | | | | 0.487 | 0.421 | 0.541 |
| Heptachlor epoxide | | | | | | | | 0.551 | **0.613** |
| Dieldrin | | | | | | | | | 0.448 |

∑-endosulfan = *α*- endosulfan + *β*-endosulfan + endosulfan sulfate, ∑-OCPs = total organochlorine pesticides, values with high correlation in bold.

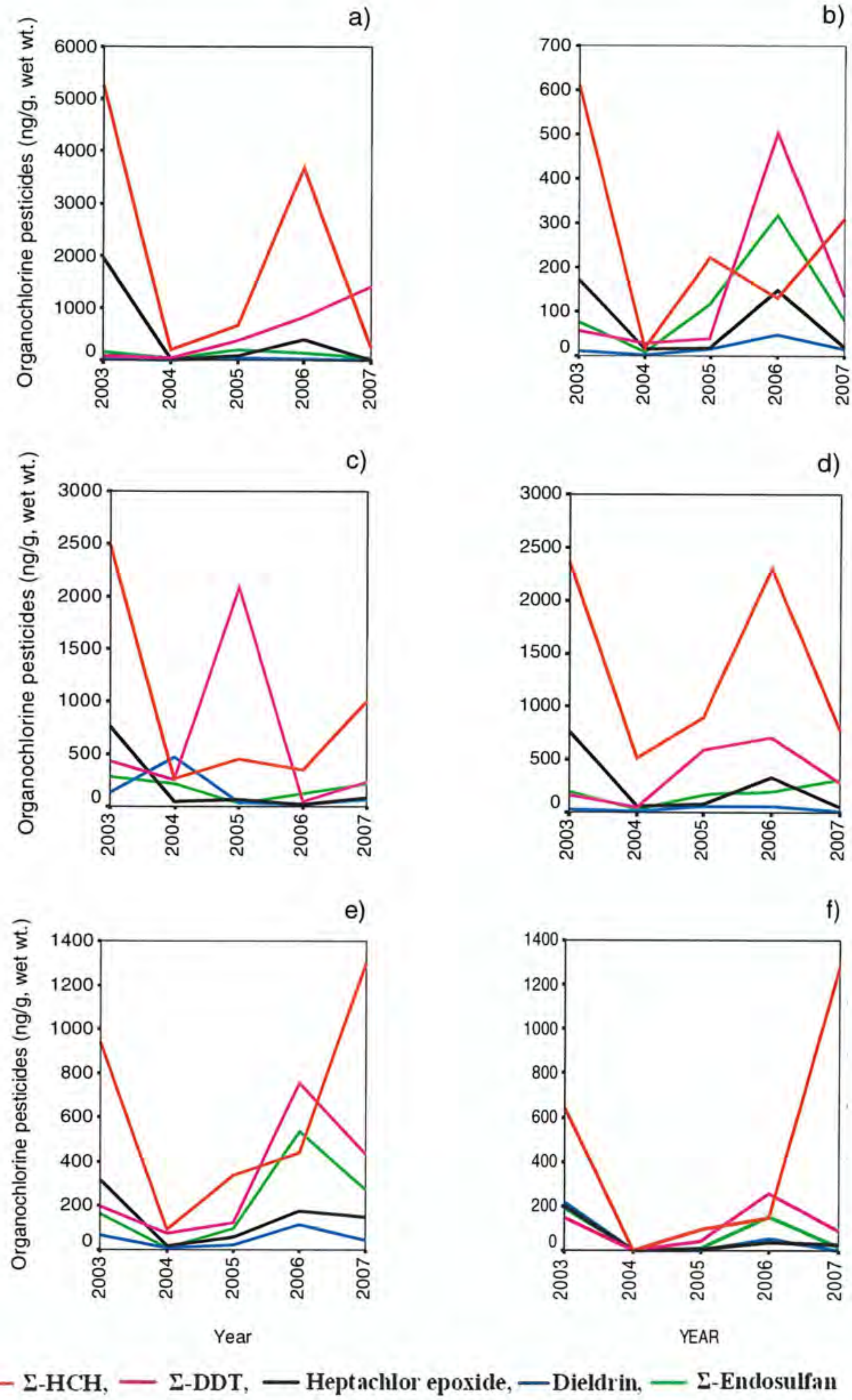



**Figure 3.7.** Temporal changes in organochlorine pesticides among various groups of birds. (a- Frugivores, b- Granivores, c- Carnivores, d- Insectivores. f- Piscivores).

Total HCH concentration recorded in the present study is much similar to the concentration reported in six species of birds, namely Flamingo, Red-wattled Lapwing, Black-winged Stilt, Large-pied Wagtail, Indian Sandgrouse and Fantail Snipe collected near Mahala water reservoir, Rajasthan (Misra, 1989), and also concordant with the results reported in Large Cormorant, Little Egret, Cattle Egret, Indian Pond-Heron, Common Myna and Jungle Babbler collected from Nilgiri, Tamil Nadu (Vijayan and Muralidharan, 1999). However, many species of birds had higher than the mean concentrations reported in Chicken (0.014 mg/kg), Pigeon (0.316 mg/kg), Crow (0.246 mg/kg), Kite (0.093 mg/kg), Vulture (1.132 mg/kg) and Cattle Egret (0.126 mg/kg) collected from Lucknow (Kaphalia *et al.,* 1981).

The HCH concentration reported in the present set of birds are several times higher than the concentration reported in the birds of Oxbow lakes of Northeastern Louisiana (Niethammer *et al.,* 1984). HCH was also one of the most common residues observed in waterfowl from Mexico, although at low levels (Mora *et al.,* 1987). Total HCH concentration in Common Myna in the present study is 4-6 times higher than the levels (HCH 250 ng/g) reported in the whole body homogenate of the same species collected from South India (Tanabe *et al.,* 1998). While HCH and DDT levels in the present study were concordant with the concentration reported in White-breasted Kingfisher (HCH 410 ng/g) and House Crow (HCH 1000 ng/g), Little Egret and Indian Pond-Heron had lower concentrations. The total HCH levels recorded in Indian White-backed Vulture included in the present study were higher than the range (BDL to 1.92 $\mu$g/g ) reported in the same species collected from different locations in India during 1999-2003 (Muralidharan *et al.,* 2008). The higher concentrations of HCH were recorded in Canadian Arctic marine wildlife because of its closer proximity to India and China, where until very recently HCH has continued to be used (Li *et al.,* 2002).

It is also reported that India is the greatest consumer of HCHs in the world (Kumari *et al.,* 2001) with approximately 36 million kg/year for agriculture purposes (Ramesh *et al.,* 1992). It has been used against sucking and biting pests and as smoking agent for control of pests in grain stores (Mathur *et al.*, 2005). Although the residue levels recorded in the current study are not indicative of toxicity, these residues can be expected to create ill effects to birds at higher concentration (Minh *et al.*, 2002). The presence of HCH residues has also been reported in human blood in Ahmedabad (Bhatnagar *et al.*, 2004) even after the ban on HCH (with effect from April 1, 1997).

Among the isomers of HCH detected in the tissues of birds, beta-HCH was the most predominant isomer. This is due to isomerization of $\alpha$- and $\gamma$- HCH into $\beta$-HCH and also due to its high stability (Jensen, 1983) and persistent nature (Chessells *et al.*, 1988; Burke *et al.*, 2003). Gamma HCH showed less accumulation. This may be due to its rapid disappearance in the tissues of birds after death (French and Jefferies, 1968). Presence of high concentrations of $\beta$-HCH has been reported in various biological components in India (Kumari *et al.*, 2001; Kole *et al.*, 2001; Muralidharan *et al.*, 2008; Muralidharan *et al.*, 2009) and in human milk in Delhi (Nair *et al.*, 1996). Moreover it is also reported that $\beta$-HCH will accumulate 10 to 30 times more than lindane ($\gamma$- HCH) in fatty tissues (Heeschen, 1980). The strong persistency of $\beta$-HCH can be attributed to its slowest rate of degradation of all the HCHs ($\delta$-HCH > $\gamma$-HCH > $\beta$-HCH) (WHO, 1992).

Comparatively, less frequency of lindane was observed in the present study. Similar observations were reported in many species of birds across the world, because lindane was not retained to any extent in tissues in unaltered form. After ingestion, lindane is readily metabolized to water-soluble chlorophenols and chlorobenzenes that are readily excreted. Its half-life in tissues and eggs of birds is much lower than the other organochlorine insecticides (Cummings *et al.*, 1967). Although lindane is less bioaccumulative than other OCPs, low levels of this insecticide were found in eggs, tissues and young ones of Ring-necked Pheasant (Burrage and Saha, 1972). The brain is usually preferred in deriving lethal diagnostic residues of organochlorine in birds, but such data are unavailable for lindane.

HCH residues as high as 1500 ng/g were observed in many species of birds belonging to carnivorous and insectivorous groups in this study. However, residue levels upto 1500 ng/g seed treatment did not have any effects on reproduction of Canada Geese *Branta Canadensis* and that no effects had been reported for any wild populations (Blus *et al.*, 1984). The toxicity of lindane to birds was demonstrated when nearly half of a flock of domestic pigeons died several days after receiving a diet that was inadvertently contaminated with lindane-treated seeds; however, the levels of contamination (2100 mg/kg) was very high (Blakely, 1982). The birds included in this current study had less than those levels reported by Blakely (1982). However, great variation in sensitivity of species to contaminants is known to exist and more studies would be necessary to determine the effects of the HCH levels observed in many species of birds.

### *3.6.1.2. DDT and its metabolites*

DDE residues in carcasses were significantly higher than DDT residues; thus, DDE: DDT ratios were high, an indication of recent and continued use of DDT in India. DDT residues were the second highest, among the residues analyzed in various tissues of birds. This feature is similar to the observation make in the birds of South India (Ramesh *et al.,* 1992) and in Indian White-backed Vulture from various places in India (Muralidharan *et al.,* 2008).

The DDE concentration was particularly elevated in some individuals, but the mean concentrations were similar to those described by Kaphalia *et al.,* (1981). The results indicated that DDE remains widely persistent in birds in India. The high DDE concentration (above 1000 ng/g) observed in some individuals of Asian Koel, Common Myna, Indian Peafowl, Pariah Kite and Shikra may be due to incidental exposure of DDT and the long persistence of DDT. Studies have demonstrated behavioral effects due to DDE in Ring Doves *Streptopelia risoria* (Haegele and Hudson, 1977). Courtship behavior was shown to be affected by 10 mg/kg DDE in the diet administered over a period of 63 days. Further this also leads to an accumulation of DDE in the brain. It was also reported that a steep decline in number of birds was observed after single DDT treatment in areas compared to unsprayed areas (Douthwaite, 1992). The total DDT levels detected in brain samples of birds in the present study are lower than the concentration (> 30 mg/kg) reported to be lethal to birds (Stickel and Stickel, 1969). In experimental studies, DDE levels in birds brain tissue that were associated with mortality were found in the range of 200-500 mg/kg (Blus, 1996).

Total DDT levels reported in the birds of the present study are concordant with the earlier reports on resident and migratory birds (DDTs 0.3 to 3,660 ng/g) from South India (Tanabe *et al.,* 1998). However, the maximum of 4742 ng/g of total DDT recorded in Shikra of present study are higher the values reported by many earlier studies (Kaphalia *et al.,* 1981; Tanabe *et al.,*1998; Muralidharan *et al.,* 2008). The DDT and its metabolites in Indian White-backed Vulture included in the present are concordant with the results (700 1140 ng/g wet wt.) reported in the same species collected from Ahmedabad and less than the concentration reported in Delhi (70-7300 ng/g) during 1999-2003 (Muralidharan *et al.,* 2008). This clearly indicates the continuous presence of these persistent chemicals in the environment of Ahmedabad.

During the DDT era about 85% of the Indian farmers utilized the organochlorine pesticides at the rate of 0.39 kg/ha. It was applied nearly over an area of 282 million hectares of agricultural land of the country. DDE also could have resulted from dicofol use, although dicofol use in India is limited. The results of lower concentration of DDT indicate that source of DDT contamination is from the aged and weathered agricultural soils with signature of recently used DDT. India has banned DDT for agricultural use in 1989, although, it is used in public health sectors for Malaria control. It is notable that in India, aldrin is completely banned, while the usage of DDT and HCH are restricted (UNEP, 2002).

### *3.6.1.3. Cyclodiene insecticides*

Cyclodiene insecticide residues recorded in the birds of the present study are at very low levels. Such levels may not pose any threat to birds. Dieldrin was found frequently, but at very low levels. Dieldrin is a highly toxic organochlorine pesticide. Although dieldrin was the least detected among the cyclodiene groups, it has been labeled as a commonly detected environmental contaminant, due to its stability (Santerre *et al.,* 1997). Dieldrin residue levels associated with mortality in birds are 5-10 mg/kg in the brain (Peakall, 1996). Aldrin in the gastrointestinal tract (89.75 mg/kg) and dieldrin at much higher quantities in the brain (4-5 mg/kg) clearly indicated that dieldrin after being metabolized from aldrin was responsible for the death of many Sarus Cranes and other granivores in Bharatpur (Muralidharan, 1993). However, the levels documented in the present study are lower than the levels expected to be leasthal. Although, there are no experimental data on dieldrin in many species of birds available, the levels detected in one Shikra appeared to be alarming. The dieldrin concentration in tissues of White-baked Vulture was the same as reported in the same species from different locations in India (Muralidharan *et al.,* 2008).

The environmental contamination by dieldrin might have been mainly from the agricultural and non-food-related uses of aldrin, and to a lesser extent from the use of dieldrin itself. Many species of wildlife have died as a result of eating food contaminated with aldrin and dieldrin (Beyer *et al.,* 1996). It was believed that about 1 mg/kg or more of dieldrin in the brain on a wet wt basis may pose hazard to some birds (Heinz and Johnson, 1982). Behavioural disturbances were observed in Bobwhite Quail with brain levels of 4 mg/kg dieldrin (Peakall, 1985). Most of the birds studied showed comparatively less dieldrin burden and are well below the levels expected to create harmful effects to avian population (Heinz and Johnson, 1981). However, it may be noted that the Long and Margan "Effects Range-Low" (ER-L) value (i.e., the contamination level above which adverse biological effects are occasionally observed), is listed as 0.02 $\mu g/kg$ for dieldrin (Long *et al.*, 1995).

Food and Drug Administration (FDA) action level for heptachlor epoxide is 300 ng/g. Projections of levels in Indian Robin, Shikra and White-headed Babbler and some individuals of Indian White-backed Vulture suggests that the estimates of heptachlor epoxide in birds may pose risk to reproduction based on studies in other species (Spann *et al.*, 1986) where the residues more than 500 ng/g were associated with observable effects. The levels recorded in the present study are higher than the $LC_{50}$ values for Japanese Quail, *Coturnix japonica,* 93 ng/g and Ring-necked Pheasant, *Phasianus colchinus,* 224 ng/g (Heath *et al.*, 1983). However, the experimental study by Henny *et al.*, (1983) concluded that residues greater than 1.5 mg/kg were most definitely associated with decreased reproduction rates in avian species. Heptachlor is readily metabolized to heptachlor epoxide, which is lipid soluble and readily stored in body fat (Burrage and Saha, 1972). Residues of heptachlor epoxide in many of the individuals in the present study are to be viewed with concern. Heptachlor epoxide seems to pose a greater risk to birds which have residue levels more than 1 mg/kg in wild birds (Blus *et al.*, 1984).

Endosulfan, a less persistent OC in birds was less than that of HCH and DDT. Bioconcentration factors in birds and mammals are less than 1 (NRCC, 1975). No-effect level for endosulfan in birds is unknown. However, data indicate young birds are more sensitive than adults (DeWitt, 1963, Hudson *et al.*, 1972). The levels reported in the present study are less than the lowest reported dietary toxicity of 805 mg/kg to birds (Hill *et al.*, 1975). The cyclodiene insecticide residues recorded in this study are less than the concentration reported in six species of birds collected from Nilgiris, Tamil Nadu, India (Vijayan and Muralidharan, 1999).

Although there were no significant differences observed among tissues in their residues loads, the maximum was observed in fat and liver than other tissues in all the bird species studied. This is due to the high lipid content and lipophilic nature of OCs. However, the differences observed in OCs among the tissue are due to combination of tissue specific retention and solubility in lipids. Changes in feeding behavior can also influence differences in OCP concentrations among tissues. It was reported that birds readily biotransform both $\alpha$- and $\gamma$-HCH (Moisey *et al.*, 2001).

The piscivorous birds that feed mainly on fishes and insects contained the highest concentrations of pesticide residues than the terrestrial birds. Further, HCH residues were higher in aquatic birds than those in terrestrial birds, which is somewhat similar to those observed in birds from South India (Ramesh *et al.*, 1992; Tanabe *et al.*, 1998) and birds of Nilgiri district, Tamil Nadu (Vijayan and Muralidharan, 1999). The relative proportions of OCPs differed among various food habits of birds providing more evidence of the influence of trophic position, migration and biotranformation on OC levels in birds.

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 that predatory and fish-eating birds have higher residues and are more sensitive when compared to other groups of birds (WHO, 1989). Next to carnivores, insectivores showed higher burden of OCPs. It was reported that if insects or food is reduced in an area, the organism that normally feed upon them also diminish in numbers (Cockbill, 1979; Rands, 1985).

Birds that eat other birds or fish usually have higher residues than those that eat seeds and vegetation. Many researchers (Cramp *et al.*, 1964; Ratcliffe, 1965; Keith and Gruchy, 1970; Prestt *et al.*, 1970) have published the comparisons of residues in birds with different food habits. Wild birds in some Latin American and African countries revealed an identical trend. It was reported that higher concentrations of insecticide residues in carnivores and insectivores than in omnivores and granivores. The ingestion of contaminated food is considered to be the primary route of exposure in birds (McCaskey *et al.*, 1967; Perry *et al.*, 1990; Driver *et al.*, 1991).

In general, results obtained in this study are comparable with earlier reports in Indian avifauna (Kaphalia, 1981; Muralidharan, 1993; Tanabe *et al.,* 1998; Muralidharan *et al.,* 2008). Concentrations of DDE and HCH residues reflect direct or indirect anthropogenic sources. A number of birds collected were victims of kite flying in Ahmedabad, Gujarat, while from Coimbatore and surrounding areas, it was mainly on an opportunistic basis. OCPs have been restricted for usage in this country. Nevertheless, OCPs continue to pervade our environment. The fact that majority of the gut content had OCPs indicate that OCPs are still widely present in our environment and actively being ingested by avian species. Their presence in liver and brain indicate that they are still being accumulated through the food chain.

### *3.6.2. Inter-site variation in organochlorine residues*

Comparison of OCPs between the two locations showed significantly higher levels in birds from Ahmedabad than Coimbatore. The higher load OCPs accumulation in birds of Ahmedabad can be explained by the extensive industrial operations and agricultural activities. The relatively lesser load of OCPs in birds of Coimbatore was due to receipt of high number of dead birds from forest areas which was considered to be less polluted. The early research showed that DDT and its metabolites were widespread in waterbirds, and residues of all organochlorine families were found in bird tissues and eggs, including cyclodienes, the most toxic members of the group.

Although present levels of organochlorine pesticides in birds from India mostly correspond to residual loads and agricultural use in the past, concern on current inputs should persist. In India although there have been studies carried out in birds and their eggs for contaminants analysis as referred elsewhere in this chapter (Regupathy and Kuttalam, 1990; Muralidharan *et al.,* 1992; Ranapratap, 2004), the present study is the first to use birds of two different cities India for the period of five years. In general the measured levels of pesticides in birds of Ahmedabad are considerably higher than the values reported in similar species in industrialized countries. Although the concentration levels found in this study were exceeding low or higher, it allowed revealing the variation in the chemical contamination among species and between sites. As the frequencies of occurrence and accumulation pattern of OCPs in birds reflect the large geographical differences. The interlocational differences in contaminant burdens reflect known geographic differences in contaminant sources.

### *3.6.3. Temporal trend and suitability of birds for monitoring studies*

HCH and DDT levels appeared to be constant or increasing over a period of time. The sudden decline during 2003 could be due to samll sample size. However, the increasing trend of HCH and DDT indicates the recent applications of these pesticides for agriculture and public health purposes. The residue pattern of cyclodiene insecticide showed declining trend among the years. It could be due to complete ban and/or restricted usage of these insecticides.

Among the various groups of birds studied for contaminants levels, carnivorous birds appeared to be good indicator of contaminants. Similar observations were reported in several studies. Birds of prey have been used to indicate local and regional contamination by OCs, as they bioaccumulate due to their high position in the trophic pyramid. Almost all the group of birds explained the trend of organochlorine pesticide load over the period of time. Based on the current study it is proposed that the species namely Pariah Kite and Blue Rock Pigeon hoods an intermediate position in the food web and is abundant enough to allow collection of samples at any growth stage and at almost every location annually. Since they have spent all their life along the same restricted areas, these birds are supposed to be valuable indicators of the particular contamination surround their living area. Therefore, the research on birds, particularly Pariah Kite and Blue Rock Pigeons or raptors could be useful in devising temporal trends in contamination, as well as pattern of local or regional differences.

### 3.7. Conclusion

Organochlorine compounds are still present in birds of India. Among the isomers of HCH detected in the tissues of birds, beta-HCH was the most predominant isomer. Comparatively, less frequency of occurance of lindane may be due to its rapid metabolism. Although HCH residues were as high in many species of birds, the levels do not fall within toxic levels, but indicative of exposure. DDT residues were the second highest, among the residues analyzed in various tissues of birds. Concentrations of DDE and HCH residues reflect direct or indirect anthropogenic sources.

The high DDE concentration (above 1000 ng/g) in some individuals of Asian Koel, Common Myna, Indian Peafowl, Pariah Kite and Shikra might be due to incidental exposure. The total DDT levels detected in brain samples of birds in the present study are lower than the lethal concentration reported in birds and less than the level associated with mortality of birds. The results of lower concentration of DDT indicate that source of DDT contamination is from the aged and weathered agricultural soils with signature of recently used DDT. India has banned DDT for agricultural use in 1989, although, it is used in public health sectors for malaria control.

Cyclodiene insecticide residue levels are lower than the lethal level expected to be create risk to birds. The levels detected in one Shikra appeared to be alarming. Most of the birds studied showed comparatively less dieldrin burden and are well below the levels expected to create harmful effect to avian population. However, many occasions dieldrin was higher than the "Effects Range-Low" levels, which has created biological effects. Heptachlor epoxide detected in Indian Robin, Shikra and White-headed Babbler and some individuals of Indian White-backed Vulture are above the FDA action levels. This study suggests that heptachlor epoxide in birds may pose risk to the referred birds if exposure continued. Hence it has to be viewed with concern. Endosulfan levels reported in the present study are less than the lowest dietary toxicity levels reported for birds.

Although there was no significant difference observed among tissues, the differences observed in OCs could due to combination of tissue specific retention and solubility in lipids. The carnivorous birds that feed mainly on small birds contained the highest concentrations of pesticide residues than the birds with different food habits. The relatively high pesticide residue levels in the predatory birds, namely Shikar 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 from Ahmedabad than in Coimbatore. The higher load OCP accumulation in birds of Ahmedabad can be explained the extensive industrial operations and agricultural activities. Although present levels of organochlorine pesticides in birds from India mostly correspond to residual loads and agricultural use in the past, concern on current inputs should persist. In India, this study is the first to use birds of two different cities for the period of five years. In general, the measured levels of pesticides are considerably higher than the values reported in similar species in industrialized countries. Although the concentration levels found in this study were exceeding low or high it allowed revealing the variation in the chemical contamination among species. The interlocation differences in contaminant burdens reflect known geographic differences in contaminant sources.

Among the various groups of birds studied for contaminants levels, carnivorous birds appear to be good indicator of contaminants. Almost all the group of birds had the same trend of organochlorine pesticide load during the study period. The residue pattern of cyclodiene insecticide showed declining trend among the years. It could be due to complete ban and/or restricted usage of these insecticides. The current study propose that the species, namely Pariah Kite and Blue Rock Pigeon could be used to understand temporal trends in contamination, as well as pattern of local or regional differences.

Chapter 4

# POLYCHLORINATED BIPHENYLS (PCBs) AND POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN BIRDS OF AHMEDABAD AND COIMBATORE

## 4.1. Introduction

Polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants due to their persistence and lipophilicity. PCBs are industrial chemicals composed of many isomers and congeners, which highly differ in toxicity, toxic effects and persistence. PCB mixtures, because of their inflammability, chemical stability and insulating properties are used in many industrial applications (Falandysz *et al.*, 2003).

PCBs being highly lipophilic, accumulate in lipid fraction of cells and tissues of living biota including human beings and birds. Individual PCB congeners exhibit different physicochemical properties that result in different profiles for environmental distribution and toxicity. Primary sources of PCBs are from industrial applications as dielectric oils in electromagnetic equipment such as transformers and capacitors. The widespread occurrence of PCBs are reported in abiotic and biotic samples including air, water, soil, sediment, food stuff and human breast milk (Minh *et al.*, 2002). They readily accumulate in the biological tissues through food chain (Hoffman *et al.*, 1996), so that high concentrations are found in top predators (Falandysz *et al.*, 2003).

Since birds occupy a wide range of tropic levels in different food chain, they are exposed to different concentrations of PCBs. Accumulation and their impact on the population of many species of birds have been well documented in several western countries (Flickinger and King, 1972; Stickel, 1973; Custer *et al.*, 1990). Adverse effects of PCBs in birds include increase in embryo mortality, decrease in hatching ratio, congenital deformity, reduced bursa of fabricius weight, porphyrin accumulation, and decline in vitamin A and thyroid hormone (Tillitt *et* *al.*, 1992; 1993; Yamashita *et al.*, 1993; Van den Berg *et al.*, 1994; Barron *et al.*, 1995; Falandysz *et al.*, 2003).

Altered reproduction in wild passerine (Bishop *et al.*, 1999) and raptorial birds (Kozie and Anderson, 1991; Bowerman *et al.*, 1994; Custer *et al.*, 1999) was reported to be related to exposure to multiple contaminants, including PCBs. From the environmental health point of view, an understanding of contaminant exposure in higher-trophic animals is of importance because bioaccumulation potential can cause adverse effects in these animals. Researches conducted on birds in USA and Europe reported adverse effects due to PCBs most of them indicated dioxin-like compounds including non-ortho and mono-ortho PCBs as the main cause (Ankley *et al.*, 1993; Yamashita *et al.*, 1993).

Polycyclic Aromatic Hydrocarbons (PAHs) enter the environment through natural sources such as forest fires and anthropogenic activities including combustion of fossil fuels, wood, petroleum refining (Douben, 2003; Latimer and Zheng, 2003) and incomplete combustion of organic compounds (Camargo and Toledo, 2003). The detectable levels of PAHs have been reported even in remote and rural environment due to atmospheric transport. As a consequence environmental contamination by PAHs has steadily increased in recent years (Metre *et al.*, 2000).

Early studies across the world have indicated widespread occurrence of PAHs in abiotic and biotic components including air (Viau *et al.*, 2000, Lin *et al.*, 2001; Ren *et al.*, 2006), water (Claudia *et al.*, 1992; Said and Hamed, 2006), soil (Yuan *et al.*, 2002; Hu *et al.*, 2006), sediments (White and Triplett, 2002), fishes (Deena *et al.*, 2004; Barron *et al.*, 2004) and birds (Walters *et al.*, 1987; Troisi *et al.*, 2006). However, studies on birds are very limited.

Many studies have revealed that the PAHs are hazardous to aquatic organisms (Readman *et al.*, 1982; Masoero *et al.*, 1985; Kelly, 1993; MPMMG, 1998; White and Triplett, 2002; Ruddock *et al.*, 2003), birds (Miller and Connel, 1980; Jessup and Leighton, 1996; Custer *et al.*, 2000; Honey *et al.*, 2000; Taniguchi *et al.*, 2003; Balsiero *et al.*, 2005;

Troisi and Borjesson, 2005; Lee and Anderson, 2005; Troisi *et al.*, 2006) and man (Samiullah, 1985). Since birds occupy a wide range of trophic levels in different food chain, they are exposed to different concentrations of environmental pollutants including PAH (Falandysz *et al.*, 2003).

Studies have also reported that the birds have deleterious effects both externally and internally when they are exposed to spilled or waste petroleum products. PAHs contribute to the toxicity of crude petroleum and refined petroleum products, but the amount of PAH in petroleum products vary greatly (Rocke, 2001). Although the PAHs are rapidly metabolized in birds (Naf *et al.*, 1992), the residues of parent PAHs have been detected in bird tissues in relation to source of incomplete combustion of petroleum products (Mineau *et al.*, 1984; Custer *et al.*, 2000).

In India a few studies have reported the elevated levels of PCBs in a few species of migratory birds collected from Lake Baikal, Russia (Kunisue *et al.*, 2002) and Southern India (Tanabe *et al.*, 1998). Levels of polychlorinated dibenzo- p-dioxin, dibenzofurans, and polychlorinated biphenyls in human tissue, meat, fish and wildlife samples from India were reported (Senthilkumar *et al.*, 2001). Although there are some information on the levels of organochlorine pesticides in many species of birds as has been explained in Chapter 3, information available on the levels of PCBs is very limited and no information is available on PAHs in birds in India. Hence, it is very important to elucidate the levels of PCBs and PAHs and their accumulation pattern in avian species in India. The present study will also generate baseline information on the residue levels of PCBs and PAHs in birds in India.

### 4.2. Background information

PCBs were first synthesized in Germany at the end of nineteenth century and the commercial production began in 1929, but large scale production started in 1945. In Western Europe and North America PCBs production was banned in 1970s (Falandysz *et al.*, 2003). The use of advanced analytical techniques led to recognition of PCBs as an environmental pollutant in 1966 (Jensen, 1966) and further their ubiquitous environmental occurrence, including human (Yakushiji, 1988) and wildlife (Colborn *et al.,* 1993; Hoffman *et al.,* 1996), as well as numerous other environmental matrices have been extensively reported (Burnstrom *et al.,* 1990; Fensterheim, 1993; EPA, 2000).

Their persistence and affinity for fats have enabled them to penetrate nearly in all living organisms and to concentrate in consumers high in the food chain. In vertebrates, such as birds, chronic exposure of relatively high concentrations of PCBs induce hepatic microsomal cytochrome P450 (Ronis and Walker, 1989). Patterns of exposure may vary substantially among individuals of a species with a varied diet. Persistent contaminants are unlikely to be evenly distributed throughout a food web; Short-lived consumer species will bioaccumulate the persistent contaminants at lower levels than longer-lived predators (Gervais and Anthony, 2003).

Metabolic rates are generally lower for congeners with high chlorine content, but substitution position of chlorine is also important. The toxicity of PCBs is being assessed based on selected indicative congeners. It is also characterized that the PCB congeners, namely 201, 209, 172/192, 194 and 195 have greatest bioaccumulation potential (Falandysz, 2003). Among the large number of PCBs, intensively studied are the non- ortho substitutes, which are also reported to induce toxic effects, such as neurobehavioural changes, inhibition of intracellular communications and induction of "Phenobarbital- type", which are non- dioxin- like toxic effects, caused by congeners with different substitution pattern (Andersson *et al.,* 1997; Giesy and Kannan, 1998).

In general, PCBs have high avian oral lethal toxicity, with median lethal dose values being 604, 1,091 and 2,699 $\mu$g/g in Bobwhite Quail *Colinus virgianus*, Ring-necked Pheasants *Phasianus colchicus* and Mallards *Anas platyrhyncos*, respectively (Heath *et al.,* 1972; Brown *et al.,* 1987). PCBs are a mixture of chlorinated biphenyl congeners (CBs) that differ in the amount of chlorine attached to the biphenyl molecule. Chlorinated Biphenyls (CBs) having no or only one chlorine

chlorine in the ortho positions are responsible for the most acute toxicity (Hoffman *et al.,* 1998). Little direct mortality has been associated with PCBs, but decreased reproductive success from PCB exposure has been demonstrated in many avian species (Fernie *et al.,* 2001; Falandysz *et al.,* 2003; Kunisue *et al.,* 2003).

Among OC concentrations detected in Japanese Common Squid *Todarodes pacificus*, PCBs were the highest, and other OCs were in the order of DDT > CHCs > HCHs >HCB (Ueno *et al.,* 2003). In a study conducted by Falandysz *et al.,* (2003), the toxicity of PCBs was assessed based on their total PCB concentrations or select indicative congeners in Black Cormorants breeding at the coast of the Gulf of Gdansk, Black Sea.

PCBs have been shown to affect mortality of embryos and chicks and cause morphological aberrations in chicks (Gilbertson *et al.,* 1991). Not withstanding the adverse effects of organochlorine chemicals, little is known about their contamination and toxic impacts on birds in developing countries (Tanabe *et al.,* 1998), including India.

Although the effects of PCBs on avian reproduction are clear from experimental work and field studies documenting, consistently high levels of the compounds, the effects of low and variable levels of exposure to OCs consistent with declining environmental concentrations are much less obvious (Gervais and Anthony, 2003). Organochlorine pesticides, PCBs and certain other persistent toxic chemicals are widely dispersed in the environment and are commonly found in tissues and eggs of wild birds from Arctic to Antarctic (AMAP, 1998; Bard, 1999; Li *et al.,* 2002).

From the ecosystem health point of view, an understanding of OC exposure in higher-trophic animals is of importance because organochlorines have high bioaccumulation potential and can cause adverse effects in these animals. Long range transport of these contaminants into different geographical areas has been recognized and chlorinated hydrocarbon residues are found to be widely dispersed in different ecosystems although these chemicals were banned or severely restricted globally.

Although many persistent organochlorine compounds have been banned in many if not all countries; lingering residues and global redistribution of low concentrations of these compounds may still have negative ecological effects when combined with other stressors (Gervais and Anthony, 2003). Considerable data have been amassed over the last 20 years regarding persistent organic pollutants in a variety of ecosystems around the world.

Decline in the populations of a few species of birds, such as Common Tern, Forsters Tern, Greater Blue Herons and Bald Eagles has been attributed to embryo toxic effects of organochlorine compounds (Wiemeyer *et al.,* 1993; Cobb *et al.,* 1994; Giesy *et al.,* 1994). Reproductive deficiencies were noticed when levels of PCBs were in Double-crested Cormorants in the Great Lakes, USA (Gilbertson *et al.,* 1991; Tillitt *et al.,* 1992; Yamashitha *et al.,* 1993) and in the Netherlands (Van den Berg *et al.,* 1992). Most of the toxicity was believed to be caused by dioxin- like compounds including planar PCBs (Braune and Norstrom, 1989; Van den Berg *et al.,* 1994). However, only a few studies assessed differences or similarities in terms of toxicity contribution by various PCB congeners. Only in recent years, there have been increased interests in examining environmental samples for individual PCB congeners. Some PCB congeners and related compounds elicit a reverse spectrum of toxic and biochemical responses including body weight loss, dermal disorders, liver damage, thymic atrophy, reproductive toxicity, immunotoxicity, and the induction of CYPIA 1 and CYPIA 2 gene expression. The mono- ortho substituted PCB congeners, namely 105, 118, 123, 114 and 167 are thought to have high toxicological and biochemical activities (Safe, 1990).

Predominant accumulation of PCB isomers (having IUPAC numbers 99, 105, 118, 138, 153 and 180) were found in birds of Japan (Guruge and Tanabe, 1997; Guruge *et al.,* 2000), England (Boumphrey *et al.,* 1993), Poland (Falandysz *et al.,* 1994) and America (Mora, 1996). It is also reported that the isomer of PCBs in avian species is contributed mainly through their food (Boumphrey *et al.*, 1993; Mora, 1996).

The actual mechanisms of PCB congeners' accumulations are complicated because of their differential biochemical, toxicological and interactive effects based on their structure. Guruge and Tanabe (1997) and Guruge *et al.*, (2000) pointed out, that the preferential accumulation of PCB congeners among bird species is due to the adjacent meta- para chlorine unsubstituted position in either rings of biphenyls and has lower biodegradability for CYPIA enzyme as a result of estimated MI (Metabolic Index) values. An experimental study (Love *et al.*, 2003) has determined that exposure to elevated levels of PCBs is associated with significant impairment of the corticosterone response system in captive birds. The persistence of PCBs, combined with the sensitivity of the hypothalamo - pituitary - adrenal (HPA) axis to organochlorine insult, suggest that exposure to these contaminants may cause delayed functional deficits later in development or life.

Investigation on PCB congeners in Black Cormorants *Phalacrocorax carbo sinensis* (Falandysz *et al.*, 2003) breeding at the coast of the Gulf of Gdansk, Baltic Sea and in eggs of Black-crowned Night-Heron *Nycticorax nycticorax* (Rattner *et al.*, 2001) collected from Baltimore Harbor revealed the accumulation of toxic congeners. Total PCB concentrations in birds were in the range of 750 to 5200 ng/g wet weight in liver of Black Cormorant. This study also found that penta, hexa and hepta CBs, were the dominating homologue classes both in the liver and muscle tissue. PCB exposure suppresses egg laying in females and delays clutch initiation, reduces the clutch size and fledging success in both males and females of American Kestrel *Falco spareverius* (Fernie *et al.*, 2001).

The accumulation of PCB congeners IUPAC 138, 153 and 180 was associated with diet of herons rather than metabolism (Boumphrey *et al.*, 1993). Eggs of several water birds showed different compositions of PCB congeners, which reflected differences in diet; lower chlorinated biphenyls were predominant in birds feeding on invertebrates and plant materials and higher chlorinated biphenyls were predominant in fish-eating birds. Some of the birds such as Spotted Owlet, Short-billed Mongolian Plover and Gull-billed Tern accumulated traceable levels of lower chlorinated PCBs. These observations reflect that a part of their diet may be invertebrates and plant material. In contrast, Ormerod and Tyler (1992) suggested that the PCB congener patterns in eggs of birds from Italy and Canada were similar, which reflected generalities in the assimilation or metabolism of PCBs by this species.

Concentrations of PCBs including non- mono and di-ortho congeners, were determined in migratory and resident birds collected from India and Lake Baikal in Russia (Kunisue *et al.,* 2003). Among the 11 different species examined, total PCB concentrations were in the range of 11- 4500 ng/g. IUPAC 105, 118, 138, 153 and 180 were the predominant congeners in almost all the birds (Kunisue *et al.,* 2003).

In birds, the levels of PCBs in the brain needed to cause behavioral changes are generally about one order of magnitude below that cause mortality (Peakall, 1985). However the brain residues of PCB and DDE necessary to cause death (310 vs 300-400 $\mu$g/g respectively) and behavioural effects (5-6 Vs 3-8 $\mu$g/g respectively) are the same (Peakall, 1985). The PCB isomer 153 was the most dominantly accumulated congener in all the species collected from India (Tanabe *et al.,* 1998), while Kentish Plover, Cheeked Tern and Common Tern recorded slightly higher ratio of PCB 99, 105, 118 (Kunisue *et al.,* 2003).

During the last few decades, contamination by POPs such as PCBs and other organic pollutants has spread all over the world as evidenced by their detection in a wide range of environmental media and biota (Tanabe, 2001). Senthilkumar *et al.*, (1999) reported isomer-specific pattern of accumulation of polychlorinated biphenyls in resident, and wintering migrant birds collected from South India. The residue concentrations of PCBs were 120-1000 ng/g in strict resident birds, 190-890 ng/g in local migrants, 80-1900 ng/g in short-distance migrants and 100-2000 ng/g in long-distance migrants.

PAHs are known carcinogens and exposure to these compounds may result in adverse effects on survival, growth, metabolism, blood and tissue chemistry, and tumor formation (Eisler, 1986). Troisi *et al.*, (2006) reported PAHs and hydroxylated PAHs in liver samples from oiled Common Guillemots *Uria aalge* stranded along the East Coast of England. The mean parent and metabolite PAH concentrations were 0.25 ± 0.09 (range 0.04-0.97) and 0.52 ± 0.14 (range 0.05-1.48) $\mu$g/g, respectively. The PAH composition was in the order of pentacyclic < tri- and tetracyclic < tricyclic < dicyclic PAHs.

Besides the physical effects due to oiling, chronic toxicity of PAHs contamination is an important factor influencing long term recovery of oiled sea birds following oil spills. Chronic toxicity effects were reported when birds ingest oil contaminated food items regularly. It leads to subtle effects on reproduction such as decreased egg production, reduced fertility and hatchability and decreased sperm production as well as reduced immunologic functions (immunodepressive drugs) and impaired disease resistance (Rocke, 2001).

Birds exposed to oil during their reproductive season can also transfer lethal doses of the contaminants to their eggs during incubation. Even small quantities of oil on eggshell can kill embryo (Rocke, 2001). Induction of hepatic monooxygenase activity after exposure to PAHs has been documented in birds both in laboratory and field (Walters *et al.*, 1987; Peakall *et al.*, 1989; Trust *et al.*, 1994).

Three hepatic monooxygenase activities; ethoxyresorufin-O-dealkylase (EROD), benzylosyresorufin-O-dealkylase (BROD), and methoxyresorufin-O-dealkylase (MROD), have been significantly correlated with PAH concentrations in wild Lesser Scaup carcasses (Custer *et al.*, 2000). In a study of Tree Swallows located at a refinery site on the North Platte River, Casper, Wyoming, average hepatic monoxygenase activities in Tree Swallow livers were more than nine times higher than in Tree Swallow livers from birds located at a nearby uncontaminated reference site (Custer *et al.*, 2001).

Ludwig *et al.,* (1996) demonstrated the consistent relationship between egg mortality in Cormorants and Caspian Terns and exposure to certain planar polychlorinated diaromatic hydrocarbons, and that the new evidence of a dose-response relationship between the incidence of deformities in cormorants and exposure to these same compounds infers a causal relationship. Based on their knowledge of laboratory and field studies, Giesy *et al.,* (1994) concluded that the lethality and deformities in embryos of colonial fish-eating birds of the Great Lakes are caused by the toxic effects of multiple compounds which express their effects through a common mode of action, mediated by the Ah-receptor.

In Indian context there are studies on the levels of organochlorine pesticides as referred in chapter 3 on many species of birds. However, studies on the levels of PCBs and PAHs in birds are scares. Even in the international context information on the ill effects of physical deposition of oil in sea birds is available, but accumulation of PAH in internal organs and the impact on birds are very limited. The present study was carried out with following objectives;

## 4.3. Objectives

- Assessing the PCB and PAH concentrations among various species of birds in Ahmedabad and Coimbatore.
- Document the variation in residue concentration among tissues, years and between sexes.
- Create baseline information on PCB and PAH residues in birds of the referred locations in India.

## 4.4. Materials and Methods

### *4.4.1. Sample collection*

All samples were collected between 2005 and 2007 from Ahmedabad (Gujarat) and Coimbatore (Tamil Nadu) (Details about study area and methodology are given in chapter 2). A total of 330 individuals comprising 13 spices were included for this chapter (Table 4.1). Soon after the death, postmortem examination was conducted and tissues

**Table 4.1.** List of species included for PCB and PAH analyses

| S. No. | Species | Scientific Name | Ahmedabad | Coimbatore | Total |
|---|---|---|---|---|---|
| 1 | Little Cormorant | *Phalacrocorax niger* | NA | 8 | 8 |
| 2 | Indian Pond-Heron | *Ardeola grayii* | NA | 12 | 12 |
| 3 | Indian Peafowl | *Pavo cristatus* | 5 | 13 | 18 |
| 4 | Indian White-backed Vulture | *Gyps bengalensis* | 13 | NA | 13 |
| 5 | Pariah Kite | *Milvus migrans govinda* | 63 | 3 | 66 |
| 6 | Grey Partridge | *Francolinus pondicerianus* | NA | 11 | 11 |
| 7 | Blue Rock Pigeon | *Columba livia* | 72 | 8 | 80 |
| 8 | Asian Koel | *Eudynamys scolopacea* | 7 | 15 | 22 |
| 9 | Small Green-billed Malkoha | *Phaenicophaeus tristis* | NA | 16 | 16 |
| 10 | Crow Pheasant | *Centropus sinensis* | NA | 11 | 11 |
| 11 | Common Myna | *Acridotheres tristis* | 6 | 12 | 18 |
| 12 | House Crow | *Corvus splendens* | 6 | 21 | 27 |
| 13 | White-headed Babbler | *Turdoides affinis* | NA | 28 | 28 |
| | Total | | 172 | 158 | 330 |

NA – Not available

were transported over ice to the laboratory at SACON, Coimbatore by air.

### *4.4.2. Sample processing*

About 10g of the tissue was ground with anhydrous sodium sulphate and the mixture was packed in a thimble, which was dissected overnight prior to extraction to remove moisture. The desiccated thimbles were loaded in soxhlet extractors and the samples were extracted with 250 ml of hexane and dichloromethane mixture in the ratio 1:1 for a period of 8 hrs. The extracts were condensed in a rotary flask evaporator to a specific aliquot (5 ml) and the same was separated in to two portions for PCB and PAH analyses.

#### *4.4.2.1. Processing of samples for PCBs*

The condensed aliquot with high lipid content was transferred to a separating funnel and digested with 1-2 ml of concentrated sulphuric acid. The digested extract was fractionated by passing through a column packed with silica (60-120 mesh) adsorbent eluting with 100 ml of hexane for the first fraction and then with a mixture of 75 ml hexane and 25 ml dichloromethane for the second fraction. Both the fractions were separately collected and condensed in rotary flask evaporator to about 1 ml. As per the standard protocol PCB residues are expected to elute in the first fraction while the second fraction is expected to have organochlorine pesticides.

#### *4.4.2.2. Processing of samples for PAHs*

The condensed aliquot with high lipid content was digested overnight with 10 ml of potassium hydroxide (0.5 N). Using separating funnel the lipid was removed from the samples. Further cleaning was performed by using an activated silica gel in a column packed with 1g of sodium sulphate (silica gel and sodium sulphate were previously heated at 130°C for 16 hours) and eluted with 100 ml of dichloromethane to elute PAH residues. The eluants were concentrated using rotary flask evaporator. The eluants were blown using nitrogen and redissolved in 2 ml acetonitrile (CAN) and transferred into HPLC auto sampler vials for PAH analysis (Hu *et al.*, 2006).

### *4.4.3. Chemical analysis*

Quantification of PCBs was performed by injecting an aliquot (1$\mu$l) of the final extract in to a gas chromatography (Hewlett Packard: 5890 series II) equipped with a $Ni^{63}$ electron capture detector in a splitless mode. The column (J & W Scientific, CA, USA) was a fused silica capillary (0.32 mm i.d x 30m length) coated with a 0.5 $\mu$m thickness (DB-608) 5% diphenyl and 95% dimethyl polysiloxane as stationary phase. The oven temperature was programmed from 100°C (1 min hold) at a rate of 10°C/min to 300°C (5 min hold). Injector and detector temperatures were maintained at 250 and 300°C respectively. Nitrogen was used as carrier gas with a flow rate of 1.5 ml/min. A mixture of known PCB congeners' composition was used as a standard. In the present study 32 congeners of PCBs were identified (IUPAC Nos. 8, 28, 52, 49, 44, 37, 74, 67, 60, 66, 101, 99, 87, 77, 82, 118/114, 153, 179/105, 138, 126/158, 166, 183, 128, 156, 180, 169, 170 and 189). All the peaks were quantified by comparing individual peak area of the samples with the corresponding peak area of the standard. The detection limits were in the range of 1ng/g. The average recoveries varied between 75 and 105 per cent. Results were not corrected for the recovery.

A mixture 15 PAHs (Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenz(a,h)anthracene, Benzo(g,h,i)perylene, Pyrene, Benz(a)anthracene, Indeno(1,2,3-cd)pyrene) were quantified using HPLC with programmable fluorescence detector at excited and emission wavelength of 260 and 500nm respectively. About 10 $\mu$l of sample was injected through an autosampler into C18 column (Zorbax 4.6 x 250 mm) of 5 $\mu$m particle size. The temperature of the column was maintained at 20°C. Water/Acetonitrile (ACN) was used as mobile phase with a flow rate of 1 ml/min. The initial content of ACN was 50% and then increased into 60% (0-3 min) and 95 % (3-14 min). This

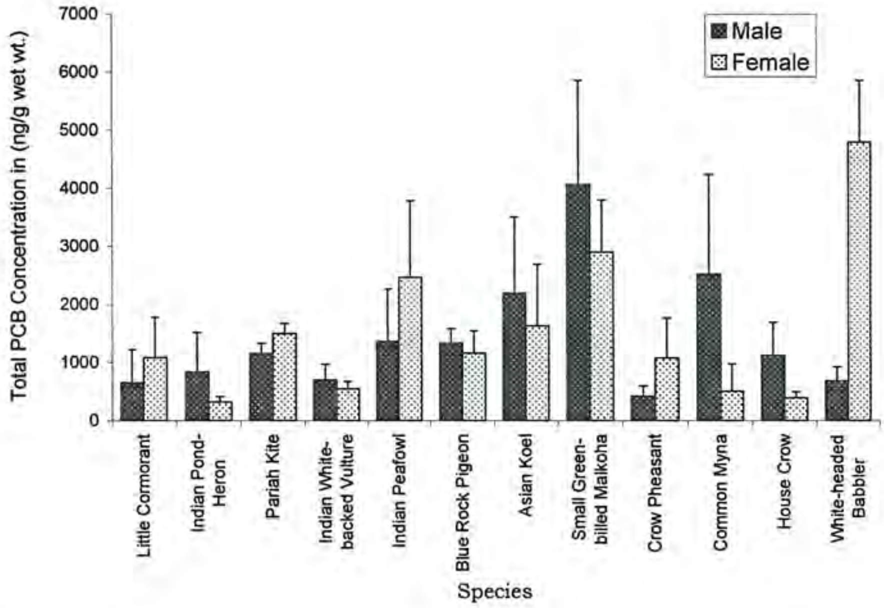



**Figure 4.1.** Variation in total PCB concentrations (mean ±SE) between female and male of various species of birds collected from Ahmedabad and Coimbatore during 2005 -2007.

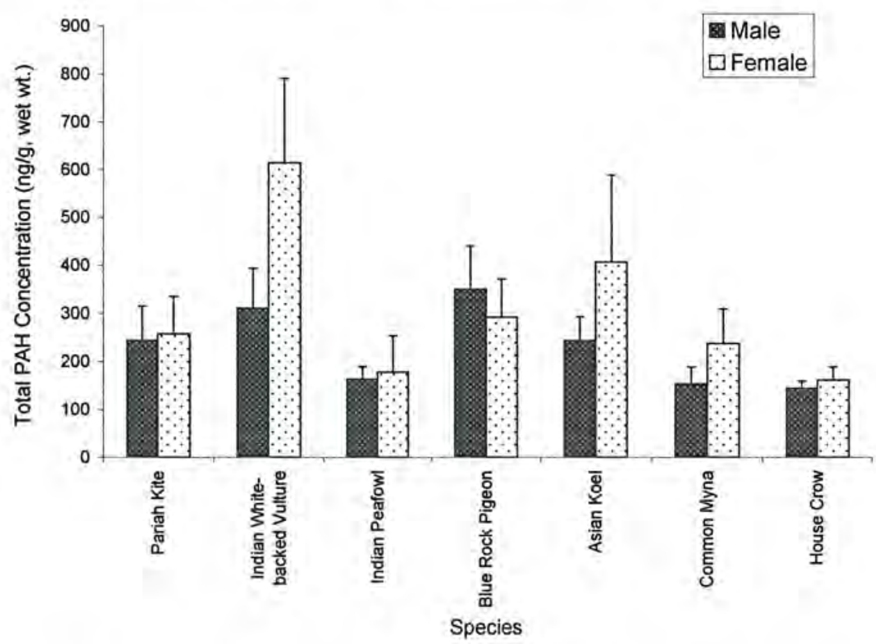



**Figure 4.2.** Variation in total PAH concentrations (mean ±SE) between female and male of various species of birds collected from Ahmedabad and Coimbatore during 2005 -2007.

Species specific comparison showed that Indian Peafowl (3648 ± 1791 ng/g) and Common Myna (3038 ± 2124 ng/g) collected from Ahmedabad had significantly higher PCB concentrations compared to those in Coimbatore (two-way ANOVA, $P < 0.05$). PCB concentrations were significantly higher in birds of Ahmedabad, than Coimbatore. It may be noted that species which were not received from both locations were excluded for species specific comparison between locations.

The total PAH concentration among various species of birds received from Ahmedabad and Coimbatore are presented in figures 4.3 and 4.4. Indian White-backed Vulture and Blue Rock Pigeon received from Ahmedabad had the highest concentration of total PAH (Figure 4.4). Although the maximum of total PAH was recorded in Indian White-backed Vulture and Blue Rock Pigeon received from Ahmedabad, it was not significantly different from other birds (two-way ANOVA, $P > 0.05$). Indian Peafowl had the least concentration of total PAHs of all the birds. There was no significant variation in total PAH was observed among various species of birds collected from Gujarat and Tamil Nadu (two-way ANOVA, $P > 0.05$).

### *4.5.4. Temporal variation in total PCBs and PAHs in birds*

The maximum mean total PCBs was recorded in Small Green-billed Malkoha (5333 ± 2300 ng/g) and White-headed Babbler (5080 ± 370 ng/g) during 2006 and minimum in Grey Partridge collected during 2005 (129 ± 56 ng/g) (Table 4.4). The mean total PAHs was the maximum in tissues of Indian White-backed Vulture collected during 2007 (838 ± 208 ng/g) and Blue Rock Pigeon collected during 2005 (422 ± 138 ng/g) (Table 4.5).

Mean total PCBs were significantly different among the species with respect to years (two-way ANOVA, $P < 0.05$). In 2006, the total PCBs was high among various species, while it was low in 2007. Species wise accumulation pattern of mean total PAHs did not vary, but year wise variation was significant (two-way ANOVA, $P < 0.05$). The minimum mean total PAHs was recorded in Indian Peafowl collected during 2005 (106 ± 29 ng/g) (Table 4.5).

**Table 4.4.** Total PCBs (ng/g wet wt.) among the birds collected in Ahmedabad and Coimbatore between 2005 and 2007.

| **Year ▶** | **2005** | | | | **2006** | | | | **2007** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species ▼** | **Mean** | **SE** | **Min** | **Max** | **Mean** | **SE** | **Min** | **Max** | **Mean** | **SE** | **Min** | **Max** |
| Little Cormorant | 178 | 100 | 30 | 369 | 1911 | 1221 | 690 | 3131 | 962 | 823 | 139 | 1784 |
| Indian Pond-Heron | 638 | 448 | 34 | 2862 | 356 | 129 | 78 | 702 | NA | NA | NA | NA |
| Pariah Kite | 1542 | 202 | 79 | 7480 | 1679 | 251 | 30 | 8656 | 613 | 117 | 59 | 3384 |
| Indian White-backed Vulture | 132 | 33 | 30 | 1047 | 855 | 447 | 57 | 2080 | 1268 | 385 | 75 | 7441 |
| Grey Partridge | 129 | 56 | 123 | 235 | 4882 | 3900 | 982 | 8781 | 167 | 119 | 48 | 285 |
| Indian Peafowl | 1560 | 701 | 224 | 2597 | 1723 | 1404 | 48 | 12883 | 1990 | 1135 | 76 | 15362 |
| Blue Rock Pigeon | 793 | 202 | 43 | 4524 | 1790 | 448 | 108 | 9156 | 1284 | 361 | 44 | 5453 |
| Asian Koel | 1091 | 863 | 35 | 8845 | 4325 | 1990 | 1596 | 8198 | 2254 | NA | 2254 | 2254 |
| Small Green-billed Malkoha | 2288 | 515 | 87 | 7461 | 5333 | 2300 | 205 | 19695 | NA | NA | NA | NA |
| Crow Pheasant | 316 | 243 | 73 | 559 | 585 | 494 | 91 | 1078 | 690 | 325 | 365 | 1014 |
| Common Myna | 4872 | 4453 | 419 | 9324 | 313 | 271 | 42 | 584 | 1071 | 457 | 336 | 1908 |
| House Crow | 1351 | 687 | 51 | 5711 | 342 | 83 | 110 | 758 | NA | NA | NA | NA |
| White-headed Babbler | 682 | 240 | 442 | 921 | 5080 | 370 | 4346 | 5524 | 4500 | 2305 | 899 | 8792 |

SE- Standard Error; Min- Minimum, Max- Maximum, NA – Not Available

**Table 4.5.** Total PAHs (ng/g, wet wt.) among the birds collected in Ahmedabad and Coimbatore between 2005 and 2007.

| **Year ▶** | **2005** | | | | **2006** | | | | **2007** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species ▼** | **Mean** | **SE** | **Min** | **Max** | **Mean** | **SE** | **Min** | **Max** | **Mean** | **SE** | **Min** | **Max** |
| Indian Peafowl | 106 | 29 | 59 | 198 | 112 | 29 | 60 | 205 | 260 | 67 | 65 | 526 |
| Indian White-backed Vulture | 130 | 15 | 77 | 204 | 390 | 132 | 60 | 1103 | 838 | 208 | 57 | 2037 |
| Common Myna | 172 | 61 | 60 | 365 | 172 | 67 | 60 | 477 | 227 | 73 | 60 | 529 |
| House Crow | 206 | 20 | 167 | 297 | 117 | 23 | 60 | 203 | 137 | 24 | 60 | 250 |
| Asian Koel | 341 | 152 | 60 | 2278 | 149 | 37 | 60 | 485 | 389 | 104 | 60 | 1155 |
| Pariah Kite | 369 | 180 | 67 | 2278 | 135 | 23 | 60 | 485 | 314 | 84 | 60 | 1155 |
| Blue Rock Pigeon | 422 | 138 | 60 | 1870 | 372 | 109 | 55 | 1527 | 171 | 29 | 56 | 349 |

SE- Standard Error; Min- Minimum, Max- Maximum.

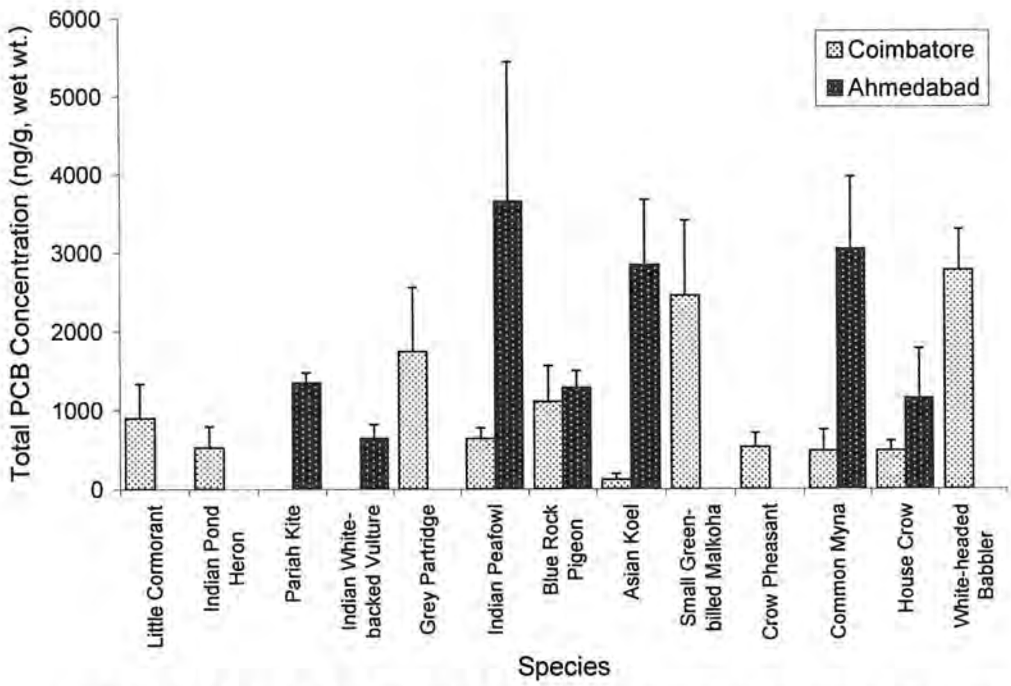



**Figure 4.3.** Variation in total PCB concentration (mean ±SE) among various species of birds collected from Ahmedabad and Coimbatore during 2005-2007.

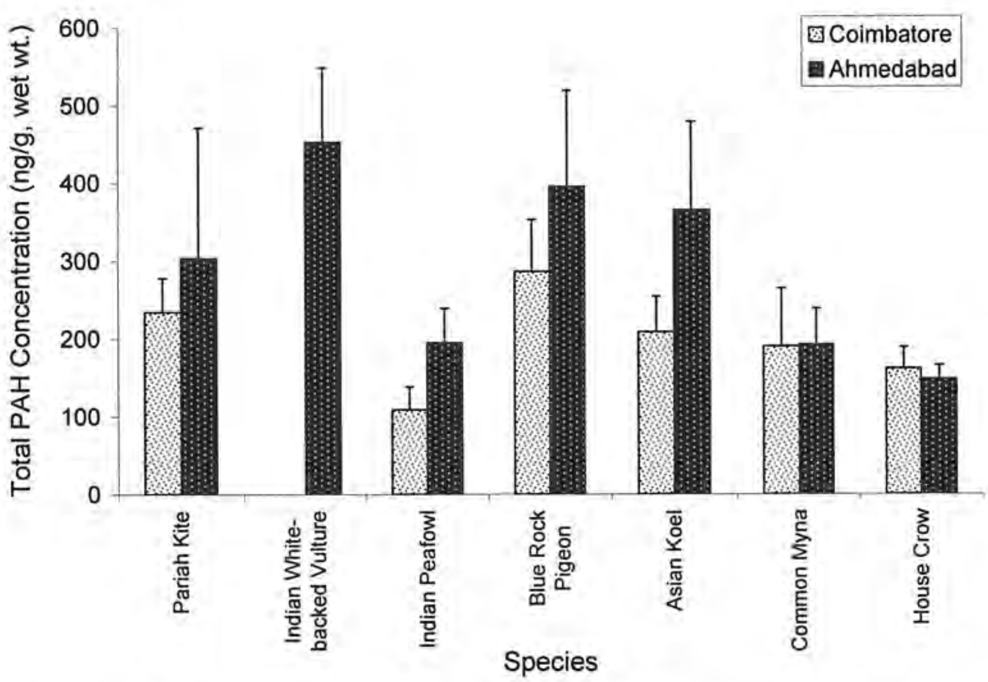



**Figure 4.4.** Variation in total PAH concentration (mean ±SE) among various species of birds received from Ahmedabad and Coimbatore during 2005-2007.

However, the residue pattern did not differ by the combined effect of place and year (two-way ANOVA, $P > 0.05$). Both contaminants showed lower concentration in 2005 and gradual increasing trend was observed in subsequent years among species.

## 4.6. Discussion

### *4.6.1. PCBs in birds*

Many of the birds had high concentrations of all higher chlorinated PCBs, which are well known for their persistence and low excretion rate in birds. However, differences in age classes were not considered. Species, namely Common Myna, Small Green-billed Malkoha, Grey Partridge, and White-headed Babbler exceeded 4000 ng/g of total PCBs. It is almost equal to the threshold values (4000-1500 ng/g wet wt.) of PCBs that experimentally produced reproductive problems in several bird species (Rice and O'keefe 1995).

The differences in contamination levels among species may be due to different feeding habits. This pattern of high accumulation of PCB congener in avian species is contributed mainly through their food (Boumphrey *et al.,* 1993; Mora 1996). The observed mean PCB concentrations were lower than those reported in birds of other countries (Goldberg, 1991; Fensterheim, 1993; Hoffman *et al.,* 1998). Considerably higher concentrations of total PCBs in birds of Ahmedabad than Coimbatore could be attributed to contamination in industrial areas. The differences in congener patterns of PCBs among bird species have been associated with differences in diet. Numerous investigations on Bald Eagles in North America have reported the OC exposure to be declining since the 1960s till now. This indicates a negative correlation between declining total OC residues in organs or egg contents, and the population recovery of Bald Eagles throughout the past decades (Grier, 1982). Biotransformations are unlikely for the PCBs, as majority of PCB congeners constituting total PCBs are recalcitrant in birds (Drouillard *et al.,* 2001). Hence, the reason for higher concentration of PCBs in birds in the present study may be due to the lower biodegradation capacity of this species.

### *4.6.2. Comparison of PCB concentrations with earlier studies*

The total PCB concentration recorded in various tissues of birds in this study are thirty to forty folds higher than the concentration reported in Black-winged Kite *Elanus caeruleus* (29 ng/g) (Tanabe *et al.,* 1998). While it is two to four folds higher than the levels (1600-2100 ng/g) which had showed reproductive impairment in Black Cormorant *Phalacrocorax carbo sinensis* (Dirksen *et al.,* 1995), almost similar to the levels (3600-7300 ng/g) reported to have caused tissue malformation in Double-crested Cormorant *Phalacrocorax auritus* (Yamashita *et al.,* 1993).

The total PCB concentration in these birds are greater than the concentration reported in the whole body concentration of total PCB in Crested Kingfisher *Megaceryle lugubris* (160 ng/g), the resident bird collected from South India and comparable with the levels reported in a short-distance migrant, White-cheeked Tern (430-4400 ng/g). It is also reported that the concentration of PCBs in resident birds of India were less than levels reported from other parts of the world. However, there has been a sign of increase in PCB concentration in birds from both locations over the years and it is assumed that PCB contamination might increase in India in the years to come due to rapid industrialization and development (Tanabe *et al.,* 1998).

Generally, birds positioned at the top of the food chain, such as kites, gulls, and eagles, accumulate elevated levels of OC contaminants (Newton, 1988). Frank *et al.,* (1977) documented greater residue levels in carnivorous birds from agricultural watershed areas in Kenya. Precisely bird species that primarily prey on fish and other birds accumulate PCBs to a greater extent than the birds feed on lower trophic level organisms (Ram and Gillet, 1993). In the present study, omnivorous species had higher load of total PCBs. In birds, PCB accumulation is related to the content and composition of prey, age, and residence time at contaminated sites (Struger and Weseloh, 1985). Additionally, unlike OC pesticides, PCB usage has been more in highly industrialized areas. Therefore, contamination by PCBs is more in birds found in industrial areas.

Although the tissues wise variation was not significant, liver tissues showed higher load of total PCBs. Individual PCB congeners exhibit different physico-chemical properties resulting in different profile of environmental distribution and toxicity (Giesy and Kannan, 1998). This may occur with HCH, as reported by Moisey *et al.,* (2001) in seabirds that are known to readily biotransform both $\alpha$- and $\gamma$-HCH but this would seem unlikely for the PCBs, as majority of PCB congeners constituting $\sum$PCB are recalcitrant in birds (Drouillard *et al.,* 2001). Hence it may be expected that the concentration of PCBs in liver can be at higher levels.

In the present study differences in the concentrations of PCBs between male and female were not significantly different. While a few studies reported the presence of elevated concentrations of OCs in males (Larsson and Lindergren, 1987; Duursma *et al.,* 1989), several studies reported comparable or greater concentrations in females than in males (Lemmetyinen *et al.,* 1982; Elliott and Shutt, 1993). Although some exceptions were found in the present study, difference between sexes could be perceived better if the sample size was larger.

The total PCB residues in various tissues of birds were in the range of 300 to 180,000ng/g as reported in Little Egrets *Egretta garzetta* which were found dead or dying in Tokyo Bay (Doguchi, 1973). Generally, PCBs were present at higher levels than DDE, and high concentrations of these compounds occurred in biota from waters adjacent to the city (Ohlrndorf *et al.,* 1978). It can be suggested that the presence of petroleum based industries in Ahmedabad might have influenced the excessive exposure of PCBs in this species. In addition, a relatively high concentration of PCB 118 was observed in this species which implies that there may be input of technical PCBs into the Ahmedabad region. The concentration reported in the present study is suspected to affect embryo development in Pariah Kite as reported by many researchers (Barron *et al.,* 1995; Falandysz *et al.,* 2003).

### *4.6.3. Comparison of PAH concentration with earlier studies*

In the present study, detectable concentrations of total PAH were found in the tissues of many of the species of birds tested. The total PAH concentration reported in the tissues of present study species were higher than the concentration reported in the whole body concentration of total PAHs (0.12-0.16 $\mu$g/g) in Lesser Scaup *Aythya affins* colleted from Indiana Harbor Canal, USA (Custer *et al.*, 2000) and wild juvenile Common Eider Ducks *Somateria mollissima* from the Baltic Sea (Naf *et al.*, 1992).

Although the present PAHs levels do not reflect the full extent of exposure, the residues of parent PAHs indicate that the sites are contaminated with PAHs. The source of PAHs in the tissue of birds studied in the present investigation could be through food chain accumulation (Kayal and Connel, 1995; Custer *et al.*, 2000; Aas *et al.*, 2000). The similar observations were reported in bird tissues from contaminated sites by Custer *et al.*, (2000 and 2001). The PAH concentration recorded in the present study is an order of magnitude lower than those reported in environmentally exposed (oil spill on sea birds) Common Guillemots *Uria aalge* stranded on the East Coast of England (2001-2002) (Troisi *et al.*, 2006).

The PAH concentration in bird tissues will create many biochemical changes and lead to certain diseases. Significant correlation was reported between PAH contamination and changes in hepatic mono oxygenase activities, ethoxyresorufin-o-dealkylase (EROD), benzylosyresorufin-o-dealkylase (BROD) and methoxyresorufin-o-dealkylase (MROD) (Custer *et al.*, 2000a). The hepatic monoxygenase activity was increased nine times higher in the liver of Tree Swallows located at a refinery site than the uncontaminated reference site in the North Platte River, Casper, Wyoming (Custer *et al.*, 2001). PAHs are also reported to be responsible for the somatic chromosomal damage in blood samples of exposed individuals (Custer *et al.*, 2000a). However, the present study did not included blood for this analyses.

The specific pattern of PAH accumulation depend on the source of contamination. It has been observed that concentration of total PAHs are considerably higher in brain than those in other tissues. The mean total PAH concentration recorded in birds included in the present study is found to be similar to the levels reported in the liver tissues of oiled Common Guillemots of East Coast in England during 2001-2002 (Troisi *et al.*, 2006).

Generally the total PAHs in liver relate to the number of benzene rings in the molecular structure and indicate the petrogenic source of contamination. Such observations were noted in aquatic bird species, namely Lesser Scaup, Redheads, Silver Gulls, Australian Pelicans (Kayal and cannel, 1995; Custer *et al.*, 2000; Aas *et al.*, 2000) and Common Guillemots (Troisi *et al.*, 2006). Biotransformation may be a reason for the detection of lower concentration of total PAHs in liver than other tissues. Liver acts as an active site for biotransformation than other tissues.

Benzo(a)pyrene and chrysene have been shown to induce embryotoxicity in Mallards *Anas platyrhynchos*. Topical applications in eggs (0.0336 x $10^{-3}$ $\mu$g bexzo(a)pyrene/g and 0.273 x $10^{-3}$ $\mu$g chrysene/g) had caused deformities, growth reductions, and doses of 2.0 mg/kg/egg, benzo(a)pyrene/g and 0.273 x $10^{-3}$ $\mu$g chrysene/g had caused deformities, growth reductions, and mortality (Hoffman and Gay, 1981, Eisler, 1987). However, the PAH concentration in the tissues of present study are less than the levels reported to be creating deformities in birds. Toxicity can vary with the amount and types of individual contaminants in the tissues of birds (Ramirez, 1997). There is no much of information available to compare the present finding. However, the levels can also be treated as references values, as these birds are collected from Ahmedabad which were victims to kite flying.

Sublethal effects of PAHs include gastrointestinal irritation, pneumonia, damage to red blood cells, immune system suppression, hormonal imbalance, impaired reproduction and reduced growth as reported by Albers (1995) in oil ingested birds. Based on the mean total PAH concentration, the risk of acute effects to birds is low.

Ahmedabad is one of the most polluted cities in Gujarat, with various industries including refining operations, industrial and municipal discharges, waste oil disposal and urban runoff. Ingestion of oil either through food or during preening is considered to be the primary source of PAHs to birds (Taniguchi *et al.*, 2003).

### 4.7. Conclusion

This is first study that compares the concentrations of PCB congeners and PAH among various tissues of birds in India. Among various tissues analysed for PCBs and PAHs, it is observed that the liver tissue recorded the maximum concentration. It can be suggested that the petroleum-based industries and electroplating industries located in Ahmedabad might have influenced the excessive exposure of PAHs and PCBs in these species. Although the birds collected for the present study were victims to kite flying in this region, it is suspected that the concentrations recorded may affect embryo development. Comparatively fewer loads of PCBs and PAHs in the birds of Coimbatore is due to less oil based industries in the city and its surroundings.

Although, the PCB contaminants detected in these individuals are not likely to have an impact on the demographic performance of the birds as a whole, the residues may reach levels causing decreased reproduction or survival over the years, particularly when combined with other non-anthropogenic stressors such as food scarcity. In addition, the birds normally would have more exposure to the contaminants due to their position in the food chain. This study also proved that PCB, 118, 138, 153, 180, 99 and 182 are the dominant congeners among the tissues of birds. Incidentally, none of the tissues tested is free from PCB congeners. Variation in PCB concentration among the various tissues and between sex are not significant in many except some specific congeners.

The total PAHs in brain tissues of many of the study species appeared to be higher than the levels reported elsewhere. However, there was no significant variation observed in total PAH contamination among tissues and species. There are no studies on PAH concentration in specific tissues in relation to whole body load. Hence, no comparison could be made. Total PAH concentration was high in male Blue Rock Pigeon while in Pariah Kite it was in females. However, the variation in contamination was not significant between sex and among years.

Although a few experimental studies have shown the effects of Polycyclic Aromatic Hydrocarbons (PAHs) in 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, particularly petrochemical industries to the environment. This study recommends a continuous monitoring for PCBs and PAH and their metabolites in various species of birds in Ahmedabad.

**Chapter 5**

# ORGANOCHLORINE PESTICIDE RESIDUES IN EGGS OF BIRDS IN TAMIL NADU

## 5.1. Introduction

Birds associated with agro ecosystem and their eggs have been proved as suitable tools for monitoring the impact of environmental contaminants. Eggs are particularly advantageous sample units for analysis of organochlorines as they decompose slowly and so are easily handled. The toxic effects related to exposure to high levels of environmental contaminants have been reported in avian species living particularly in polluted ecosystems (Newton, 1988; Bernes, 1998). The population level effects of pesticides through reduction in survival at different states of the life cycles on many species of birds have been reported across the world (Mateo *et al.,* 2000; Lotter and Bouwman, 2001; Krol *et al.,* 2002).

Pesticide residues in the eggs reflect the levels in the female birds at the time the eggs were laid. Added to this, concentrations in eggs give an indication of reproductive success of the birds (Ohlendorf *et al.,* 1978; Medvedev and Markova, 1995). The organochlorine pesticides have been creating adverse effects on wild bird population by means of two mechanisms; increased mortality and reproductive failures (Newton, 1979; Mateo *et al.,* 2000).

Among the various environmental contaminants, DDE a metabolite of DDT is the most widespread pollutant and accounts for the major parts of pollutants in eggs (Fitzner *et al.,* 1988). The reported adverse effects of organochlorine pesticides in birds are eggshell thinning, hatching failure, decreased reproductive success, disrupted physiological function (Bishop *et al.,* 2000; Tabassum *et al.,* 2003) embryo mortality, chick edema (Gilbertson *et al.,* 1991; Yamashita *et al.,* 1993) and teratogenic dysfunction. It was reported that the Brown Pelican *Pelicanus occidentals* has been one of the most sensitive bird species to the eggshell thinning and the levels of DDE approximately 3

$\mu g/g$ wet weight in eggs increased egg breakage and decreased productivity (Mora *et al.*, 2003).

About 70% of the species in various agro-ecological zones in India reported a significant decline in population of natural enemies of pests and also a large number of beneficial birds and other soil flora and fauna (Shetty, 2002). However, the information on the residue levels in eggs and the impact on birds are scarce in India. There are some information on the levels of organochlorine available in eggs of a few species of colonial water birds breeding at Keoladeo National Park, Bharatpur (Muralidharan *et al.*, 1992) and few avian eggs in Tamil Nadu (Regupathy and Kuttalam, 1990).

However, studies are not adequately replicated through time and number of individuals. Hence, it becomes essential to understand the species wise variation in contamination load and identify both the source and levels of contaminants in eggs of birds in agro ecosystem to interpret the demographic impact of exposure. It is also heartening to note that India is the second largest manufactures of basic pesticides and consumer of pesticide in South Asia next to Japan. Despite the proliferation of different types of pesticides, organochlorine pesticides such as HCH and DDT still account for two-third of the total consumption in the country because of their low cost and versatality in action against various pests (Yakushiji, 1988; Mukherjee and Gopal, 1996).

**5.2. Background Information**

Environmental contaminations in avian species lead to eggshell thinning, and ultimately result in lowered population recruitment. This phenomenon has been demonstrated in several species by controlled ingestion studies. DDE inhibit formation of carbonic anhydrous in the shell gland (Stickel, 1973; Haseltine *et al.*, 1975) leading to impaired eggshell formation and reproductive failure (Ratcliffe, 1970).

Eggshell thickness and the physical dimensions of embryo brains were measured in Double-crested Cormorants nesting in Green Bay and

Lake Michigan, Wisconsin, USA. Concentrations of organochlorine, including *p,p'*-DDE, PCBs and PCB congeners were generally an order of magnitude higher in eggs and chicks from Wisconsin than from reference location (Custer *et al.*, 2001).

The pesticides need not kill the individual birds unless exposure level is high, but do affect their bodies and they lay eggs with very thin shells. These thin-shelled eggs, break or the birds are unable to reproduce. Pesticides also affect bird's physiological function (Tabassum *et al.*, 2003). Fasola *et al.*, (1987) reported eggshell thickness reduction in Night Heron when the concentration of DDE was 3.3 $\mu$g/g. Blus *et al.*, (1974) reported a maximum of 2.37 $\mu$g/g DDE in an egg of Brown Pelican from a successful nest and 8.48 $\mu$g/g in an egg from an unsuccessful nest. In contrast, eggs of the Dalmatian Pelican *Pelecanus crispus* with a concentration of 6.91 $\mu$g/g of DDE showed relationship with the eggshell thickness, and did not affect the reproductive success of this species (Crivelli, 1989).

Henny and Herron (1989) conducted a study on the reproduction of White-faced Ibis at Carson Lake, Nevada. The results revealed the fact that as DDE in eggs increased to > 4 $\mu$g/g (wet wt), and especially > 8 $\mu$g/g, productivity decreased significantly ($P < 0.05$) and the incidence of cracked eggs increased and 4 $\mu$g/g of DDE as critical level. The correlations between concentrations of chlorinated hydrocarbons in eggs and biological effects measured in the field are established both on colony and individual clutch level. It is also reported that the long term toxicity in birds are often observed when the organochlorine pesticide concentration in eggs are above 0.5 $\mu$g/g (Fox, 1976; Hall *et al.*, 1989; Castillo *et al.*, 1994).

Organochlorine concentrations in the egg are about equal to whole body concentrations found in the female at the time the egg was laid (Keith and Gruchy, 1972). During incubation, however, the developing embryo appears to metabolize DDT to DDD and DDE (Blus *et al.*, 1974). Lindane metabolites instead of lindane were found in all samples probably due to its short life in the environment (Blus *et al.*, 1985) and the elevated amounts of beta HCH may indicate that lindane is still in use in the referred region. Lindane is not harmful to birds, in contrast to heptachlor and especially its metabolite heptachlor epoxide being lethal for birds in concentrations ≤ 9 $\mu$g/g (Blus *et al.*, 1985).

Dieldrin above 1 $\mu$g/g in the eggs of Golden Eagle *Aquila chrysaetos* may cause reproductive problems (Stickel, 1973). Dieldrin at a level of 1 or more $\mu$g/g in the eggs of the Brown Pelican was associated with reproductive impairment (Blus, 1982) and 0.54 $\mu$g/g was lethal to the embryos (Blus *et al.*, 1974). According to King *et al.*, (1978), in view of all the great variation in toxicity of dieldrin to different species, residues in eggs greater than 1 $\mu$g/g must be treated as hazardous and viewed with concern.

Muralidharan *et al.*, (1992) conducted a study on organochlorine residues in the eggs of select colonial water birds breeding at Keoladeo National Park, Bharatpur, India. The study recorded higher concentrations of dieldrin in eggs of the Large Cormorant (1.54 $\mu$g/g), Indian Shag (2.94 $\mu$g/g), Darter (1.52 $\mu$g/g), Grey Heron (5.95 $\mu$g/g), Cattle Egret (2.52 $\mu$g/g), Painted Stork (5.78 $\mu$g/g) and Spoonbill (1.3 $\mu$g/g). As the concentrations in all the eggs were higher than 1 $\mu$g/g, it was suggested that the concentrations would have been associated with reproductive impairments as suggested by Stickel (1973).

Vijayan (2003) reported the population decline in House Sparrow, one of the common birds in the entire country. House sparrow is the fast disappearing 'common' bird and this species can still be spotted at over two thirds of world's land surface. Moreover, the information on the declining trend of House Sparrow was reported all over India and around the world. Although, reductions in population of many species of birds have been reported in India, no study attempted to figure out if pesticides were responsible. However, circumstantial evidences could point towards pesticides. Systematic studies on the impact of agricultural chemicals on any species of bird are absent in this country. The present study is not a complete ecological investigation, but efforts are made to document levels of some of the persistent organic contaminants in eggs of 25 species of birds which are collected from different districts of Tamil Nadu on opportunistic basis.

### 5.3. Objectives

- Estimate organochlorine pesticide residues in eggs of select species of birds.
- Identify the possible risks on the birds, and
- Help contribute to the existing information base whereby any impending threat could be understood.

### 5.4. Materials and Methods

#### *5.4.1. Study Sites*

Egg samples were collected from seven districts in Tamil Nadu (Figure 5.1).

***Coimbatore District***

Coimbatore districts is comprised of variety of agricultural activity and equipped with electroplating and textile industries. It is located between $10^{\circ}55'$ and $11^{\circ}10'$ N, and $77^{\circ}50'$ and $76^{\circ}50'$ E at an approximate altitude of 470m. Although it is an industrial town, agriculture is practiced in vast areas in the district. The major crops cultivated are cotton and sugarcane. The pesticide, namely quinolphos, endosulfan, fenthion, carbaryl and sulphur compounds are used on crops to control pests. Many species of common terrestrial birds are observed in this area. Karamadai, a village located north of Coimbatore and Anaikatty reserve located at the foothills of Western Ghats were chosen as the study area.

***Dharmapuri District***

Industrial development in Dharmapuri district is confined only to a few pockets like Hosur, Krishnagiri and Bargur blocks. The strength of the district lies in its horticulture resources and agricultural products like paddy, cotton, sugarcane, groundnut and millets etc. Samples were collected from Kallipattai, a village located 50 kms north–east of Salem on the Thiruppathur highway.

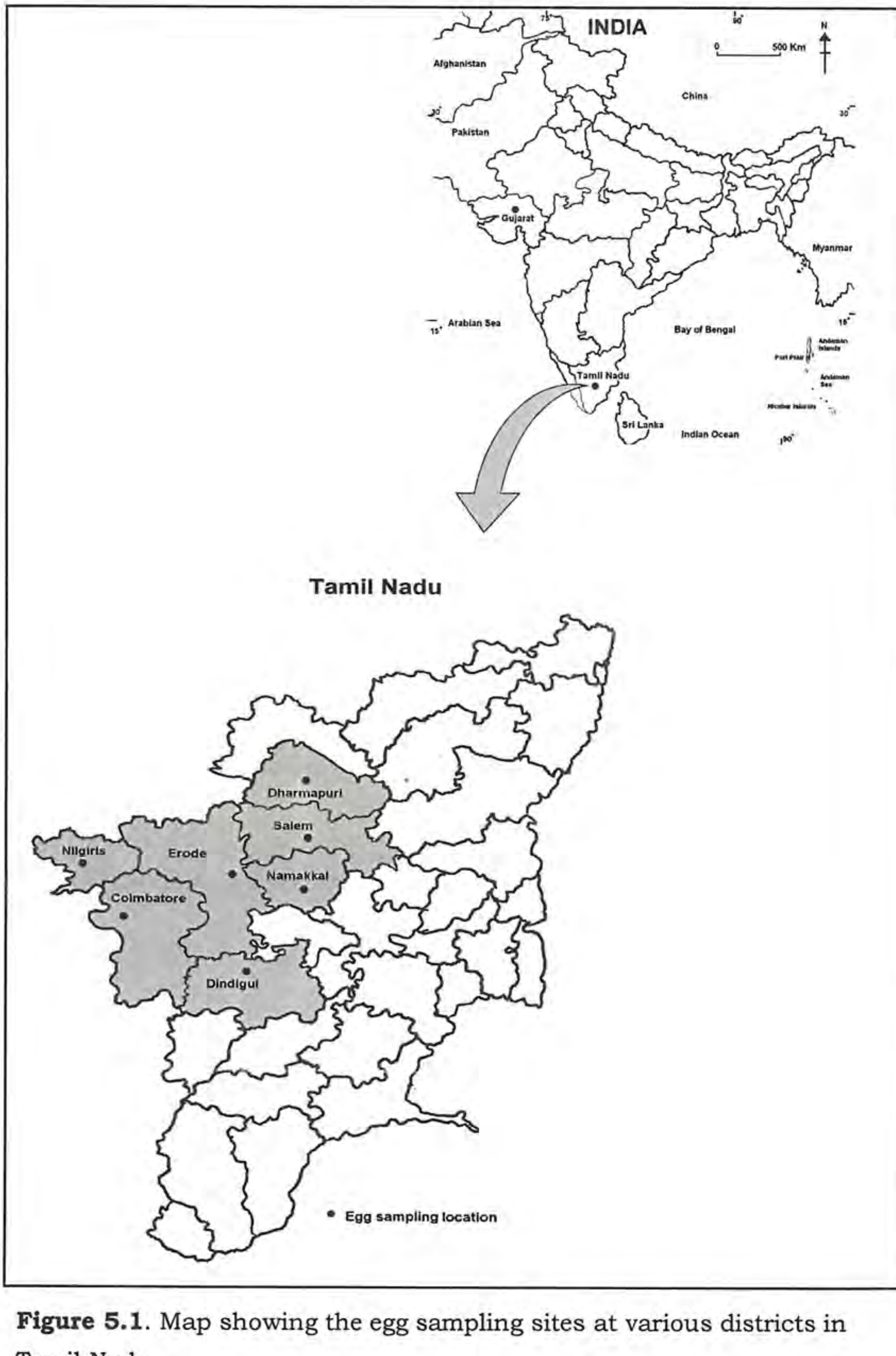



**Figure 5.1**. Map showing the egg sampling sites at various districts in Tamil Nadu

Ekalus, endosulfan, sevin powder, demecron are the most common pesticides used to protect the crops from pest with the help of mechanical and hand sprayer. Farmers are ignorant of the ill effects of pesticides on birds although they are aware of the fact that there are a few beneficial birds.

***Nilgiri District***

Kotagiri in the District of Nilgiris was chosen as a representative area for plantation crops. The study area was largely confined to two tea estates, namely Curzon and Kodanad in Kotagiri taluk. While Curzon is about 130 acres, Kodanad is about 120 acres in extent. In the vicinity of the estates, vegetables, namely carrot, beetroot, pea, potato, tomato, brinjal, cauliflower, cabbage, beans etc., are cultivated. The dominant tree species found in the area are Custard Apple *Annona reticulata,* Eucalyptus *Eucalyptus globulus,* Bamboo *Bamboo arundinaceae,* Naval *Syzygium cumini.* While use of many organochlorine compounds such as DDT is stopped due to regulation, and export restrictions, enormous amount of chemicals, namely quinolphos, dimethoate, endosulfan, fenthion, carbaryl and sulphur compounds are used on tea as well as vegetable crops.

***Salem District***

Eggs were collected from Kullamudayanur located 14 kms east of Mettur in Salem district which is yet another study area. Intensive study was carried out in about 100 acres of land where ragi, maize, beans, groundnut, black gram, green gram, horse grams and tomato were the major crops. Scrub jungle is the major habitat around the agricultural land. The most common pesticide used in this area was endosulfan, ekalus and sevin powder.

***Dindigul District***

Kukkal, located in Poomparai reserve forest, Dindigul district is 35 Km northwest of Kodaikanal town. Elevation of the places is about 1850m; this area is now considered as important bird area in India (Islam and Rahmani, 2004). The primary vegetation now is confined to isolated pockets such as Kukkal (330 ha) and Mathikettan (100 ha), both are listed as Nature Reserves. Wattle, Eucalyptus and Pine are the major plantation which alone accounted for 23,037 ha in 1986-87. Expansion of agriculture, requirements of local people for timber and fuel wood and cattle grazing made a significant effect on the natural vegetations.

***Erode District***

Sathiyamangalam division lies in the northern part of Erode district and is bound on the north by Karnataka state, on the east and south by Erode forest division and in the west by Nilgris division. The tract falls between the latitude 11°29'15" to 11°48'4" and longitude 8°76'50" to 77°27'22". Sathiyamangalam is blessed with rich variation of wild fauna. Hassanur plateau, Kinchigull valley are some of the places for observing wildlife and avifauna. Agriculture is the main activity of the rural people of Sathiyamangalam Taluk. Main crops in the wetlands are paddy, sugarcane and banana.

***Namakkal District***

In Tamil Nadu, Namakkal District is prominent in poultry farming and also in generating large amount of poultry wastes. The issue of the death of hundreds of Crows and Pariah Kites at the poultry waste disposal sites in September 2003 and the outbreak of avian flu in poultry industry in India in January 2004 and its various impacts over the environment and economy made to choose this district as one of the study sites.

### *5.4.2. Sample collection and storage*

Between 2001 and 2006, unhatched eggs were collected during nest monitoring visits in the study areas as soon as it became evident that they were not going to hatch. Totally 202 eggs belonging to 25 species of birds (Table 5.1) were collected from nests which were deserted. Efforts were made not to disturb any active nests. The collected eggs were wrapped in aluminum foil transported to laboratory and refrigerated at 3–4°C until they were opened for chemical analysis. Egg samples were examined for crack and other abnormalities. Each egg was cleaned with detergent in warm water and air dried prior to processing.

### *5.4.3. Sample processing*

Each egg was weighed using top loading electronic balance, Metler AE420. Length and diameter were measured by precision, 0.1 mm; Scienceware 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 four weeks prior to measuring thickness. Known quantity of egg contents were dried with required amount of 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 x 1.5 cm) for extraction and clean up (Wardall, 1977) 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 50-100 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 organochlorine pesticide were reconstituted in 2 ml of hexane. Processed samples were stored in deep freezer at -20°C until gas chromatography (GC) analysis.

### *5.4.4. Organochlorine residues analysis*

The eggs collected from 2001 to 2006 were analyzed at Sálim Ali Centre for Ornithology and Natural History (SACON), Coimbatore, India. The final extracts of 202 egg samples collected from different sites in Tamil Nadu were analyzed for the following organochlorine pesticide residues; an isomer mixture of Hexachlorocyclohexane (ΣHCH) consisting of *α*-, *β*-, *δ*- and *γ*-HCH (lindane), DDT metabolites (ΣDDT) including the basic isomers, namely *p,p'*-DDE, *p,p'*-DDE and *p,p'*-DDD, heptachlor, heptachlor epoxide, dieldrin, *α*-endosulfan, *β*-endosulfan and endosulfan sulfate. Gas Chromatography (GC) Hewlett Packard 5890 series II equipped with a $Ni^{63}$ electron capture detector with Nitrogen as carrier gas was used for the measurements.

The column oven temperature was programmed from 180°C, held for 3 min, then increased to 270°C at the rate of 10°C $min^{-1}$ and held for 20 min. Injector and detector temperatures were set at 250°C and 280°C respectively. The GC column employed was DB-608 fused silica capillary column (30 m x 0.32 mm; J & W Scientific Inc., Folsom CA) coated with 35% phenyl methyl polysiloxane. An equivalent mixture provided by Dr. Ehrenstorfer Laboratories (Germany) was used as the standard. 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 (50 ng/g) ranged from 94 to 103% and the results are not corrected for per cent recovery and the results are expressed in wet weight basis. Procedural blanks were run for every set of ten samples and 5% of the samples were run in duplicates. The minimum detection limit for all the compounds analysed was 1 ng/g.

### *5.4.5. Eggshell measurements*

An index of eggshell thickness was obtained by weighing and measuring whole eggshells and calculating the Ratcliffe Index (RI), where RI = Weight (mg)/(Length (mm) x Breadth (mm) (Ratcliffe, 1970). Eggshells were gently washed with tap water before opening to remove attached debris and the excrement. Sometimes it was necessary to scrape the debris material off using a soft tissue. Eggshell thickness was measured to the nearest 0.001 mm with a digital micrometer (Mitutoyo, IP65, No.395-271) after the empty shells had dried at room temperature for at least one month. Six measurements of the shell and shell membranes were taken at the equator; a mean thickness value for each egg was derived from these measurements (Capein, 1977).

### *5.4.6. Statistical analysis*

Organochlorine residue concentrations were log transformed to satisfy the homogeneity of variance assumption of analysis of variance (ANOVA). Means were compared between locations and years only when more than one-half of the samples had detectable contaminant concentrations of a particular contaminant. For purpose of analysis, samples below the detection value were given one-half the detection limit before comparison of the means. Correlation among variables and between eggshell thickness and contaminants were determined using Pearson's rank correlation coefficient. The probability level determining significance was $P < 0.05$ for all statistical tests. All statistical calculations were performed using statistical software SPSS student version 10.

## 5.5. Results

### *5.5.1. Organochlorine insecticides in eggs of birds*

Totally 202 eggs comprising 25 species of birds collected from various places in Tamil Nadu (Table 5.1) between 2001 and 2006 were analyzed for organochlorine residues. Invariably, all the samples had one or more pesticide residues. Means and range are presented in Table 5.2. HCH residues were detected in 85% of samples analyzed, while 82% of the eggs had *p,p'*-DDE residues. 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, heptachlor, endrin and endrin aldehyde were not detected in any of the sample analyzed.

Among various HCH isomers, *β*-HCH was the most predominant contaminant in almost all the birds' eggs analyzed (Figure 5.2). Similarly, *p,p'*-DDE, the most stable metabolite of DDT, was found in highest levels in the eggs of all species studied (Figure 5.3).

**Table.5.1.** Details of eggs received from various places in Tami Nadu (2001-2006).

| S.No. | Species | Scientific Name | I | II | III | IV | V | VI | VII | VIII | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Grey Partridge | *Francolinus pondicerianus* | 5 | 11 | - | - | 4 | 3 | 7 | - | 30 |
| 2 | Jungle Bush-Quail | *Perdicula asiatica* | - | 3 | - | - | - | - | - | - | 3 |
| 3 | Red Junglefowl | *Gallus gallus* | 1 | - | - | - | - | - | - | - | 1 |
| 4 | Indian Peafowl | *Pavo cristatus* | - | 6 | - | - | - | - | - | - | 6 |
| 5 | Small Buttonquail | *Turnix sylvatica* | 11 | - | - | - | - | - | - | | 11 |
| 6 | White-breasted Waterhen | *Amaurornis phoenicurus* | - | 7 | - | - | - | - | - | 3 | 10 |
| 7 | Blue Rock Pigeon | *Columba livia* | 3 | 9 | - | - | - | - | - | - | 12 |
| 8 | Asian Koel | *Eudynamys scolopacea* | 1 | - | - | - | - | - | - | - | 1 |
| 9 | Rufous-backed Shrike | *Lanius schach* | 1 | - | - | - | - | - | - | - | 1 |
| 10 | Red-whiskered Bulbul | *Pycnonotus jocosus* | - | - | - | 1 | 2 | - | - | - | 3 |
| 11 | Red-vented Bulbul | *Pycnonotus cafer* | 11 | - | - | - | 3 | - | - | - | 14 |
| 12 | White-headed Babbler | *Turdoides affinis* | 11 | 3 | - | - | - | - | - | - | 14 |
| 13 | Nilgiri Flycatcher | *Eumyias albicaudata* | 1 | - | - | 2 | - | - | - | - | 3 |
| 14 | Plain Prinia | *Prinia inornata* | 7 | 5 | - | - | - | - | - | - | 12 |
| 15 | Common Tailorbird | *Orthotomus sutorius* | 2 | - | - | - | - | - | - | - | 2 |
| 16 | Pied Bushchat | *Saxicola caprata* | - | - | - | 4 | - | - | - | - | 4 |
| 17 | Indian Robin | *Saxicoloides fulicata* | 16 | - | - | - | - | - | - | - | 16 |
| 18 | Paddyfield Pipit | *Anthus rufulus* | - | - | - | - | 1 | - | - | - | 1 |
| 19 | Nilgiri Pipit | *Anthus nilghiriensis* | - | - | - | - | 1 | - | - | - | 1 |
| 20 | Purple Sunbird | *Nectarinia asiatica* | - | 4 | 4 | - | - | - | - | - | 8 |
| 21 | Spotted Munia | *Lonchura punctulata* | 13 | 5 | - | - | - | - | - | - | 18 |
| 22 | House Sparrow | *Passer domesticus* | 3 | 3 | 6 | - | 9 | 4 | - | - | 25 |
| 23 | Baya Weaver | *Ploceus philippinus* | - | - | - | - | - | 4 | - | - | 4 |
| 24 | Eurasian Blackbird | *Turdus merula* | - | - | - | 1 | - | - | - | - | 1 |
| 25 | White-bellied Shortwing | *Brachypteryx major* | - | - | - | 1 | - | - | - | - | 1 |
| | **Total** | | **86** | **56** | **10** | **9** | **20** | **11** | **7** | **3** | **202** |

I- Anaikatty Forest, Coimbatore, II– Coimbatore (Rural), III– Dharmapuri, IV- Kukkal, Dindigul, V- Ooty, VI- Mettur, VII-Namakkal, VIII- Erode, - Not received.

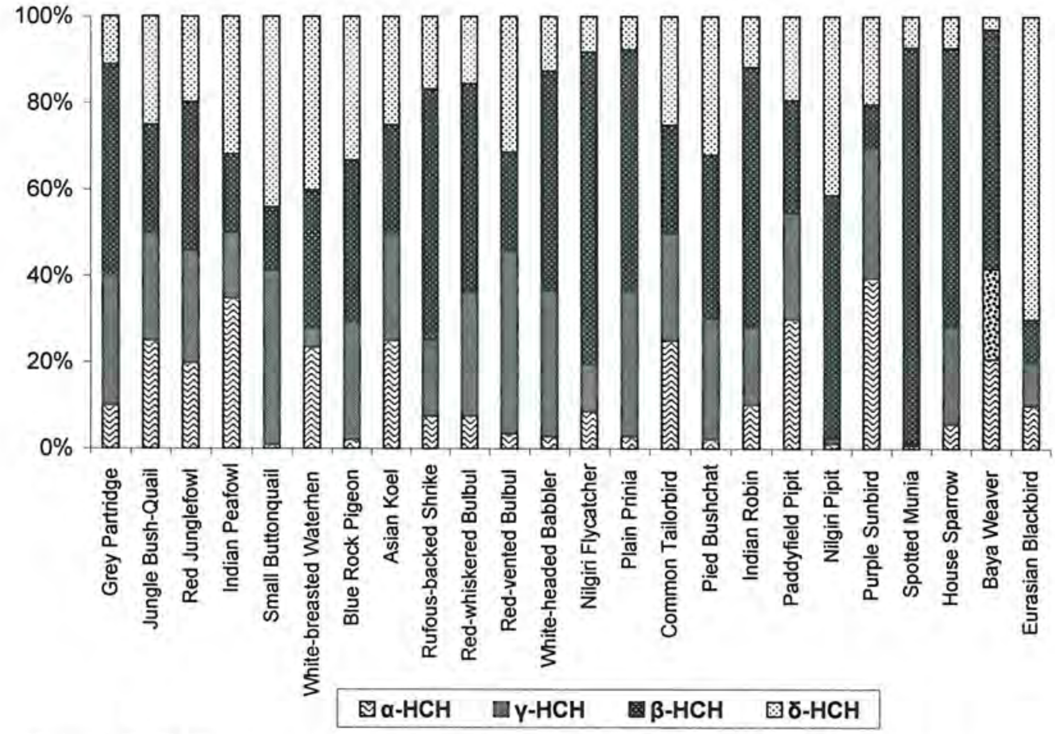



**Figure 5.2.** Percentage composition of HCH isomers in eggs of various species of birds.

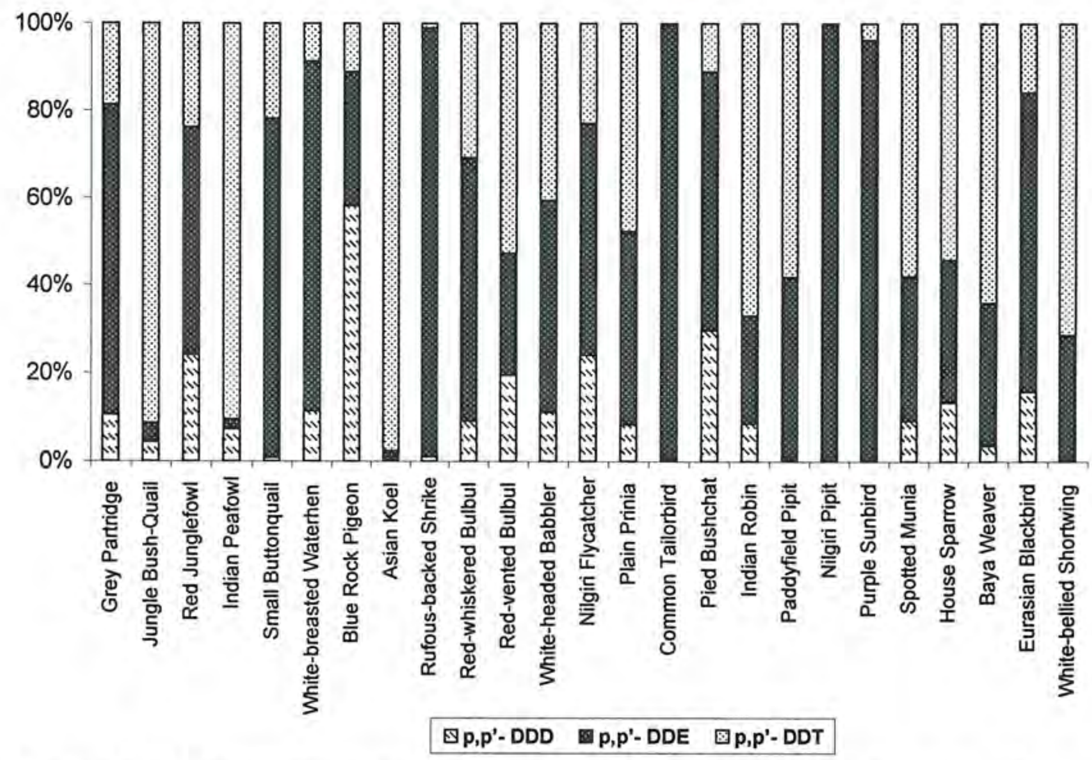



**Figure 5.3.** Percentage composition of DDT metabolites in eggs of various species of birds.

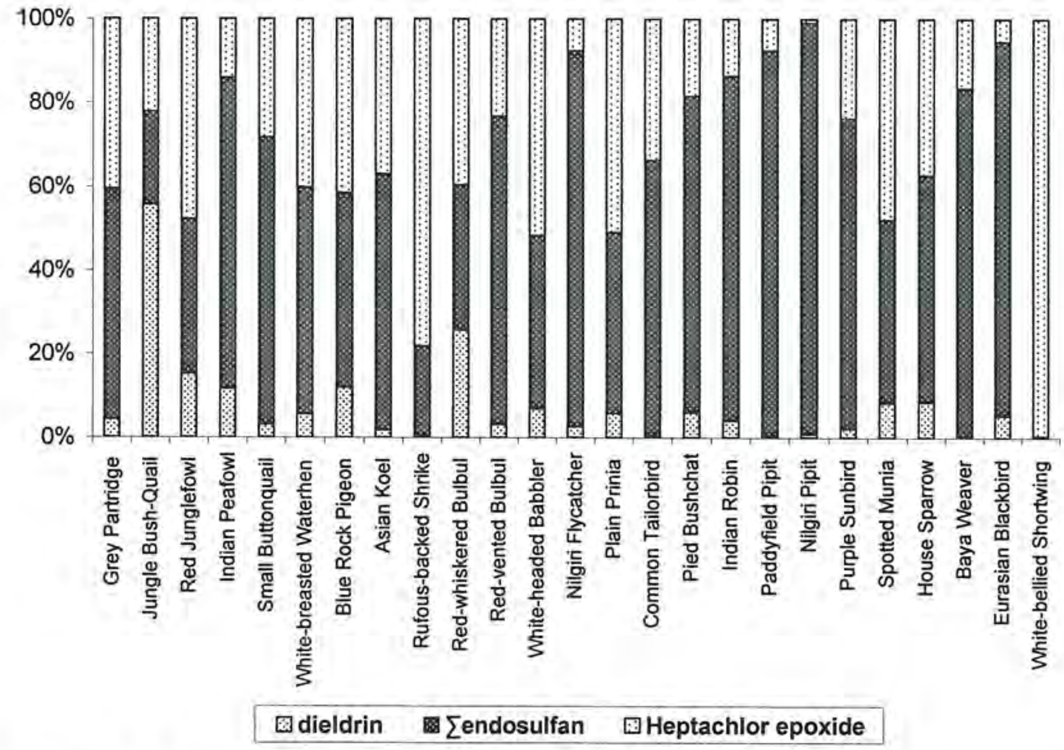



**Figure 5.4.** Percentage composition of cyclodiene insecticide residues in eggs of various species of birds.

Similarly, Among cyclodiene insecticides, endosulfan appeared to be the maximum (Figure 5.4), while dieldrin and heptachlor epoxide were the minimum.

### *5.5.2. Inter-species variation in organochlorine residues*

Among the various species studied, eggs of Spotted Munia had high levels of total HCH (5574 ng/g) and heptachlor epoxide (1072 ng/g) followed by other species, while the total OC residues were < 400 ng/g in, Jungle Bush-Quail, Small Buttonquail, Common Tailorbird, Nilgiri Pipit and Eurasian Blackbird (Table 5.2).

Bird species could be differentiated by their burden of HCH, heptachlor epoxide, DDE and endosulfan which had the highest load and variation ($P < 0.05$). However, there were no significant differences observed in *p,p'*-DDD, *p,p'*-DDT and total DDT and dieldrin among the species (Table 5.3).

**Table 5.2**. Organochlorine pesticide residues (ng/g, wet wt.) in the eggs of birds collected from various places in Tamil Nadu during 2001-2006 (Range in parentheses).

| Species | N | Σ-HCH | HE | *p,p'*-DDE | *p,p'*-DDD | *p,p'*-DDT | Σ-DDT | Dieldrin | Σ-Endosulfan |
|---|---|---|---|---|---|---|---|---|---|
| Grey Partridge | 30 | 340 (<DL -1841) | 101 (<DL -701) | 137 (0.4-881) | 20.24 (0.1-148) | 35.9 (<DL -287) | 192 (0.4-1168) | 10.6 (0.2-144) | 138 (<DL -1312) |
| Jungle Bush-Quail | 3 | <DL | <DL | <DL | <DL | 10.5 | 10.5 | 1.3 | 0.5 |
| Red Junglefowl | 1 | 2028 | 700 | 244 | 248 | 541 | 1035 | 223 | 547 |
| Indian Peafowl | 6 | 18.27 (<DL -37.9) | 16.27 (<DL -27.1) | 16.4 (2.8-35.2) | 50.5 (<DL -150) | 626.2 (<DL -1438) | 692 (2.8-1600) | 13.5 (<DL -39) | 86.1 (5-158) |
| Small Buttonquail | 11 | 57.8 (<DL -221) | 67.4 (<DL -265) | 48.6 (<DL -186) | 0.5 (<DL -0.78) | 13.63 (0.5-105) | 61.7 (<DL -186) | 7.39 (<DL -48.4) | 164 (<DL -863) |
| White-breasted Waterhen | 10 | 240 (<DL -971) | 238 (<DL -1767) | 12.8 (<DL -56.6) | 16.9 (<DL -57.4) | 119 (<DL -847) | 148 (12-868) | 32.6 (<DL -102) | 325 (1.8-1649) |
| Blue Rock Pigeon | 12 | 444 (<DL -2282) | 164.86 (<DL -644) | 170 (<DL -957) | 322.39 (<DL -2765) | 61.05 (<DL -414) | 553 (<DL -2876) | 47.3 (<DL -339) | 186 (<DL -1165) |
| Asian Koel | 1 | <DL | 10.5 | 9.8 | <DL | 411 | 421 | <DL | 17.5 |
| Long-tailed Shrike | 1 | 1371 | 322 | 46.1 | <DL | <DL | 46.1 | <DL | 90.1 |
| Red-whiskered Bulbul | 3 | 1702 (8.4-5080) | 372 (4.1-1107) | 551 (20.3-1612) | 83.9 (<DL -250) | 281.53 (15.8-796) | 916 (36.1-2659) | 243 (<DL -729) | 330 (37.2-904) |
| Red-vented Bulbul | 14 | 1119 (<DL -3533) | 618 (<DL -5146) | 115 (<DL -1010) | 81 (<DL -569) | 218 (<DL -902) | 414 (<DL -1763) | 85.3 (<DL -854) | 1975 (<DL -11603) |
| White-headed Babbler | 14 | 1217 (<DL -6326) | 444 (<DL -3601) | 181 (<DL -2172) | 41.72 (<DL-536) | 151 (<DL -680) | 373 (<DL -3263) | 59.2 (<DL -425) | 360 (<DL -2154) |

**Table 5.2.** *Continued...*

| Species | N | ∑-HCH | HE | *p,p'*-DDE | *p,p'*-DDD | *p,p'*-DDT | ∑-DDT | Dieldrin | ∑-Endosulfan |
|---|---|---|---|---|---|---|---|---|---|
| Nilgiri Flycatcher | 3 | 347 (48.2-640) | 22.17 (11.4-28.8) | 53.9 (<DL -160) | 56.6 (5.1-83) | 125.8 (59.1-164) | 236 (224-246) | 7.73 (<DL -22.2) | 263 (<DL -479) |
| Plain Prinia | 12 | 1678 (<DL -6677) | 118 (<DL -513.4) | 24.3 (<DL -94.3) | 4.58 (<DL -20.9) | 26.2 (<DL -96) | 54.2 (<DL -211) | 13.54 (<DL -54.3) | 101 (28.3-190) |
| Common Tailorbird | 2 | (<DL -0.5) | (<DL -306) | (100-108) | (0.4-0.56) | (0.5-0.5) | (100-108) | (<DL -0.5) | (287-319) |
| Pied Bushchat | 4 | 1559 (23.7-2607) | 125 (10.5-274) | 24.2 (<DL -48) | 65.1 (<DL -164) | 130 (<DL -305) | 219 (3.7-427) | 41.2 (18.8-83.5) | 517 (30.3-1718) |
| Indian Robin | 16 | 855 (20.9-5959) | 135 (<DL -1015) | 58.9 (<DL -316) | 20.5 (<DL -269) | 161 (<DL -894) | 240 (<DL -979) | 40.3 (<DL -252) | 819 (<DL -8996) |
| Paddyfield Pipit | 1 | 141 | 16 | 54.1 | <DL | 75.7 | 129 | <DL | 196 |
| Nilgiri Pipit | 1 | 40.8 | <DL | 298 | <DL | <DL | 298 | 0.5 | 53.7 |
| Purple Sunbird | 8 | 290 (<DL -937) | 37.3 (<DL -84.8) | 26.06 (<DL -119) | <DL | 680 (<DL -2633) | 706 (<DL -2752) | 3.58 (<DL -15.9) | 117 (<DL -727) |
| Spotted Munia | 18 | 5574 (329-14943) | 1072 (79.3-3163) | 86.3 (<DL -610) | 24.07 (<DL -212) | 542 (<DL -1777) | 565 (8.6-1777) | 60.9 (<DL -352) | 1246 (<DL -4753) |
| House Sparrow | 25 | 1294 (8.2-15091) | 103 (<DL -65) | 91.3 (<DL -1298) | 37.3 (<DL -748) | 151.66 (<DL -1477) | 279 (<DL -2402) | 23.8 (<DL -360) | 152 (<DL -1726) |
| Baya Weaver | 4 | 347 (16.2-1105) | 55.1 (3.9-159) | 24.6 (1.7-73.4) | 2.7 (<DL -8.3) | 48.4 (<DL -161) | 75.3 (15.2-164) | 0.53 (<DL -0.6) | 280 (<DL -1095) |
| Eurasian Black bird | 1 | 3.5 | <DL | 2.2 | <DL | <DL | 2.2 | <DL | 8.5 |
| White-bellied Shortwing | 1 | 287 | 238 | 30.5 | <DL | 77 | 107 | <DL | <DL |

HE- Heptachlor Epoxide, <DL- Below Detectable Limit

**Table 5.3.** Analysis of variance (ANOVA) among eggs of different species of birds' eggs analysed for organochlorine residues.

| **Pesticide residues** | **df** | **F** | ***P* value** |
|---|---|---|---|
| $\alpha$-HCH | 24 | 2.594 | 0.05 |
| $\gamma$-HCH | 24 | 2.677 | 0.05 |
| $\beta$-HCH | 24 | 1.817 | 0.01 |
| $\delta$-HCH | 24 | 1.762 | 0.02 |
| $\sum$-HCH | 24 | 3.472 | 0.05 |
| $\sum$-heptachlor epoxide | 24 | 2.083 | 0.05 |
| *p,p'*-DDE | 24 | 3.096 | 0.05 |
| *p,p'*-DDD | 24 | 1.191 | 0.25 |
| *p,p'*-DDT | 24 | 1.217 | 0.23 |
| $\sum$-DDT | 24 | 0.743 | 0.80 |
| dieldrin | 24 | 1.198 | 0.24 |
| $\sum$-endosulfan | 24 | 1.672 | 0.03 |

The variation in HCH levels in eggs of birds was highly significant ($P < 0.05$). Total HCH level was high and it ranged below detectable limits and 5574 ng/g. Ten species of birds exceeded 500 ng/g (Table 5.2). Asian Koel and Jungle Bush-Quail did not have any of the isomers of HCH. $\beta$-HCH was high in the eggs of Spotted Munia, Nilgiri Flycatcher, House Sparrow, Indian Robin, Nilgiri Pipit and Rufous backed Shrike. It constituted more than 50% of the total HCH (Figure 5.2).

Inter specific differences in total DDT were not significant among all the species tested ($P > 0.05$). Red Junglefowl (1035 ng/g) recorded the maximum of total DDT followed by Red-whiskered Bulbul (916 ng/g), Purple Sunbird (706 ng/g) and Indian Peafowl (692 ng/g). The lowest level was detected in the eggs of the Eurasian Blackbird and Jungle Bush-Quail and. *p,p'*-DDE, the most toxic metabolite of *p,p'*-DDT ranged between 2.2 ng/g in Eurasian Blackbird and 551 ng/g in Red-whiskered Bulbul. It may be noted that *p,p'*-DDE and beta HCH contributed the maximum to the total DDT and HCH respectively.

Among the cyclodiene group of pesticides, heptachlor epoxide and total endosulfan showed significant difference among species.

**Table 5.4.** Mean organochlorine pesticide residues (ng/g, wet wt.) in the eggs of birds collected from various districts in Tamil Nadu (Range in the parenthesis).

| Pesticides | Anaikatty | Coimbatore | Ooty | Metur | Namakkal |
|---|---|---|---|---|---|
| **Grey Partridge** | | | | | |
| N | 5 | 11 | 4 | 3 | 7 |
| Σ-HCH | 103 (<DL-409) | 1009 (329-1842) | 502 (34.4-1450) | 26 ( 21.1-31) | 166 (<DL-913) |
| Σ-DDT | 48 (0.4-193) | 74 (31.2-139) | 698 (3.3-1168) | 18 (<DL-35.3) | 255 (5.6-1055) |
| Σ-Endosulfan | 35 (0.9-125) | 265 (40.6-991) | 143 (16.7-245) | 19 (<DL-19.7) | 214 (<DL-1313) |
| Heptochlor epoxide | 28.2 (<DL-88) | 217 (49.1-364) | 104 (3.6-246) | 19 (17.8-20) | 138 (<DL-701) |
| Dieldrin | 1.1 (0.2-3.8) | 2.6 (0.4-11.7) | <DL | <DL | <DL |
| **Blue Rock Pigeon** | | | | | |
| N | 3 | 9 | NA | NA | NA |
| Σ-HCH | 2283(100-3083) | 240 (<DL-1495) | - | - | - |
| Σ-DDT | 120 (<DL-250) | 602 (<DL-2877) | - | - | - |
| Σ-Endosulfan | 128 (<DL-600) | 193 (<DL-1167) | - | - | - |
| Heptochlor epoxide | 334 (<DL-865) | 112 (<DL-513) | - | - | - |
| Dieldrin | <DL | 53.5 (<DL-340) | - | - | - |
| **Red-vented Bulbul** | | | | | |
| N | 11 | NA | 3 | NA | NA |
| Σ-HCH | 1021 (<DL-3533) | - | 1662 (1173-2150) | - | - |
| Σ-DDT | 478 (<DL-1764) | - | 67 (51.3-83.5) | - | - |
| Σ-Endosulfan | 1435 (<DL-11603) | - | 4943 (3823-6062) | - | - |
| Heptochlor epoxide | 725 (<DL-5147) | - | 34.3 (22.4-45.0) | - | - |
| Dieldrin | 100 (<DL-854) | - | <DL | - | - |
| **White-headed Babbler** | | | | | |
| N | 11 | 3 | NA | NA | NA |
| Σ-HCH | 180 (130-210) | 1529 (<DL-6327) | - | - | - |
| Σ-DDT | 17 (<DL-46.0) | 481 (0.6-3264) | - | - | - |
| Σ-Endosulfan | 689 (207-850) | 261 (<DL-2154) | - | - | - |
| Heptochlor epoxide | 144 (4.6-397) | 535 (<DL-3601) | - | - | - |
| Dieldrin | 2.7 (<DL-689) | 76.2 (<DL-426) | - | - | - |

**Table 5.4.** *Continued...*

| Pesticides | Anaikatty | Coimbatore | Ooty | Metur | Namakkal |
|---|---|---|---|---|---|
| **Plain Prinia** | | | | | |
| N | 7 | 5 | NA | NA | NA |
| ∑HCH | 65 (23.7-105) | 2755 (<DL-6678) | - | - | - |
| ∑DDT | 4.5 (<DL- 6.0) | 88 (19.3-211) | - | - | - |
| ∑Endosulfan | 109 (28.3-90) | 54 ( 56-141) | - | - | - |
| Heptochlor epoxcide | 11 (<DL-22.1) | 190 ( 22.6-513) | - | - | - |
| Dieldrin | <DL | 22.2 | - | - | - |
| **Purple Sunbird** | | | | | |
| N | NA | 4 | NA | NA | 4 |
| ∑HCH | - | 115 (<DL-457) | - | - | 446 (34-937) |
| ∑DDT | - | 41 (<DL-124) | - | - | 1373 (<DL-2752) |
| ∑Endosulfan | - | 222 (<DL-727) | - | - | 14 (<DL-25.9) |
| Heptochlor epoxcide | - | 34 (<DL-85.6) | - | - | 40 (<DL-74.3) |
| Dieldrin | - | 2.8 (<DL-10.5) | - | - | 4.4 (<DL-16.4) |
| **Spotted Munia** | | | | | |
| N | 13 | 5 | NA | NA | NA |
| ∑HCH | 1 (<DL-4.0) | 5575(329-14943) | - | - | - |
| ∑DDT | 27 (<DL-112) | 565 ( 8.6-1777) | - | - | - |
| ∑Endosulfan | 19 (<DL-88) | 1246 (<DL- 4754) | - | - | - |
| Heptochlor epoxcide | 2 (<DL-8.0) | 1073 (79.3- 3164) | - | - | - |
| Dieldrin | 4.9 (<DL-12.0) | 2.3 ( <DL- 9.0) | - | - | - |
| **House Sparrow** | | | | | |
| N | 3 | 3 | 9 | 4 | 6 |
| ∑HCH | 1226 (343-2936) | 7678 (264-15091) | 901 (36.8-2709) | 233 ( 8.2- 872) | 500 (2-2039) |
| ∑DDT | 178 (<DL-476) | 236 (155-317) | 113 (12.5-296) | 601 (<DL-2402) | 382 (<DL-1492) |
| ∑Endosulfan | 109 (<DL-327) | 429 (413-444) | 56 (32.6-120) | 432 (<DL-1726) | 39 (<DL-91.0) |
| Heptochlor epoxcide | 201 (<DL-439) | 58 (48.7-67.1) | 73 (17.8-186) | 166 (<DL-657) | 75 (<DL-248) |
| Dieldrin | <DL | 77.6 (<DL-155) | 2.7 (<DL-9.0) | 90.8 (<DL-360) | 5.1 (<DL-16.0) |

<DL- Below Detectable Limit; NA- Not Available.

0.05). The total HCH (1662 ng/g) and total endosulfan residues (4943 ng/g) were comparatively higher in the eggs collected from Kotagiri. Dieldrin was not detected in the eggs collected from Kotagiri, Ooty, while it was in trace level in the eggs from other districts.

**White-heated Babbler:** There was no significant difference between the sites where from the eggs came in (*t*-test, $P > 0.05$). However, one of the eggs collected from Coimbatore showed high concentration of organochlorine pesticides.

**Plain Prinia:** The variation in organochlorine pesticide residues was not significant between the study areas (*t*-test, $P > 0.05$). However, eggs collected from various places in Coimbatore showed higher levels than the levels recorded in Anaikatty forest.

**Purple Sunbird:** Residues of total HCH and total DDT in the eggs collected from Dharmapuri were higher than in the eggs from Coimbatore. Except total HCH and total DDT, other pesticide residues were not significantly different between the two sites. Heptachlor epoxide and dieldrin concentrations in eggs collected from both the places showed almost equal concentration, and the variation was not significant (*t*-test, $P > 0.05$).

**Spotted Munia:** All the eggs had very low concentrations of organochlorine residues except one egg collected from Coimbatore which had very high concentrations of organochlorine residues. The total HCH, total DDT, total endosulfan and heptachlor epoxide residues in the egg were 5575, 565, 1246 and 1073 ng/g respectively. Except dieldrin, all other residues were significantly different between the study sites (*t*-test, $P < 0.05$).

**House Sparrow:** Comparatively high concentrations of organochlorine residues were detected in samples collected from Coimbatore followed by Mettur district. The total DDT in eggs of Mettur and Dharmapuri was 601 and 382 ng/g respectively. Total HCH was high (7678 ng/g) in the eggs collected from Coimbatore. Dieldrin was not detected in the eggs collected from Anaikatty forest, Coimbatore. Significant variations in residue levels were observed among sites (Anova, $P < 0.05$).

### *5.5.4. Eggshell thickness and contaminants*

Egg measurements and eggshell parameters (eggshell thickness, shell weight, length and width and shell index) of all the 25 species of birds are tabulated (Table 5.5). The variation in eggshell thickness and shell index was highly significant among the species (Anova, $P < 0.05$). Quantitative relationship among organochlorine residue levels in various species of birds (n=202) is described in the correlation matrix (Table 5.6). Since the number of samples in each species was not adequate enough to be considered separately, all the eggs were considered together and all the contaminant data were transformed to $\log_{10}$ prior to analysis.

Thickness showed negative correlation with organochlorine residues. HCH, heptachlor epoxide, DDE and endosulfan had significant positive correlation ($P < 0.05$) among them. It is common for organochlorine residues in biological samples collected in the field to be positively correlated as all are lipophilic and tend to concentrate in fatty tissues (Klass *et al.*, 1978). However, further study of contaminants in each species and their prey is needed.

Linear regression analysis revealed the negative relationship between eggshell thickness and DDE concentration in eight species of birds (Table 5.6). Although, there was no strong correlation between other organochlorines and eggshell thickness, the negative influence on the eggshell thickness were observed.

**Table 5.5.** Egg measurements and eggshell parameters in various species of birds in Tamil Nadu.

| Eggshell parameters ▶ | N | Shell Thickness (mm) | | Shell Weight (mg) | | Length (mm) | | Width (mm) | | Shell Intex ($mg/mm^2$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species ▼ | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Grey Partridge | 30 | 0.08 | 0.01 | 1035 | 390.5 | 32.55 | 3.39 | 25.16 | 2.55 | 1.23 | 0.37 |
| Indian Peafowl | 6 | 0.08 | 0.02 | 7957 | - | 69 | - | 54 | - | 2.14 | - |
| Small Buttonquail | 11 | 0.08 | - | 130.1 | 31.24 | 22.33 | 1.25 | 18.52 | 1.07 | 0.32 | 0.08 |
| White-breasted Waterhen | 10 | 0.12 | 0.02 | 71.78 | 25.1 | 18.38 | 8.42 | 10 | 7.75 | 0.73 | 0.56 |
| Asian Koel | 1 | 0.23 | - | - | - | - | - | - | - | - | - |
| Red-whiskered Bulbul | 3 | 0.16 | - | 171.87 | 33.11 | 19.67 | 4.04 | 14.33 | 2.89 | 0.71 | 0.48 |
| Red-vented Bulbul | 14 | 0.06 | 0.01 | 47.99 | 19.57 | 21 | 1.87 | 14.69 | 1.49 | 0.16 | 0.06 |
| White-headed Babbler | 14 | - | - | 80.63 | 32.11 | 23.23 | 3.61 | 18.85 | 2.76 | 0.19 | 0.09 |
| Plain Prinia | 12 | 0.38 | 0.03 | 26.27 | 2.81 | 16.29 | 1.65 | 11.23 | 0.56 | 0.15 | 0.03 |
| Common Tailorbird | 2 | 0.2 | 0.08 | 67.65 | 0.5 | 12.5 | 2.12 | 13 | 2.83 | 0.42 | 0.02 |
| Pied Bushchat | 4 | 0.07 | 0.01 | 55.95 | 8.5 | 18 | - | 14 | - | 0.22 | 0.03 |
| Indian Robin | 16 | 0.32 | 0.04 | 53.76 | 14.18 | 18.75 | 1.16 | 13.71 | 1.2 | 0.21 | 0.05 |
| Purple Sunbird | 8 | 0.1 | 0.03 | 46.62 | 15.74 | 14.2 | 1.1 | 8.44 | 0.9 | 0.4 | 0.14 |
| Spotted Munia | 18 | 0.18 | 0.05 | 57.88 | 14.46 | 17.44 | 2.24 | 12.19 | 0.99 | 0.28 | 0.1 |
| House Sparrow | 25 | 0.52 | - | 51.07 | 15.15 | 17.58 | 2.41 | 12.79 | 1.91 | 0.24 | 0.09 |
| Baya Weaver | 4 | 0.11 | 0.02 | 156.9 | 2.88 | 21 | - | 15.25 | 0.5 | 0.49 | 0.02 |

- Not available

**Table 5.6**. Correlation matrix among organochlorine residues in the eggs of birds collected from various districts in Tamil Nadu (Significant at < 0.05) unless otherwise mentioned.

| | **γ-HCH** | **β-HCH** | **δ-HCH** | **∑-HE** | ***p,p'*-DDE** | ***p,p'*-DDD** | ***p,p'*-DDT** | **Dieldrin** | **∑-endo-sulfan** | **Thickness** |
|---|---|---|---|---|---|---|---|---|---|---|
| α-HCH | 0.645 | 0.612 | 0.489 | 0.491 | 0.396 | 0.164 | 0.211 | 0.172 | 0.284 | -0.368 |
| γ-HCH | | 0.631 | 0.675 | 0.703 | 0.535 | 0.315 | 0.145* | 0.345 | 0.454 | -0.561 |
| β-HCH | | | 0.492 | 0.490 | 0.464 | 0.254 | 0.014* | 0.295 | 0.397 | -0.342 |
| δ-HCH | | | | 0.562 | 0.496 | 0.337 | 0.151 | 0.256 | 0.303 | -0.366 |
| ∑-HE | | | | | 0.641 | 0.465 | 0.090* | 0.478 | 0.662 | -0.466 |
| *p,p'*-DDE | | | | | | 0.402 | 0.276 | 0.298 | 0.661 | -0.483 |
| *p,p'*-DDD | | | | | | | 0.245* | 0.424 | 0.455 | 0.018* |
| *p,p'*-DDT | | | | | | | | 0.346 | 0.105 | -0.021* |
| Dieldrin | | | | | | | | | 0.379 | -0.107* |
| ∑-endosulfan | | | | | | | | | | -0.441 |

Not Significant ($P > 0.05$)

**Table 5.6**. Correlation matrix among organochlorine residues in the eggs of birds collected from various districts in Tamil Nadu (Significant at < 0.05) unless otherwise mentioned.

| | **γ-HCH** | **β-HCH** | **δ-HCH** | **HE** | ***p,p'*-DDE** | ***p,p'*-DDD** | ***p,p'*-DDT** | **Dieldrin** | **∑-endo-sulfan** | **Thickness** |
|---|---|---|---|---|---|---|---|---|---|---|
| *α*-HCH | 0.645 | 0.612 | 0.489 | 0.491 | 0.396 | 0.164 | 0.211 | 0.172 | 0.284 | -0.368 |
| *γ*-HCH | | 0.631 | 0.675 | 0.703 | 0.535 | 0.315 | 0.145* | 0.345 | 0.454 | -0.561 |
| *β*-HCH | | | 0.492 | 0.490 | 0.464 | 0.254 | 0.014* | 0.295 | 0.397 | -0.342 |
| *δ*-HCH | | | | 0.562 | 0.496 | 0.337 | 0.151 | 0.256 | 0.303 | -0.366 |
| ∑-HE | | | | | 0.641 | 0.465 | 0.090* | 0.478 | 0.662 | -0.466 |
| *p,p'*-DDE | | | | | | 0.402 | 0.276 | 0.298 | 0.661 | -0.483 |
| *p,p'*-DDD | | | | | | | 0.245* | 0.424 | 0.455 | 0.018* |
| *p,p'*-DDT | | | | | | | | 0.346 | 0.105 | -0.021* |
| Dieldrin | | | | | | | | | 0.379 | -0.107* |
| ∑-endosulfan | | | | | | | | | | -0.441 |

HE- heptachlor epoxide, Not Significant ($P > 0.05$)

## 5.6. Discussion

In India there have been studies carried out in birds and their eggs for contaminants. However, no studies were systematic. The present study is the first to use eggs of terrestrial bird for contaminant analysis in India. In general, the measured levels of pesticides in eggs in the present study comparable to the values reported in similar species of birds in industrialized countries. Nevertheless, this study allowed revealing variations in the chemical contamination among species.

### *5.6.1. Organochlorine pesticide among species and between places*

The observed differences in the contamination by HCH and DDE among the bird species in this study may be explained by the variation in their metabolism (ability to accumulate, catabolism, and excretion of contaminants), diets, position in food chain, foraging strategies and feeding places, mobility and local migration as has been explained by many studies (Becker *et al.,* 1998; Mattig *et al.,* 2000; Thyen and Becker, 2000).

Of all the 25 species studied, Red-whiskered Bulbul, Purple Sunbird and Indian Peafowl were very much different from other species in terms of high concentration of total DDT levels. Although, Red Junglefowl had higher load of residues, it is difficult to make any conclusion because of small sample size. This might be explained by the fact that these species feed on insects, seeds and grains from the terrestrial food chain, especially from agricultural areas. Although DDT is banned for agriculture, it is not possible to explain the source unless DDT's use for malaria in the referred sites is confirmed. Nevertheless, DDT's persistence cannot be discounted.

The presence of *p,p'*-DDE in all the eggs could be associated with "old" DDT contamination (Tanabe *et al.,* 1998). Eggs of the Indian species were approximately eight times highly contaminated with these chemicals (Muralidharan *et al.,* 1992). Further investigation using eggs as bioindicators could help estimate the magnitude of recent input of DDT in to the terrestrial food chain in India.

HCH has been restricted in India for agricultural usage. Furthermore, a possible source of these compounds might be related to the use of gamma HCH, which is commercially available as lindane. The relatively high levels of HCH in the eggs of Spotted Munia, Red-whiskered Bulbul and Plain Prinia may be through consumption of contaminated food although they are not mainly associated with agricultural ecosystem. The levels of contamination varied not only among species, but also sites in response to site specific chemical contaminations.

### *5.6.2. Effects of the contaminants on bird population in India*

As chemical substances considered in this study are toxic, persistent, and they biomagnify in lipid-rich tissues throughout the food chain (Moriarty, 1990; Furness, 1993), they can negatively influence the birds' health, survival and reproduction (Ohlendorf *et al.*, 1978; Scheuhammer, 1987; Walker, 1994; Grassman *et al.*, 1998). In order to estimate the potential risk for birds, the threshold levels known and mentioned in the literature were considered carefully, although threshold levels vary among chemicals compounds, bird species, and other environmental factors. Additionally, low concentrations of individual toxicants could damage organisms by possible interactions and synergistic effects (Wachs, 1994; Busch, 1996).

The most predominant occurrence of $\beta$-HCH in eggs reflects its stable nature than the other isomers, namely $\alpha$-, $\gamma$- and $\delta$- isomers. Some of the eggs showed higher proportion of $\alpha$- and $\gamma$-HCH suggesting recent exposure to technical HCH mixture (containing 70% $\alpha$-, 15% $\gamma$-, 6% $\beta$ and 9% $\delta$- HCH) used in India (Tanabe *et al.*, 1998). Similarly, Nygard (1999) noticed the presence of elevated proportion of *p,p'*-DDE and $\beta$-HCH in Merlin eggs in Norway. As reported by Goutner *et al.*, (1997), lindane has not received much attention as far its effects on eggs of birds are concerned. Although several studies have been carried out, investigations pertaining to the levels of lindane are few (King *et al.*, 1978; Becker *et al.*, 1993). Lindane used as a heptachlor substitute for seed treatment, is safer to wild birds (Blus *et al.*, 1985) and thus, searching for it may not be useful when effects of chemicals on birds are investigated. However, varying levels of contaminants by lindane and its metabolites in eggs of birds reflect local contamination (Becker *et al.,* 1993) and recent exposure.

Many studies have associated high concentrations of DDT (between 500 ng/g and 6000 ng/g) specifically *p,p'*-DDE in bird eggs with impaired reproduction (Castillo *et al.,* 1994) and eggshell thickness with organochlorine contaminants (Blus *et al.,* 1974; Nygard, 1999; Ewins *et al.,* 1999). Similarly association between organochlorine pesticide usage and decline of raptor species were also reported (Newton, 1979; Mateo *et al.,* 2000). Since the levels found in the eggs of Grey Partridge, House Sparrow, Red-vented Bulbul and White-headed Babbler are above the threshold levels, the negative effects could be anticipated. Relatively, larger portion of *p,p'*-DDE, which is the major constituent (80%) of the technical mixture of DDT suggests recent exposure of these species to DDT in the study areas (Figure 5.3). HCH and DDT have been reported as the predominant OC pesticides in resident and local migratory birds in South India (Tanabe *et al.,* 1998). This is quite similar to the measurements in eggs of birds from other countries (Custer *et al.,* 1999; Nygard, 1999).

Frequency of occurrence of *p,p'*-DDE was the highest (87%) of all the pesticides tested. This confirms the continued presence of DDE in the environment. This could be largely due to the chemicals persistence and also its use in malaria control. The highest concentration (1.97 $\mu$g/g) of *p,p'*-DDE recorded in one of the eggs of House Sparrow collected from Mettur, Salem District showed that this species is at risk. It indicates the greater exposure of this species to the pesticide contamination. Area surrounding study sites are being subjected to massive application of pesticides for agriculture. However, use of DDT for agriculture could not be confirmed.

The relationship between DDE concentration and eggshell thinning have been reported in many species of birds, namely Dalmatian Pelican *Pelicanous crispus* (6.91 $\mu$g/g) (Crivelli *et al.,* 1989), Night Heron (3.3 $\mu$g/g) (Fasola *et al.,* 1987) and Great-crested Grebe (10 $\mu$g/g) (Galassi *et al.,* 2002) and Little Tern (Goutner, 1997) elsewhere.

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 population of any species of bird are unknown in Indian context, effects would depend on both concentration and species sensitivity. Regarding *p,p'*-DDT and *p,p'*-DDD the concentrations recorded in the present study were below the levels which created reproductive failure in wildbirds (Fox, 1976; Hall *et al.*, 1989; Castillo *et al.*, 1994) elsewhere.

In contrast to many studies in various countries on the levels of DDE, in the eggs of many species of birds, the levels reported in Indian birds are low. May be because of this, the present study did not find any relationship between organochlorine and eggshell thickness except *p,p'*-DDE. *p,p'*-DDE exceeded the threshold values of 0.5 μg/g proposed by Castillo *et al.*, (1994) in wildbirds. The concentration of *p,p'*-DDE and heptachlor epoxide in House Sparrow eggs collected from Mettur, Salem District and two eggs from Dharmapuri are above the threshold value of 0.5 μg/g. Levels of *p,p'*-DDE in Grey Partridge from Kotagiri, Ooty and heptachlor epoxide in one of the eggs of Plain Prinia from Coimbatore exceeded the threshold value of 0.5 μg/g. Although, the levels of *p,p'*-DDT in eggs of Purple Sunbird were higher than other species, not high enough to cause any adverse effects on reproduction.

Residues of heptachlor epoxide detected in the eggs of the present study species (non detectable level to 4.76 μg/g) are lesser than the levels reported by Muralidharan *et al.*, (1992) in the eggs of Large Cormorant in Keoladeo National Park, Bharatpur. However, this study does not show any direct evidence that heptachlor epoxide residues in the eggs was related to reduced eggshell thickness. Although, the concentration detected is less than the reported levels in Canada Goose *Branta canadensis* (> 10 μg/g, Blus *et al.*, 1984) and American Kestrel (>1.5 μg/g, Henny *et al.*, 1983). The maximum residues of heptachlor epoxide in the eggs of Grey Partridge, House Sparrow, Spotted Munia and Red-whiskered Bulbul in this study were above the levels which were associated with impaired reproductive success.

The levels of dieldrin in the eggs of House Sparrow, Plain Prinia, Purple Sunbird, Grey Partridge, Red-whiskered Bulbul and Baya Weaver are very low when compared with levels reported in eggs of the select species of colonial water birds breeding at Keoladeo National Park, Bharatpur, India (Muralidharan *et al.,* 1992). All the species in the present study recorded less than 1 $\mu$g/g of dieldrin, which is not expected to create ill effects to the bird populations (Stickel, 1973). King *et al.,* (1978) while summarizing the effects of dieldrin on various species of birds, reported considerable variation in the resistance. They proposed that amounts greater than 1 $\mu$g/g must be viewed as hazardous. Hence, dieldrin levels found in this study pose no damage to the birds.

The presence of elevated amount of beta HCH, the isomeric form of lindane may indicate that lindane is used in this region. Lindane is not harmful to birds, in contrast to heptachlor and especially its metabolite heptachlor epoxide which is lethal for birds in concentration $\geq$ 9 $\mu$g/g (Blus *et al.,* 1985). Among the various species of birds, eggs of Plain Prinia (mean 3.24 $\mu$g/g) and House Sparrow (mean 1.03 $\mu$g/g) recorded higher concentration of HCH. One of the eggs of House Sparrow, Spotted Munia, Red-whiskered Bulbul and Plain Prinia collected from Maruthamalai food hills, Coimbatore had higher concentration of total HCH, which reflects its use in the surrounding agricultural area. These values are higher than the levels recorded in the eggs of the same species from Anaikatty forest, Coimbatore. However, eggs of Plain Prinia could not be collected from rest of the three study locations. Further, varying levels of total HCH and its metabolites in eggs of six species of birds 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 selected colonial water birds of Keoladeo

National Park in Bharatpur, India (Muralidharan *et al.,* 1992). Although varying levels of HCH and its isomers recorded in many species of birds, their effects on reproductive success are not clear.

The concentrations of organochlorine residues recorded in House Sparrow were high. It is also reported that the population of House Sparrow, one of the common birds in the entire country is declining. However, this species can still be spotted at over two thirds of world's land surface (Vijayan, 2003). Moreover, declining population of House Sparrow is being reported all over India and around the world. Since levels of *p,p'*-DDE and heptachlor epoxide are above the threshold levels, the present situation should be viewed with concern. The spectacular, long tubular nests of the Baya, hanging from palm fronds, have become a sight of the past. Bayas today have become extremely rare to spot (Vijayan, 2003). Although the endosulfan concentration in the eggs of Baya collected from Mettur is high, due to the lack of historic information, no prediction is possible.

All the specie of birds had significantly different concentrations ($P < 0.05$) of all pesticide residues except DDD, DDT and dieldrin. However, it is obvious that there is consistent bioaccumulation through the food chain mechanisms. High frequency of detection of HCH, and endosulfan in all the study sites indicates the extensive application of these pesticides in agriculture fields. Additive and possibly synergetic effects may occur where many different agricultural pesticides are used. Moreover, in India information are not available in these species of birds. It may be noted that although, the survey revealed use of many pesticides belonging to organochlorine, organophosphorus and carbamate, the present study documented only the levels of organochlorines, as the other group of pesticides are not expected to get accumulated through the food chain. Agro-ecosystem in Tamil Nadu appears to be a contaminated environment with respect to HCH and endosulfan. Further, the impact of contaminants must be investigated in the light of the findings presented here. Studies in similar and other habitats are warranted.

### *5.6.3. Pesticide residues and eggshell thickness*

Concentration of *p,p'*-DDE in egg of many species of birds were negatively correlated with eggshell thickness, but not significant. This could be attributed due to smaller samples size. Even though DDT has been banned for agricultural purpose, the responses of birds' reproduction to DDE with unsuccessful reproduction believed in many species of birds. Other pollutants levels were relatively low, and were believed to have less significant influence on reproduction.

## 5.7. Conclusion

The present study showed wide range of pesticide contamination in eggs of all the species collected from various locations. Levels of *p,p'*-DDE detected in the eggs of Grey Partridge and Purple Sunbird, heptachlor epoxide in Plain Prinia, Grey Partridge, House Sparrow, Spotted Munia and Red-whiskered Bulbul are suspected of creating ill effects as the levels were higher than levels which were associated with impaired reproductive success. Considering the present status of House Sparrow, the above threshold levels of *p,p'*-in eggs of House Sparrow should be viewed with concern.

*p,p'*-DDE concentration in egg of many species of birds were negatively correlated with eggshell thickness, but not significant. Other pollutants levels were relatively low, and were believed to have a less significant influence on the reproduction. High frequency of detection of HCH and endosulfan in all the study sites indicates the extensive application of these pesticides in agriculture fields. Additive and possibly synergetic effects may 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. Further, the impact of contaminants must be investigated in the light of the findings presented here. Studies in similar and other habitats are warranted.

Although, the concentration of organochlorine residues in eggs of some species seem to be lower than the levels documented elsewhere, it is of concern as even low levels of contamination if continued, can pose serious ill effects. The results of this study could be the basis to monitor environmental chemicals in India with terrestrial birds' eggs over temporal and spatial scale in the future. Therefore, this study recommends installation of a monitoring programme in birds' eggs in India. This should done on a national scale covering agricultural industrial and wildlife hot spots.

It does not appear that the organochlorine contaminants detected in the present study species are likely to excert impact on the demographic performance of these species as a whole. However, residues may reach high levels causing decreased reproductive or survival in some years, particularly when combined with other non-anthropogenic stressors such as food scarcity. These effects can only be documented if data on these additional stressors are collected for a population as well as the contaminants.

**Chapter 6**

# ORGANOCHLORINE PESTICIDES (OCPs) AND POLYCHLORINATED BIPHENYLS (PCBs) IN BLOOD PLASMA OF BIRDS IN AHMEDABAD, GUJARAT

## 6.1. Introduction

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are well known environmental contaminants found worldwide and able to concentrate in living organisms, in particular at the top of food chain (Tanabe and Tatsukawa, 1991; Moessner and Ballshmiter, 1997). Organochlorine (OC) insecticides were introduced into the environment in large quantities beginning in the 1940s (Ware, 1989). By the early 1960s residues of many chemicals were found in tissues and eggs of birds in Europe and North America and dramatic declines in certain bird populations were also noted, particularly in fish eating birds and raptors (Risebrough, 1985). In the latter years polychlorinated biphenyls (PCBs) were found to be responsible for embryonic deformities and reduction in reproductive success in a variety of species (Hoffman *et al.*, 1998; Fernie *et al.*, 2000).

Use of blood samples as a non-destructive technique for assessing both exposure and effects of environmental toxicants in avian wildlife has become increasingly common in recent years. Advances in analytical methodology now allow for routine measurement of a variety of chlorinated hydrocarbon and trace metal contaminants in blood samples, while development of biomarker techniques permits assessment of variety of toxicological endpoints. Many studies have effectively used blood samples in avian contaminant investigations; migrating raptors (Elliott and Shutt, 1993), nestling Bald Eagles (Bowerman, 1993; Welch, 1994; Dykstra *et al.*, 1998; Elliott and Norstrom, 1998), Peregrine Falcons and Gyrfalcons Nestlings (Jarman *et al.*, 1994) and Egyptian Vultures (Gomara *et al.*, 2004).

OCPs and PCB congeners are highly lipid soluble and are thus stored primarily in body fat. They are also very slowly metabolized with halflives in the order of one year in birds. 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 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. Studies in humans and various wildlife species have shown consistent relationships between levels of OCs in blood and other tissues (Friend *et al.*, 1979; Mes, 1992; Bernhoft *et al.*, 1997; Bustnes *et al.*, 2001). It has been validated as an accurate measure of body levels of OCs in human (Burse *et al.*, 1989; Mes *et al.*, 1992).

Among the 209 possible congeners of PCBs, attention was focused only in those characterized by their relative high abundance in the environment. However, in recent years, attention has been extended to a few abundant PCBs showing toxicity similar to that of dioxin and furans (Ballschmiter and Zell, 1980; Van den Berg *et al.*, 1998), for which the World Health Organization (WHO, 1989) has established toxic equivalency factors (TEFs) for risk assessment (Van den Berg *et al.*, 1998) in humans/mammals, fish and wildlife.

In contrast to the large amount of information related to OCPs and PCBs in tissues and eggs of many species of birds available, studies on the determination of residue levels in blood or serum/plasma samples of avian species are limited in India (Kaphalia, 1981) and elsewhere (Van Wyk *et al.*, 1993, 2001; Gomora *et al.*, 2004). In the present study, residues of OCs in blood from various species of birds were monitored between 2005 and 2007 from Ahmedabad, Gujarat.

### 6.2. Background Information

Organochlorines such as DDT and PCBs are global contaminants and they exert a variety of negative effects on wildlife. At high concentrations OC are lethal, but at sublethal levels they may affect neurological (Peakall, 1985) or and endocrine systems (Colborn *et al.*, 1993). Endocrine disruption may affect reproductive behaviors because the breeding cycle is hormonally controlled (Farner and Wingfeild, 1980; Barron *et al.*, 1995). A number of experimental studies in birds have shown that exposure to various OCs, such as PCBs, DDE and dieldrin affect parental behaviour. This may often been attributed to neurological effects (McArthur *et al.,* 1983; Hoffman *et al.,* 1996).

Jarman *et al.,* (1994) evaluated the levels, trends and patterns of organochlorine contaminants in plasma samples collected from Greenland Peregrine Falcons between 1983-1989, and from adult and nestling Gyrfalcons *Falco rusticolus* from 1989 to 1990, to determine the possible sources and impact of these compounds on the populations.

DDE levels and the associated eggshell thinning in peregrines reached critical levels in Greenland during the 1970s (Walker *et al.,* 1973). Despite the potential for the effect of changes in body conditions, plasma concentrations of OCs in blood of individual incubating adult Glaucous Gulls *Larus hyperboreus* remained consistent between years at Bear Islands in the north-eastern Atlantic (Bustnes *et al.,* 2001). Year-round residents in arctic environments depend on fat reserves as a buffer against cold, stress, and periods of low food availability. This may contribute to elevated contaminant concentrations in these organisms (AMAP, 1998; Bard, 1999).

Significantly higher PCB burden was found in male than female Glaucous Gulls *Larus hyperboreus* with the concentration ranging from 52 to 1079 ng/g wet weight. Increased absence from the nest site was also observed in individual Gulls which had high concentrations of OC. This suggests possible effects on reproductive behavior, endocrine disruption or neurological effects. Kaphalia *et al.,* (1981) estimated BHC and DDT residues in plasma of a few species of birds, namely Chicken, Pigeon, Crow, Kite, Vulture and Cattle Egret captured in and around the urban area of Lucknow, India. The mean total BHC levels in blood plasma ranged from 0.004 $\mu$g/g in Cattle Egret to 0.106 $\mu$g/g in Vulture, while the total DDT ranged between 0.009 $\mu$g/g (Pigeon, Chicken) and 0.522 $\mu$g/g (Kite).

Bustnes *et al.,* (2002) measured differences in organochlorine compounds (OCs) in the blood of breeding South Polar Skuas *Catharacta maccormicki* at Svarthamaren, Dronning Maud land (Antarctica) and birds of Northern Hemisphere Gulls. It was observed that birds with high blood concentration of OCs spent more time on feeding trips than birds with low levels.

Study by Bustnes *et al.,* (2002) demonstrated the association between blood concentration of OCs and asymmetrical wings. The endocrinology behind moult and feather growth is poorly understood, but thyroid hormones are seen critical in regulating shedding and replacement of feathers. PCB congeners are known to reduce the circulating levels of thyroid hormones (Murk *et al.,* 1994; Hoffman *et al.,* 1996; Alvareg *et al.,* 2000), and this may lead to abnormal feather growth in birds with high circulating OC concentrations. Consequently negative association between basal metabolic rate and concentration of total PCBs, total DDT and chlordane were observed. However, levels of THs (thyroxin and triiodothyronine) were not associated significantly with concentrations of the compounds (Verreault *et al.,* 2007).

Nesting Bald Eagles sampled from a variety of sites on the coast of British Columbia (B.C.) had significant differences in mean lipid content of plasma samples among sites, and there was a significant positive relationship between mean plasma lipid content of nestlings and mean productivity (Elliott and Norstrom, 1998; Elliott *et al.,* 1998). The differences among sites in average lipid content of plasma were thought to be caused by variation in body condition of nestlings related to food delivery and local prey availability. Most studies on contaminants in wildlife blood have not discussed the influence of food intake and fasting on plasma lipid concentrations. In a study, Phillips *et al.,* (1989) on humans found that concentrations of OCs in serum can be affected by fasting and feeding. For birds, there are no similar laboratory studies.

Gomara *et al.,* (2004) documented the levels of PCBs and OCPs in serum samples of Egyptian Vulture *Neophron percnopterus* in Spain, and reported the recent input of DDT and PCBs. The concentrations of total PCBs were 3.2-97 ng/g, while those of total DDT ranged from 0.93 to 38 ng/g. Among the various congeners, 52, 132, 105, 138, 153 and 180 were dominant.

It was also assumed that the plasma concentration of OCs will be representative of body burden. However, there are currently no published criteria for DDE or other OCs in plasma of birds for assessment of reproductive effects. Information available on the levels of OCs in plasma of birds is very much limited in India and abroad. Hence, comparison and prediction of ill effects are difficult.

## 6.3. Objectives

- To determine the status and trend of OCPs and PCBs in plasma of birds in Ahmedabad.
- To compare the organochlorine levels among species based on their feeding habits.
- Generate a database on contaminant levels in blood in birds of India.

## 6.4. Materials and Methods

### *6.4.1. Blood sample collection and extraction*

Blood samples were obtained from live birds in Ahmedabad, India between 2005 and 2007 by jugular venipuncture using a heparinized syringe with a 28 gauge needle. A total of 137 plasma samples comprising 16 species of birds were collected (Table 6.1). Samples were kept on wet ice until centrifugation. Blood was transferred from syringe to heparinized capillary tubes and centrifuged at 4000g for 10 min. Plasma was separated and stored in microcentrifuge tubes (1.5 ml). Organochlorine chemicals 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 hours at 50 rpm on a slow speed rotating machine. In case of emulsion formation after extraction, the mixture was centrifuged at 2000 rpm for 4 to 5 minutes to effect

**Table 6.1.** List of birds from which blood plasma samples were collected in Ahmedabad during 2005-2007.

| S. No. | Species | Scientific Name | 2005 | 2006 | 2007 | Total |
|---|---|---|---|---|---|---|
| 1 | Cattle Egret | *Bubulcus ibis* | 2 | 1 | - | 3 |
| 2 | Painted Stork | *Mycteria leucocephala* | 1 | - | 2 | 3 |
| 3 | White Ibis | *Threskiornis melanocephalus* | 1 | - | - | 1 |
| 4 | Black Ibis | *Pseudibis papillosa* | 1 | - | 1 | 2 |
| 5 | Pariah Kite | *Milvus migrans govinda* | 21 | 16 | 14 | 51 |
| 6 | Besra Sparrow-hawk | *Accipiter virgatus* | - | - | 4 | 4 |
| 7 | Griffon Vulture | *Gyps fulvus* | 2 | - | - | 2 |
| 8 | Indian White-backed Vulture | *Gyps bengalensis* | 7 | 6 | 3 | 16 |
| 9 | Egyptian Vulture | *Neophron percnopterus* | 4 | - | - | 4 |
| 10 | Indian Peafowl | *Pavo cristatus* | - | 2 | 3 | 5 |
| 11 | Sarus Crane | *Grus antigone* | - | - | 4 | 4 |
| 12 | Blue Rock Pigeon | *Columba livia* | 16 | 9 | 9 | 34 |
| 13 | Little Brown Dove | *Streptopelia senegalensis* | 1 | - | - | 1 |
| 14 | Asian Koel | *Eudynamys scolopacea* | - | 3 | - | 3 |
| 15 | Barn Owl | *Tyto alba* | 1 | - | - | 1 |
| 16 | House Crow | *Corvus splendens* | 2 | 1 | - | 3 |
| | Total | | 59 | 38 | 40 | 137 |

- not available

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.

### *6.4.2. Sample analysis*

All plasma samples were analyzed in the laboratory at Sálim Ali Centre for Ornithology and Natural History (SACON), Coimbatore, India. The final extracts were analyzed for the following organochlorine pesticides; isomer mixture of hexachlorocyclohexane (∑HCH) consisting *α*, *β*, *δ* and *γ*-HCH (lindane), DDT metabolites (∑DDT) including the basic isomers, namely *p,p'*-DDE and *p,p'*-DDD, heptachlor, heptachlor epoxide, dieldrin, *α*-endosulfan, *β*-endosulfan and endosulfan sulfate, and 32 congeners of PCBs (IUPAC Nos. 8, 28, 37, 44, 49, 52, 60, 66, 70, 74, 77, 82, 87, 99, 101, 105, 114, 118, 126, 128, 138, 153, 156, 158, 166, 169, 170, 179, 180, 183, 187 and 189).

An aliquot (1$\mu$l) from the final extract was injected into a Gas Chromatography (GC) Hewlett Packard 5890 series II equipped with a $Ni^{63}$ Electron Capture Detector and splitless injection port. The GC column employed was DB-608 fused silica capillary column (30 m x 0.32 mm x 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°C 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 (Dr. Ehrenstorfer Laboratories, Germany) and a mixture of 32 PCB congeners (Accustandard, USA) 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/g) ranged from 94 to 103% and 92 to 110% for OCPs and PCBs respectively. Results are not corrected for per cent recovery and the results expressed in wet weight basis. Procedural blanks were run for every set of ten samples and 5% of the samples were run in duplicates. The minimum detection limit for all the compounds analyzed was 1 ng/g.

### *6.4.3. Statistical analysis*

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 10.

## 6.5. Results

### *6.5.1. Organochlorine chemicals in blood plasma of birds*

All plasma samples were analyzed for organochlorine chemicals residues. Invariably, all the samples detected one or more organochlorine residues. The mean and range of OCPs and PCBs are presented (Table 6.2).

The highest level of mean total DDT (147 ng/g) was detected in Painted Stork. The mean ∑-DDT calculated from plasma of Indian Peafowl (85.8 ng/g) was rather similar to that found in Asian Koel (88 ng/g). Lowest level of ∑-DDT was found in Black Ibis (19 ng/g) and Sarus Crane (21.4 ng/g) compared to other species. Among the various OCPs analyzed *p,p'*-DDE was detected most frequently. The mean concentration of *p,p'*-DDE among the birds ranged between 5.3 and 38.6 ng/g (Table 6.2). *p,p'*-DDE was the most contributing metabolite for total DDT which accounted more than 50% of total DDT in Black Ibis, House Crow, Sarus Crane and Besra Sparrow-hawk.

**Table 6.2.** Organochlorine chemical (mean and range, ng/g wet wt) in plasma of birds collected in Ahmedabad, India..

| Species | N | ∑-HCH | HE | Dieldrin | *p,p'*-DDE | *p,p'*-DDD | *p,p'*-DDT | ∑-DDT | ∑-Endosulfan | ∑- PCBs |
|---|---|---|---|---|---|---|---|---|---|---|
| Cattle Egret | 3 | 57.7 (23.9-80) | 30.2 (8.0-65) | 3 (<1-7.9) | 38.6 (6.5-101) | <DL | <DL | 38.6 (6.5-101) | 64.4 (2.7-143) | 479 (136-1109) |
| Painted Stork | 3 | 78.2 (45.6-131) | 296.2 (4.5-808) | 10.5 (6.2-19) | 22.7 (13.6-40.2) | 23.4 (<1-69) | 101 (<1-224) | 147 (13.6-333) | 41.1 (<1-70) | 824 (1-2293) |
| White Ibis | 1 | 11.4 | 28.9 | <DL | 27.5 | <DL | <DL | 27.5 | 66 | 312 |
| Black Ibis | 2 | 47 (23.7-70.2) | 15.8 (11.8-20) | 9.9 (5.1-15) | 10.8 (7.6-14.1) | 8.4 (<1-16.3) | <DL | 19 (7.6-30.4) | 48.3 (42.8-53.7) | 254 (226-282) |
| Pariah Kite | 51 | 77.2 (<1-619) | 26.2 (<1-156) | 9.1 (<1-68.5) | 15.3 (<1-137) | 8.7 (<1-57.4) | 19.2 (<1-227) | 42.5 (<1-265) | 44.4 (<1-290) | 358 (39-1571) |
| Besra Sparrow-hawk | 4 | 166.6 (11.1-541) | 21.9 (1<1-45) | 4.7 (<1-9) | 35.2 (7.0-115) | 1.1 (<1-3.1) | 8.6 (<1-23) | 44.3 (9.6-115) | 153.2 (45.3-395) | 1100 (168-3684) |
| Griffon Vulture | 2 | 43.7 (<1-87) | 12.9 (11.4-14.4) | 5.6 (5.0-6.3) | 16.2 (5.9-27) | 19.9 (<1-39) | <DL | 35.9 (26.5-45) | 52.8 (12.5-93) | 253 (164-342) |
| Indian White-backed Vulture | 16 | 136 (<1-770) | 23.9 (<1-73.4) | 6.5 (<1-16) | 16.2 (<1-39.5) | 17.6 (<1-163) | 31.6 (<1-261) | 64.8 (<1-455) | 31.7 (<1-104) | 885 (109-3776) |
| Egyptian Vulture | 4 | 76.6 (<1-215) | 14.4 (11.5-17.9) | 4.8 (<1-7.3) | 8.7 (<1-26) | <DL | <DL | 38.7 (<1-86) | 26.2 (19.1-33) | 225 (186-275) |

**Table 6.2.** *Continued...*

| Species | N | ∑-HCH | HE | Dieldrin | *p,p'*-DDE | *p,p'*-DDD | *p,p'*-DDT | ∑-DDT | ∑-Endosulfan | ∑-PCBs |
|---|---|---|---|---|---|---|---|---|---|---|
| Indian Peafowl | 5 | 49.3 (<1-127) | 35.7 (9.2-96) | 15 (<1-53) | 37.1 (<1-158) | <DL | 48.9 (<1-186) | 85.8 (<1-344) | 60.7 (5.1-223) | 494 (217-1226) |
| Sarus Crane | 4 | 286 (56-944) | 53.8 (11.0-92) | 5.7 (<1-10) | 11.9 (5.5-21) | <DL | 9.8 (<1-38) | 21.4 (8.8-43) | 45.4 (5.5-148) | 453 (128-1087) |
| Blue Rock Pigeon | 34 | 88.3 (<1-422) | 28.3 (4.8-159) | 7.8 (<1-37.7) | 13 (<1-101) | 3.3 (<1-66.9) | 38.7 (<1-1015) | 54.2 (<1-1037) | 112.8 (4.3-804) | 296 (1-865) |
| Little Brown Dove | 1 | 73.1 | 17.9 | 3.8 | 5.9 | 43.2 | <1 | 49.1 | 58.7 | 205 |
| Asian Koel | 3 | 69.6 (<1-154) | 24.6 (<1-61) | 6.7 (<1-19) | 38.3 (<1-102) | <DL | 50 (<1-149) | 88 (<1-161) | 44 (<1-84.3) | 295 (1-695) |
| Barn Owl | 1 | <DL | 28.1 | 6.3 | 5.3 | <DL | 74 | 79.3 | 58.3 | 301 |
| House Crow | 3 | 73.3 (12.1-192) | 53.9 (26.2-78) | 4.1 (2.9-6.3) | 34.2 (4.6-51) | 7.6 (4.2-12.2) | 3 (<1-8) | 44.5 (17.0-63) | 140 (31.2-267) | 689 (279-1268) |

<DL – below detectable limit, HE- heptachlor epoxide

Relatively high level of total HCH was detected in plasma of Sarus Crane (286 ng/g) an omnivorous bird followed by Besra Sparrow-hawk (166.6 ng/g) a carnivore. White Ibis (11.4 ng/g) showed generally low concentration of HCH and it was not detected in Barn Owl. However, $\beta$ and $\gamma$-isomer of HCH accounted for the major burden of total HCH (Figure 6.1).

The concentrations of cyclodiene insecticides, namley heptachlor epoxide, dieldrin and total endosulfan ranged from 12.9 to 296.2 ng/g, BDL to 15 and 26.2 to 153.2 ng/g respectively (Table 6.2). High levels of total PCBs were detected in plasma of Besra Sparrow-hawk (1100 ng/g), Indian White-backed Vulture (885 ng/g), Painted Stork (824 ng/g), House Crow (689 ng/g) and Indian Peafowl (494 ng/g).

Significantly higher concentrations of $\delta$-HCH and heptachlor epoxide were observed in plasma of piscivores birds than birds with other food habits. Total endosulfan appeared to be high ($P < 0.01$) in plasma of granivores than other birds (Figure 6.1). The pattern of total OC load generally occurred in the following order: insectivores < omnivores < carnivores < granivores < piscivores. The PCB levels were high in carnivores and piscivores, while it was lower in granivores (Figure 6.2). In general, organochlorine load was the highest in Besra Sparrow-hawk followed by Painted Stork, House Crow and Sarus Crane. It may be noted that the minimum was recorded in Egyptian Vulture, Black Ibis and Griffon Vulture (Figure 6.3).

### *6.5.2. Temporal variation in organochlorine residues*

To know temporal variation in organochlorine residues in plasma, three species, namely Blue Rock Pigeon, Pariah Kite and Indian White-backed Vulture collected in all three years were compared (Table 6.3).

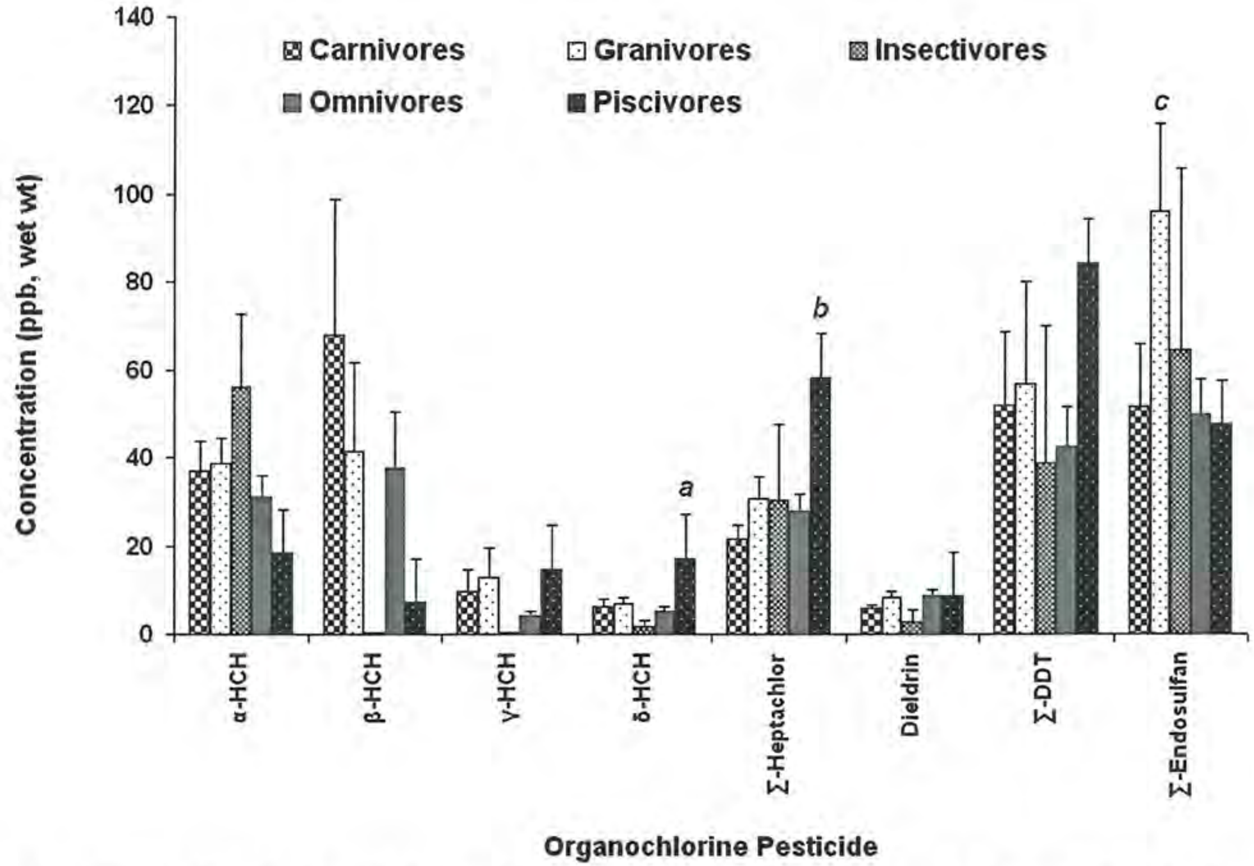



**Figure 6.1**. Organochlorine pesticide residues (mean ± standard error, ng/g, wet wt.) in plasma of birds collected from Ahmedabad, India grouped according to their feeding habits.

*a* = $P < 0.05$ vs carnivores, granivores, omnivores and insectivores; *b*= $P < 0.001$ vs carnivores, granivores, omnivores and insectivores; *c* = $P < 0.05$ vs omnivores

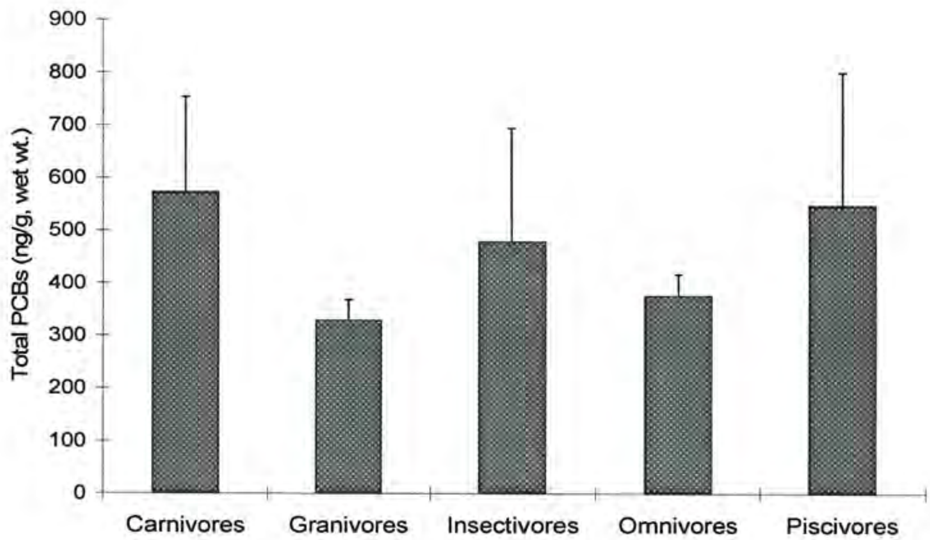



**Figure 6.2.** Comparison of total PCB residues in blood plasma of various species of birds with different food habits received between 2005 and 2007.

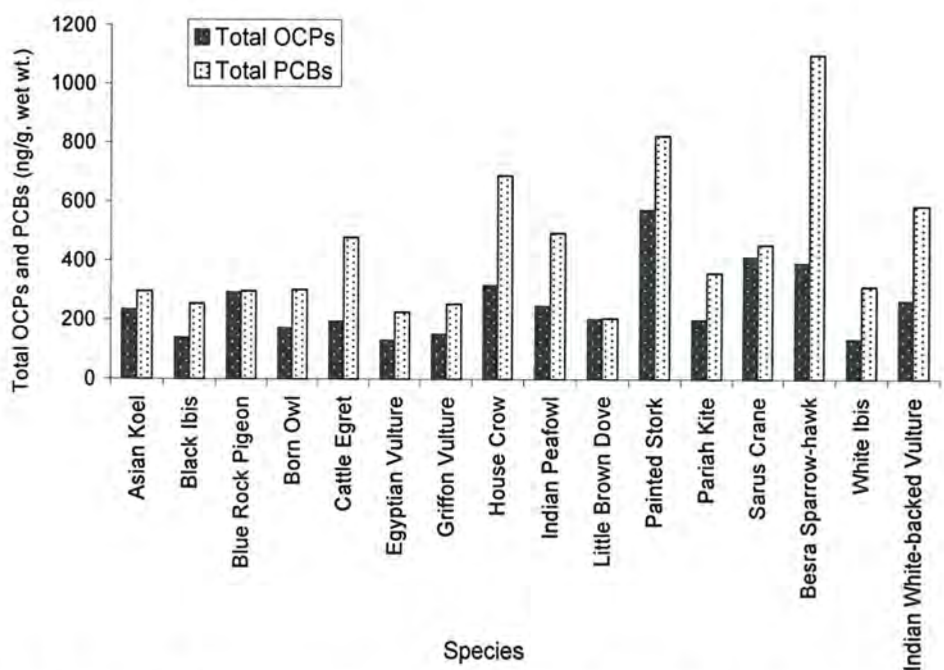



**Figure 6.3.** Comparison of total OCPs and PCBs in blood plasma of various species of birds collected from Ahmedabad, Gujarat between 2005 and 2007.

**Table 6.3.** Comparison of organochlorine residues (mean and range, ng/g wet wt) in blood plasma of select species of birds collected from Ahmedabad, Gujarat between 2005 and 2007.

| Year | 2005 | 2006 | 2007 | ANOVA $P <$ value |
|---|---|---|---|---|
| **Pariah Kite (N)** | 21 | 16 | 14 | |
| Σ-HCH | 47 (<1-201) | 85 (<1-290) | 114 (<1-619) | <0.05 |
| Σ-DDT | 20 (<1-69) | 36 (<1-172) | 83 (<1-265) | NS |
| Heptachlor Epoxide | 19 (<1-68) | 32 (<1-156) | 30 (<1-109) | NS |
| Dieldrin | 7 (<1-23) | 8 (<1-30) | 14 (<1-69) | NS |
| Σ-endosulfan | 42 (<1-290) | 44 (<1-116) | 48 (<1-210) | NS |
| Σ-PCBs | 297 (140-850) | 408 (73-1571) | 391 (39-921) | NS |
| **Indian White-backed Vulture (N)** | 7 | 6 | 3 | |
| Σ-HCH | 104 (30-179) | 228 (9-770) | 27 (<1-68) | NS |
| Σ-DDT | 40 (10-64) | 114 (<1-455) | 24 (13-33) | NS |
| Heptachlor Epoxide | 30 (5-73) | 24 (10-49) | 9 (<1-16) | <0.05 |
| Dieldrin | 7 (6-8) | 7 (<1-16) | 4 (<1-10) | NS |
| Σ-endosulfan | 38 (10-104) | 37 (14-63) | 5 (<1-8) | <0.05 |
| Σ-PCBs | 324 (109-853) | 923 (173-3776) | 517 (121-1207) | NS |
| **Blue Rock Pigeon (N)** | 16 | 9 | 9 | |
| Σ-HCH | 68 (<1-269) | 141 (14-422) | 71 (<1-332) | NS |
| Σ-DDT | 18 (<1-61) | 163 (<1-1038) | 8 (<1-32) | <0.05 |
| Heptachlor Epoxide | 31 (9-159) | 30 (<1-86) | 21 (5-100) | NS |
| Dieldrin | 6 (<1-15) | 9 (<1-38) | 8 (<1-22) | NS |
| Σ-endosulfan | 155 (6-804) | 121 (5-672) | 29 (4-68) | NS |
| Σ-PCBs | 288 (<1-756) | 375 (<1-865) | 232 (68-523) | NS |

NS= Not Significant, < 1= Values below detection limit.

Although blood samples were collected between 2005 and 2007, it was not possible to collect from the same species in all three years because the sampling was opportunistic. Birds which were fell as victims to kite flying in Ahmedabad, Gujarat was considered for the study. In general OC residues in Blue Rock Pigeon and Indian White-backed Vulture showed declining trend from 2006 to 2007, while Pariah Kite showed increasing levels. There were no differences over a period of three years in many of the organochlorine residues analysed except total HCH in Pariah Kite, total endosulfan in Indian White-backed Vulture and total DDT in Blue Rock Pigeon and heptachlor epoxide and (Table 6.3).

## 6.6. Discussion

The absence of comparable data concerning residue levels in the blood plasma samples of avian species rendered direct analogies, for majority of toxicants included in this study, extremely difficult. Similar problem was encountered for a number of compounds when concentrations in various tissues were considered. The objective was to report persistent OCP and PCB residues currently present in the Indian avifauna thus create baseline information for future research. Values cited from the literature on various species of birds are not intended for direct comparisons but to provide an indication of contamination.

### *6.6.1. Organochlorine pesticide contamination in blood plasma of birds*

Overall, DDT and its metabolites were detected in 82% of plasma samples. *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE were detected in 32, 30 and 76 per cent of plasma samples analysed. Presence of the parent compound *p,p'*-DDT in many species of birds indicates recent usage of DDT for public health purpose. Reports have indicated that DDD is the least detected of the two most common persistent metabolites of DDT (i.e DDD and DDE), present in wildlife and environments (Jiminez-Castro *et al.,* 1995). The concentrations of DDT in animal tissues get decreased as the toxin is metabolized into DDE and DDD over a number of years. The eventual excretion of DDD results in the absence of this component from areas where no DDT has been used (Van Wyk *et al.,* 1993 a, b).

The distribution pattern of DDT and its derivatives are similar to the pattern reported in the blood samples of African White-backed Vulture (Van Wyk *et al.,* 2001). Among the metabolites of DDTs, *p,p'*-DDE was found to be the most abundant (76%) in the bird plasma analysed. DDE concentration found in the plasma of House Crow, Pariah Kite, Cattle Egret, Blue Rock Pigeon and Indian White-backed Vulture 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) (Table 6.4). The mean *p,p'*-DDE documented in blood plasma of Indian White-backed Vulture was almost five-folds higher than the levels measured in blood samples of African White-backed Vulture (3.44 ng/g) (Van Wyk *et al.,* 2001). The DDE concentration detected in plasma of Egyptian Vulture was several folds higher than the levels reported in plasma of the same species reported from Spain (Gomara *et al.,* 2004). However, these levels are far below the concentrations in eggs which were correlated with toxic effects in different bird species (5 $\mu$g/g wet wt.) (Hoffman *et al.,* 1998). According to the results obtained by Iseki *et al.,* (2001) the concentration of POPs in eggs are similar to those found in blood samples. As it is well known, *p,p'*-DDE is greatly associated with eggshell thinning in many species of birds (Dirksen *et al.,* 1995; Konstantinou *et al.,* 2000).

The differences in $\sum$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 ($\Sigma$DDT) in many species of birds revealed that the region was contaminated more with DDT. DDT and derivatives are quite stable and are resistant to enzyme action; thus, residue accumulation in biological tissues (Kaphalia *et al.,* 1981). It was also reported that heavily chlorinated compounds have a relatively low potential for long range transport through the atmosphere, the higher concentrations are probably the results of the high contamination source (Wania *et*

**Table 6.4.** Comparison of organochlorine residues (ng/g, wet wt) in plasma of birds with earlier studies.

| Region | Species | Year | Tissue | N | *p,p'*-DDE | dieldrin | total PCBs | Source |
|---|---|---|---|---|---|---|---|---|
| Colorado | Bald Eagle | 1977 | Plasma | 10 | 140 (30-230) | 10 (<10-50) | 100 (<10-680) | Henny *et al.*, 1981 |
| Missouri | Bald Eagle | 1978 | Plasma | 15 | 60 (10-140) | 30 (ND-80) | 240 (ND-360) | 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) | 980 (650-1500) | Mora *et al.*, 1993 |
| Papoose Island, Great Lake | Caspian Terns | 1990 | Plasma | 10 | 180 (103-320) | 15 (10-20) | 1390(830-2340) | Mora *et al.*, 1993 |
| South Greenland | Peregrine Falcons | 1985-89 | Plasma | 5 | 140 (100-190) | - | 300 (160-570) | Jarman *et al.*, 1994 |
| West Greenland | Peregrine Falcons | 1985-89 | Plasma | 41 | 220 (63-720) | - | 130 (40-380) | Jarman *et al.*, 1994 |
| South Africa | White-backed Vulture | 1993-95 | 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 | - | 185.1-630.8 | Bustnes *et al.*, 2001 |
| Spain | Egyptian Vulture | 99-2000 | Serum | 27 | 0.5-38 | - | 3-97 | Gomara *et al.*, 2004 |
| Antarctic South Polar | Skuas | 2001-02 | Blood | 142 | 7 (0.4–40) | - | 9 (1-50) | Bustnes *et al.*, 2006 |
| Subartic | Great Black-backed Gull | 2001-02 | Blood | 39 | 230 (2-160) | - | 100 (10-780) | Bustnes *et al.*, 2006 |
| Norwegian Arctic | Gulaucous gull | 2004 | Serum | 23 | ∑DDT (180-903) | - | (190-2660) | Verreault *et al.*, 2007 |
| India | House Crow | 2005-07 | Plasma | 3 | 34 (5-50) | 4.1 (2.6-6.3) | 690 (280-1270) | Present Study |
| India | Pariah Kite | 2005-07 | Plasma | 51 | 153 (<1-137) | 9.1 (<1-6.5) | 360 (40-1570) | Present Study |
| India | White-backed Vulture | 2005-07 | Plasma | 16 | 16 (<1-40) | 6.5 (<1-16) | 590 (109-3780) | Present Study |
| India | Egyptian Vulture | 2005-07 | Plasma | 4 | 9 (<1-26) | 4.8 (<1-7.3) | 230 (190-280) | Present Study |
| India | Griffon Vulture | 2005-07 | Plasma | 2 | 16 (6-27) | 5.6 (5-6.3) | 250 (165-342) | Present Study |
| India | Cattle Egret | 2005-07 | Plasma | 3 | 39 (7-101) | 3 (<1-7.9) | 480 (140-1110) | Present Study |
| India | Blue Rock Pigeon | 2005-07 | Plasma | 34 | 13 (<1-101) | 7.8 (<1-37.7) | 296 (<1-870) | Present Study |

*al.*, 1993; 1996; Corsolini *et al.*, 2002). A, *p,p'*-DDT to *p,p'*-DDE ratio higher than one was obtained for six species of birds (Asian Koel, Blue Rock Pigeon, Indian Peafowl, Painted Stork, Pariah Kite and Indian White-backed Vulture), which could be considered as a possible indicator of recent use of this pesticide.

Of the 137 individual plasma samples analysed in this study, 84% contained residues above the lower quantifiable levels (1 ng/g) for ΣHCH. HCH also contributed relatively more to the total OCs. Relatively high levels of total HCH in plasma of Sarus Crane and Besra Sparrow-hawk may be explained by their feeding habits. Below detectable level of HCH was recorded in Barn Owl, however, the sample size was small. Among various isomers of HCH anlaysed, $\beta$- and $\gamma$-isomers accounted for the major burden of total HCH. Total HCH and lindane ($\gamma$-HCH) levels in the present study are almost two-folds higher than the levels reported in Blue Rock Pigeon (48; 16 ng/g), House Crow (30; 11 ng/g), Pariah Kite (60; 28 ng/g) and Indian White-backed Vulture (106; 66 ng/g) collected from the urban area of Lucknow, India during 1980 (Kaphalia *et al.,* 1981). However, significantly higher $\delta$-HCH burden was observed in piscivorous birds than birds with other food habits. Previous studies in Indian biota have also reported high levels of HCH (Tanabe, 1998; Senthilkumar, 1998, 2001; Vijayan *et al.*, 2004; Muralidharan *et al.*, 2008). There is also substantial use of $\gamma$ -HCH in India, which might be a contributing factor for the relatively high levels HCH metabolites found in plasma tissue of birds. HCH and their isomers are widely distributed in the ecological systems. Although HCH residues are excreted rapidly, slow accumulation does occur in the body tissues and body fat on chronic exposure.

The cyclodiene insecticide, dieldrin has been labeled as commonly detected environmental contaminants (Van Wyk *et al.,* 2001), which is accumulated by fish and other wildlife. This may be due to its stability (Santerre *et al.,* 1997). This statement could certainly serve to corroborate the results of the present study, where dieldrin occurred in 81% of the total number of plasma samples analysed. The concentration of dieldrin detected in the 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 6.4). Dieldrin levels recorded in Indian White-backed Vulture in the present study are comparable with the levels reported in blood of African White-backed Vulture (Van Wyk *et al.,* 2001). However, it is imperative to note that considerable diversity in the resistance to the effects of this agent was exhibited by various avian species and proposed that amounts grater than 0.001 ng/g must be viewed as hazardous (King *et al.,* 1978). Nevertheless, it should be noted that the Long and Morgan 'effects range-low' (ER-L) value, (i.e, the contaminant levels above which adverse biological effects are occasionally observed), is listed as 0.02 ng/g for dieldrin (Long *et al.,* 1995).

Heptachlor epoxide was found to be one of the most abundant chlorinated hydrocarbons included in the analysis and occurred in 93% of the total plasma samples examined. The over all mean heptachlor epoxide levels determined in Indian White-backed Vulture are two to three folds higher than the mean reported in whole blood of African White-backed Vulture (10.5-25 ng/g), Cape Griffon *Gyps coprotheres* (11.34-16.07 ng/g) and Lappet-faced Vulture (<nd-5.81 ng/g) (Van Wyk *et al.,* 2001). Residue level of heptachlor epoxide in plasma of Besra Sparrow-hawk, Barn Owl and three species of vultures are comparable with the levels reported in birds with similar food habits, namely Peregrine Falcon from South Green land (31; 14-71 ng/g) and West Greenland (17; 4.5-68) (Jarman *et al.,* 1994). Heptachlor epoxide detected in the present study species should be viewed with concern, because these levels (Table 6.4) are higher than the $LC_{50}$ values reported for Japanese Quail, *Coturnix japonica* (93 ng/g), Ring-necked Pheasants, *Phasianus colchinus* (224 ng/g) and Mallard (480 ng/g) (Heath *et al.,* 1983). 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.

Above detectable levels of total endosulfan were found in 75% of the birds studied. 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). 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.

### *6.6.2. PCB contamination in blood plasma of birds*

Data reported concerning PCB levels in serum or plasma of birds in India is very scarce in the literature. Total PCB levels found in our study are lower than those found in Caspian Terns from Great Lake (Mora *et al.*, 1993) (Table 6.4). More importantly, PCB levels detected in plasma samples of Egyptian Vulture of the present study are several fold higher than those reported in Spain (Gomara *et al.*, 2004) and are much lower than those reported in eggs (4.7 $\mu$g/g, Hoffman *et al.*, 1993) associated with toxic effects in birds. As it was mentioned previously, POP concentrations in eggs are similar to those found in blood samples (Iseki *et al.*, 2001).

PCBs recorded in plasma of Besra Sparrow-hawk and Indian White-backed Vulture are much greater than any other species. Concentrations of PCBs have usually been the predominant chlorinated hydrocarbons in Great lakes wildlife (Heinz *et al.*, 1985; Struger and Weseloh, 1985; Kubiak *et al.*, 1989). The greater concentrations of PCBs in plasma samples of Besra Sparrow-hawk and Indian White-backed Vulture collected from Ahmedabad suggest that birds in this region are exposed to greater concentrations of PCBs through their diet. The differences in diet may explain, in part, the differences in concentrations of PCBs in plasma.

Although this study did not include any ecological investigations, it is understood that higher contaminations of PCBs are capable of creating behavioral changes in birds (Bustnes *et al.*, 2001). Birds which have higher levels of PCBs in plasma are more often absent at the nest and spent higher proportion of their time away from the nest site. The nesting period is the most critical phase in avian reproduction; rendering both parents and offspring vulnerable to predation and starvation. Thus, factors impairing nesting behavior may cause reproductive failure.

The results obtained in the present study could be related to the feeding habits to an extent. The differences in the PCB profiles could be also associated with differences in metabolic activities caused by the presence of other contaminants. In addition to PCBs, other compounds could affect the PCB metabolism as suggested by Van den Brink and Bosveld (2001) and Gomara *et al.*, (2004). Ahmedabad is an industrial city and hence the PCB levels found in the bloods could be associated with industrial operations.

### *6.6.3. Comparison of organochlorine residues based on food habits*

When birds were grouped according to their food habits, birds belonging to piscivorous category showed higher load of organochlorine residues, followed by carnivorous. 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).

In general the smaller the animal the higher the ratio of food consumption per unit weight, regardless of food habits. Thus, within the same food habit group, small individuals are likely to ingest larger amounts of chemical residues. Age and body size of the species are also important in influencing the accumulation of chemical residues. Although it has been suggested that concentration of chlorinated hydrocarbons in plasma may not reflect concentration in adipose tissue (Monro, 1990; Schecter *et al.*, 1991), particularly in situations of continuous daily intake, Henny and Mecker (1981) found a significant correlation between concentrations of OCs in the diet and those in the blood of American Kestrel *Falco sparverius*. Furthermore, significant relationship between DDE in plasma and body lipids has been observed in field and laboratory experiments with raptors (Henny and Mecker, 1981) Mallards (Friend *et al.*, 1979) and White-faced Ibises (Capen and Leiker, 1979). Therefore, it is likely that concentrations of PCBs and DDE in blood plasma of birds were significantly correlated with those in adipose tissues.

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 residues 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.

### 6.7. Conclusion

The results from the present investigation suggest that variation in residue burden in plasma of birds could be influenced by the food habits. The detected organochlorine 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, 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.

DDE concentration was less than those of PCBs and less than the threshold concentration to cause significant eggshell thinning in bird species. 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 presence of organochlorine residues in birds over the years indicates continued exposure to organochlorine compounds. Attention should be paid, in particular, in breeding areas of different geographical regions so as to ultimately facilitate the early identification of increased risks to the survival of a 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 consequently be viewed as excellent choice for the bioindication of the occurrence of chlorinated hydrocarbon contamination within the foraging ranges covered by the birds. The current data present baseline information for a number of organochlorine pollutants, many of which have not been analysed in birds in the past. It is also 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.

## Chapter 7

# BRAIN AND PLASMA CHOLINESTERASE (AChE AND BChE) ACTIVITY IN BIRDS IN AHMEDABAD, INDIA

### 7.1. Introduction

In recent years there has been increased concern about the potential of environmental contaminants to suppress immune systems, thereby increase the incidence of disease in humans as well as wildlife. The understanding and predictions of the reactions and influences of chemicals in and on the environment have become important issues in environmental quality assessment. It is also important in the assessment of the risk or hazard associated with their presence in the environment. Until recently, monitoring and assessing of the "health" of an ecosystem was mainly based on chemicals and physical measures of the component. A limitation to this way of monitoring the environmental health is that physical and chemical information is based on the momentary condition that exists at the time of sample collection. This often means missing the short-term events that may be critical to ecosystem health (Roux, 1994; Heath and Classen, 1999).

The sub-lethal levels of pollutants usually cause biochemical or physiological effects at the sub-cellular level in an organism. Data on these sub-lethal effects would help in identifying the toxicants causing the effects before dramatic changes (eg. mass mortality) occur in the natural population of an ecosystem. Before organisms die normal physiological process are affected and death being a too extreme criteria for determining whether a substance is harmful or not, it becomes important to find out bio-indicators/biomarkers for sublethal effects of toxicant (Van Vuren *et al.,* 1999). Biomarkers can detect effects over an entire continuum; they are good measures to use for earlier exposures and more sensitive detection of toxicity in animal bioassays. Biomarkers (biochemicals) measure the exposure of organisms to environmental chemicals, and in certain cases, can also measure toxic effects.

Inhibition of brain acetylcholinesterase (AChE) activity, for example, has been the most widely used biomarker to diagnose organophosphate (OP) poisoning in a wide variety of organisms (Day and Scott, 1990; Bocquence *et al.*, 1990; Sanchez *et al.*, 1997).

A great number of field studies have assessed the exposure to, and/or effects of, OP insecticides on birds in terms of inhibition of brain AChE (Zinkl *et al.*, 1977; Busby *et al.*, 1991; Rainwater *et al.*, 1995). The varying levels of AChE activity and similar values for closely related species were determined in brain for 48 species of wild birds from North America representing 23 families (Hill, 1988) and 47 species from Europe representing 19 families (Westlake *et al.*, 1983). Non-target effects of pesticides are typically evaluated using mortality as the end point, but mortality can be an insensitive predictor of long-term adverse effects on the organisms. Measurements of impact of contaminants can act as 'early warning' signs of impending ecosystem harm.

Determination of brain AChE activity involves killing of organism which is unacceptable for endangered or protected species. Butyrylcholinesterase (BChE) and Carboxylesterase (CbE) have been used as nondestructive biomarkers in explaining mortality of many species of birds (Thompson and Walker, 1994; Bartkowiak and Wilson, 1995; Rainwater *et al.*, 1995).

Birds are thought to have only limited A-esterase and hepatic microsomal monooxygenase activities for detoxifying cholinesterase (ChE) inhibiting compounds (Walker, 1983). B-esterases, namely AChE, BChE and CbE are important determinants of toxicological response to ChE inhibitors (Leopold, 1996; Parker and Goldstein, 2000). Reference data are expected to provide an estimate of the mean and normal levels of plasma and brain ChE activity for each species of birds. Therefore, it has been suggested that development of a normal and or reference data for ChE activity under a standardized protocol for various wildlife species is essential to enhance our diagnostic capability to fix reason for mortality in the absence of concurrent controls (Westlake *et al.*, 1983; Hill and Murray, 1987).

In India, for example, large areas of agricultural lands are sprayed with OP and carbamate (CB) insecticides making it difficult to find birds that have not potentially been exposed to these AChE inhibitors. Further for rare or migratory birds, adequate number of control birds might not be available when needed. There are many advantages in using controls collected at times other than when poisoning is suspected. Hence, if brains salvaged on opportunistic manner at any point of time are used to measure ChE, healthy wild birds need not be sacrificed to serve as controls.

Although scattered information on the residue levels of pesticides are available in India (Kaphalia *et al.*, 1981; Siddiqui and Saxena, 1983; Misra, 1989; Reghupathy and Kuttalam, 1990; Kulashrestha *et al.*, 1990; Senthilkumar *et al.*, 1999, 2001; Muralidharan, 1993, Tanabe *et al.*, 1998; Muralidharan *et al.*, 1992, 2004, 2008), a little is known about the normal levels of cholinesterase activity in birds. Even though brain AChE was measured in Indian Bustard (1.046 $\mu$moles/min/g), identification of the cause was possible only based on the residue levels of methyl parathion and chlorpyrifos (Muralidharan and Dhananjayan, unpublished data). Hence, it becomes essential to generate data on normal levels of enzyme activity in a variety of free ranging birds in India.

Although, birds have been experimentally exposed to OPs and CBs in order to evaluate the use of B-esterase activities as non destructive biomarkers elsewhere in the world, there are no reports on the normal levels of AChE and BChE activity in brain and plasma of birds in India. In this study normal activities of two esterases, namely AChE and BChE were evaluated to be used as biomarkers in free ranging birds in Ahmedabad, India.

### 7.2. Background Information

The concept of "Biomarkers" has recently received considerable attention among ecotoxicologists as a new and powerful tool for detecting and documenting exposure and effects of environmental contaminants (McCarty and Secord, 1990). One of the most appropriate definitions formulated by the National Academy of Science (National Research Council, 1989) is "A biomarker is a xenobiotically induced variation in cellular or biochemical components or process, structure, or functions that is measurable in a biological system or sample".

The biomarker most frequently used for diagnosing exposure of wildlife to OP and CB compounds is the measurements of the inhibitions of the B type cholinesterase (Ludke *et al.,* 1975; Busby *et al.,* 1987). The esterase is a member of a large and varied group of enzymes. "A" esterase, hydrolyzes organophosphates, and "B" esterase are inhibited by them (Aldridge, 1953). "A" esterase of serum and plasma showed much lower activities in birds than mammals (Brealey *et al.,* 1980; Peakall, 1992). The use of biochemical biomarkers may allow early warning interventions with the objective of protecting wild population exposed to chemical agents (Newman, 1998). The cholinesterase enzymes fall broadly into two types depending on their substrate preference. AChE is an essential component of the cholinergic synapses, where it rapidly cleaves the neurotransmitter acetylcholine. BChE has wider substrate specificity, but no known physiological function (Silver, 1974).

Based on observations in a large number of bird species, namely European Starling *Sturnus vulgaris,* Common Grackle *Quiscalus quiscula,* Red-winged Blackbird *Agelaius phoeniceus,* House Sparrow *Passer domesticus* and Barn Swallow *Hirundo rustica* (Yawetz *et al.,* 1979; Niethammer and Baskett, 1983; Hill and Murray, 1987), Ring-necked Pheasants *Phasianus colchicus,* Rock Doves *Columba livia,* Mallards *Anas platyrhynchos,* Mourning Doves *Zenaida macroura,* Northern Bobwhite Colinus virginianus and Japanese Quail *Coturnix coturnix japonica* (Bunyan *et al.,* 1968; Ludke *et al.,* 1975; Franson *et* *al.,* 1983), Rock Doves and Bald eagles (Fairbrother, 1990), it was reported that there were no sex differences in plasma or serum ChE activity in birds.

AChE inhibition can cause various kinds of toxic effects in birds, ranging from lethargy (Grue *et al.,* 1991) and anorexia (Grue *et al.,* 1983) to reproductive impairment. Depression of AChE activity can cause infection, anemia, malnutrition and liver disease (Mayer *et al.,* 1992). During acute poisoning it affects the parasympathetic nervous system, the neuromuscular junction as well as the central nervous system (Yawetz *et al.,* 1993). The inhibition of AChE will thus result in tremors, convulsions and finally death (Frank *et al..,* 1977; Fernandez-vega *et al.,* 1999).

Inhibition of brain AChE activity has been used extensively to monitor exposure and as one of several criteria for establishing cause of death in free ranging wildlife presumed to be exposed to ChE inhibiting pesticides (Bunyan *et al.,* 1968; Hill and Fleming, 1982). Cause of death is best diagnosed by detection of ChE inhibiting pesticides or metabolites in ingesta or tissues, in conjugation with inhibition of brain AChE activity. Inhibition of brain AChE in excess of 50-70% of the normal value has been used as a threshold for diagnosis of death from anticholinesterase pesticide (Ludke *et al.,* 1975; Hill and Fleming, 1982). However, some instances of natural variation and stress could falsely yield data suggestive of pesticide exposure (Grue and Hunter, 1984; Custer and Ohlendorf, 1989). Ideally, use of appropriate unexposed controls should minimize misclassification errors in exposure assessments.

The inhibition of AChE has been widely used as a biomarker for the evaluation of the potential risk to bird population due to the extensive use of OPs (Hill and Fleming, 1982; Robinson *et al.,* 1988). However, since it is destructive biomarker, it cannot be used always, especially in endangered species. As an alternative approach, it is therefore important to investigate the possibility of using non-destructive biomarkers such as BChE and CbE which are considered as sublethal means. It may be noted that to calculate the percentage of reduction normal activity in healthy birds has to be assessed.

Inhibition of brain cholinesterase activity will remain for several weeks following exposure and correlate with the effects, while inhibition of blood cholinesterase activity is indicative of exposure rather than harm and is short lived. The advantage of blood sampling is that the organisms need not be scarified for sampling and serial samples can be collected from the same organisms, and measurements of continuing exposure is thus possible (Melancon, 1995).

AChE is an enzyme that is essential for the normal functioning of the central and peripheral nervous system (Hart, 1993) and is widely distributed in the neural and non-neural tissues (Fribroulet *et al.*, 1990). The transmitter substance, acetylcholine, is responsible for the transmissions of signals from terminal of motor nerves to the muscles as well as synaptic transmission between nerve cells (Schmidt-Nielsen, 1990). The hydrolysis of acetylcholine is catalyzed by AChE and choline is produced as a metabolite (Nwsou *et al.*, 1992). AChE is thus vital for normal functioning of the sensory, integrative and neuromuscular system.

It is also reported that larger species have lower brain AChE activity than smaller species with few exceptions (Wang and Murphy, 1982) and brain AChE activity of apparently normal wild bird (ie, unexposed to ChE-inhibiting insecticide) may vary up to three-folds in adults of the same species (Hill, 1988). However, whole brain AChE activity does not appear to vary among seasons (Mitchell and White, 1982; Hill and Murray, 1987).

Plasma and brain ChE activities were measured by Westlake *et al.*, (1983) in 27 avian species. Many species of birds had plasma BChE activity less than plasma AChE values. Interestingly, there has been no further documentation of this observation till date. Recently, a few studies have been conducted to determine the relative amounts of AChE and BChE in mammals (Walker and Thompson, 1991) and avian serum (Leopold, 1996; Parker and Goldstein, 2000; Gill *et al.*, 2004; Roy *et al.*, 2005). For diagnostic interpretation, AChE activity of affected birds is compared to that of normal birds (controls). Hence, collection of control becomes very important. When OPs or CBs poisoning is not suspected brains salvaged could be used, and healthy wild birds need not be sacrificed to serve as controls.

### 7.3. Objective

- Document species specific variations in AChE and BChE activities in brain and plasma of birds.

### 7.4. Materials and Methods

#### *7.4.1. Study area and sample collection*

Detail description about the study area is given in chapter 2. Number of species and individuals included for the study between 2005 and 2007 are presented in Table 7.1. Brain and plasma of birds were collected between 2005 and 2007 with the help of Animal Help Foundation at Ahmedabad, Gujarat. All the samples were collected from birds which were victims to kite flying in Ahmedabad. The brain of each bird was dissected out from the cranium immediately after death and stored at -20°C until further processing. Blood samples were obtained by jugular venipuncture using heparinized syringes fitted with a 28 gauge needle. After collection, blood was transferred to heparinized capillary tubes and centrifuged at 5000 rpm for 10 min for plasma separation. Separated plasma was stored in microcentrifuge tubes (1.5 ml) and kept at -20°C until analysis.

#### *7.4.2. Sample processing*

Brain tissues were thawed, weighed up to 1 g with a minimum of 100 mg and then homogenized in 4 ml of 0.05 M phosphate buffer (pH-8.0) (250 mg/ml, w/v) using a glass homogenizer. The homogenate was centrifuged at 14000 rpm for 10 minutes, supernatant decanted slowly into labeled test tubes and stored at -20°C until analysis.

**Table 7.1.** Birds included for documenting brain and plasma AChE and BChE activities.

| S. No. | Species | Scientific Name | No.of Plasma tissues* | No. of Brain tissues* |
|---|---|---|---|---|
| 1 | Cattle Egret | *Bubulcus ibis* | 1 | 10 |
| 2 | Painted Stork | *Mycteria leucocephala* | 1 | 1 |
| 3 | Asian Openbill | *Anastomus oscitans* | - | 1 |
| 4 | White Ibis | *Threskiornis melanocephalus* | - | 1 |
| 5 | Black Ibis | *Pseudibis papillosa* | 2 | 2 |
| 6 | Pariah Kite | *Milvus migrans govinda* | 42 | 59 |
| 7 | Besra Sparrow-hawk | *Accipiter virgatus* | 5 | - |
| 8 | Griffon Vulture | *Gyps fulvus* | 4 | - |
| 9 | Indian White-backed Vulture | *Gyps bengalensis* | 15 | 8 |
| 10 | Egyptian Vulture | *Neophron percnopterus* | 4 | - |
| 11 | Indian Peafowl | *Pavo cristatus* | 5 | 5 |
| 12 | Sarus Crane | *Grus antigone* | 4 | - |
| 13 | Blue Rock Pigeon | *Columba livia* | 20 | 41 |
| 14 | Indian Ring Dove | *Streptopelia decaocto* | - | 3 |
| 15 | Little Brown Dove | *Streptopelia senegalensis* | 1 | - |
| 16 | Asian Koel | *Eudynamys scolopacea* | - | 6 |
| 17 | Crow Pheasant | *Centropus sinensis* | - | 2 |
| 18 | Barn Owl | *Tyto alba* | - | 3 |
| 19 | Common Myna | *Acridotheres tristis* | - | 3 |
| 20 | Bank Myna | *Acridotheres ginginianus* | - | 1 |
| 21 | House Crow | *Corvus splendens* | 10 | 3 |
| | Total | | 114 | 149 |

* Plasma and brain samples do not necessarily belong to the same birds.

\- Not Available

Long-term storage was avoided in order to minimize the problems associated with dehydration of the samples and stability of the inhibited enzymes. Plasma was thawed and immediately assayed for ChE activity without dilution.

### *7.4.3. Cholinesterase (AChE and BChE) activity estimation*

Cholinesterase activities were measured following the method of Ellman *et al.,* (1961), and adapted by Hill (1988). Cholinesterase assays were performed as soon as possible after the collection of plasma and brain samples. A nonenzymic blank was included to assess background levels of hydrolysis of the substrate. About 3 ml of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB)/buffer solution was pipetted out into a cuvette with a micropipette and 20 $\mu$l of sample was added, mixed gently with a probe and the blank absorbance measured at 406 nm in an UV-Vis Spectrophotometer (PerkinElmer Lambda 35 UV/Vis Spectrophotometer). After addition of 0.1 ml of the substrate, Acetylthiocholine Iodide (ATCI- freshly prepared), into the sample cuvette, it was mixed immediately and allowed to settle for 15 seconds and then allowed to stabilize for 60 seconds. Subsequently, the change in absorbance with a light path of 1 cm width was recorded following time drive kinetic spectrophotometer. Each assay was run for 4 minutes to ensure that the linear phase of the reaction was measured at a time lag of 30 sec. All samples were assayed in at least duplicate. All activities were converted from optical density units/minute to $\mu$moles AThCh hydrolyzed/min/ml plasma or g brain tissue. BChE activity was determined in the same manner with butyrylthiocholine iodide as the substrate.

### *7.4.4. Data analysis*

All enzyme activity data were log transformed before statistical analysis to obtain homogenous variation among species. One way analysis of variance (ANOVA) was used to test variation in enzyme activity among species. Students *t'*-test was used to understand differences between sex. All the statistical tests were performed using SPSS student version 10.

## 7.5. Results

AChE and BChE activity levels in brain and plasma were determined in 149 individuals comprising 16 species and 114 individuals comprising 13 species of birds respectively. Cholinesterase (AChE and BChE) activities measured in various species of birds are presented in tables (Table 7.2 and 7.3).

### *7.5.1. Brain cholinesterase (AChE, BChE)*

The highest levels of brain AChE activity was recorded in Asian Openbill (5.52 $\mu$moles/min/g) followed by House Crow (4.06 $\mu$moles/min/g) and Painted Stork (3.01 $\mu$moles/min/g). Of all the species studied low levels of AChE activity was measured in Bank Myna, White Ibis and Barn Owl (0.51, 0.65 and 0.93 $\mu$moles/min/g respectively) (Table 7.2). The variation in brain AChE activity among the species was highly significant (ANOVA, $P < 0.05$). However, the mean brain AChE activities between sexes are almost similar. The variation in AChE activity between sexes was not significant except Pariah Kite (Table 7.3).

Brain BChE activity among the species ranged between 0.09 $\mu$moles/min/g in Asian Openbill and 0.48 $\mu$moles/min/g in House Crow. In general, BChE activity was similar in all the bird species included in the study and the variation was not significant (ANOVA, $P > 0.05$). To know the temporal variation in brain AChE and BChE activities, the samples collected between 2005 and 2007 were compared (Figure 7.1). The mean brain AChE and BChE values among years were not varying in any of the species studied (ANOVA, $P > 0.05$).

### 7.5.2. Plasma cholinesterase (AChE, BChE)

Plasma AChE activity was significantly different among species (ANOVA, $P < 0.05$). The highest AChE activity was observed in Blue Rock Pigeon (1.09 $\mu$moles/min/ml) followed by Painted Stork (1.06 $\mu$moles/min/ml) and House Crow (0.96 $\mu$moles/min/ml) (Table 7.4.). The lowest activity was measured in Besra Sparrow-hawk (0.1 $\mu$moles/min/ml).

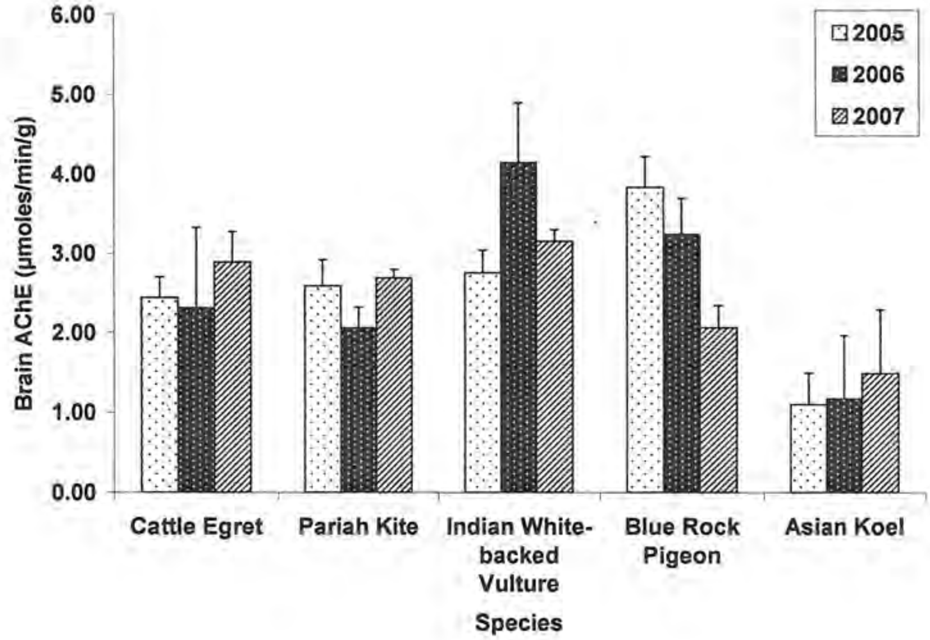
**Table 7.2.** Brain cholinesterase activity ($\mu$moles/min/g) in various species of birds collected in Ahmedabad, Gujarat (2005-2007).

| S. No. | Species | N | AChE Mean | AChE Min-Max | BChE Mean | BChE Min-Max |
|---|---|---|---|---|---|---|
| 1 | Cattle Egret | 10 | 1.62 | 0.14-4.17 | 0.25 | 0.07-0.51 |
| 2 | Painted Stork | 1 | 3.01 | - | 0.20 | - |
| 3 | Asian Openbill | 1 | 5.52 | - | 0.09 | - |
| 4 | White Ibis | 1 | 0.65 | - | 0.10 | - |
| 5 | Black Ibis | 2 | 1.43 | 1.05-1.80 | 0.22 | 0.11-0.32 |
| 6 | Pariah Kite | 59 | 1.72 | 0.01-5.12 | 0.24 | 0.01-1.06 |
| 7 | Indian White-backed Vulture | 8 | 2.50 | 0.89-4.89 | 0.22 | 0.05-0.78 |
| 8 | Indian Peafowl | 5 | 1.77 | 1.21-2.33 | 0.30 | 0.03-0.71 |
| 9 | Blue Rock Pigeon | 41 | 2.30 | 0.02-6.37 | 0.21 | 0.01-0.88 |
| 10 | Indian Ring Dove | 3 | 1.32 | 1.03-1.61 | 0.39 | 0.11-0.66 |
| 11 | Asian Koel | 6 | 1.24 | 0.37-2.80 | 0.10 | 0.08-0.12 |
| 12 | Crow Pheasant | 2 | 2.47 | 2.33-2.61 | 0.27 | 0.27-0.27 |
| 13 | Barn Owl | 3 | 0.93 | 0.81-1.04 | 0.29 | 0.16-0.41 |
| 14 | Common Myna | 3 | 2.08 | 0.03-3.44 | 0.29 | 0.07-0.72 |
| 15 | Bank Myna | 1 | 0.51 | 0.51-0.51 | 0.18 | 0.18-0.18 |
| 16 | House Crow | 3 | 4.06 | 1.68-5.51 | 0.48 | 0.08-0.87 |

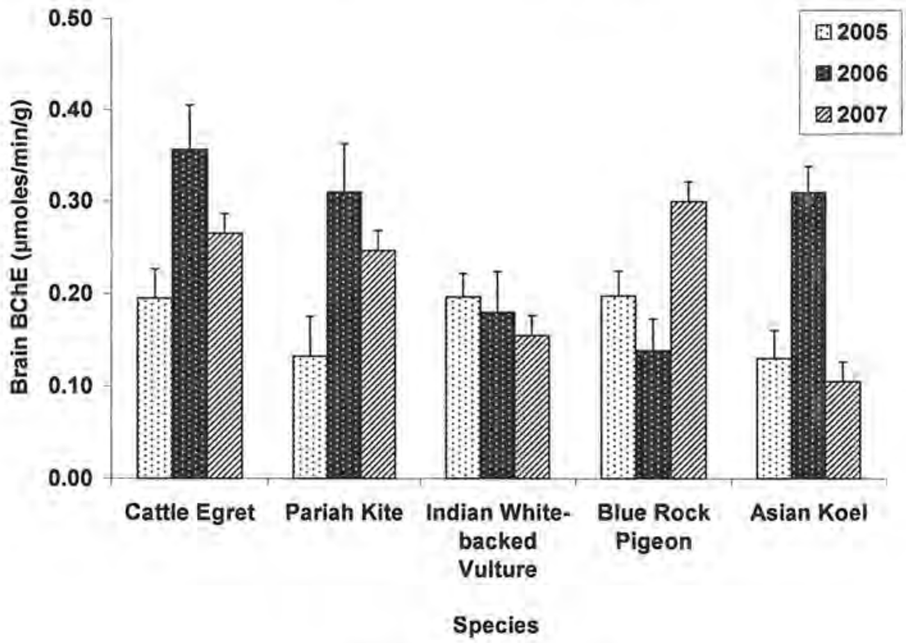
- Not available

**Table 7.3.** Variation in brain AChE activities between sexes.

| Species | Mean AChE activities ($\mu$moles/min/g) Male | Mean AChE activities ($\mu$moles/min/g) Female | *t*- test *P*- Value |
|---|---|---|---|
| Asian Koel | 1.15 | 1.26 | 0.51 |
| Blue Rock Pigeon | 2.46 | 2.07 | 0.22 |
| Cattle Egret | 1.39 | 1.72 | 0.86 |
| Pariah Kite | 1.19 | 1.99 | 0.00 |
| Indian White-backed Vulture | 2.38 | 2.61 | 0.09 |




a) Brain AChE activity




b) Brain BChE activity

**Figure 7.1.** Variation in brain AChE and BChE activities ($\mu$moles/min/g) in apparently healthy wild birds collected from Ahmedabad, Gujarat (2005-2007).

There was no variation in plasma BChE activity among species (ANOVA, $P > 0.05$). Documenting variation in plasma cholinesterase activity between sexes was not possible in species where sex differentiation was difficult unless cut open. The year wise plasma cholinesterase activity was not attempted due to small sample size.

**7.6. Discussion**

As incident of wildlife mortality is essentially unique, it was believed initially that a set of concurrent control was required for each case to negate influences of variables such as storage and assay conditions on ChE activity (Hill and Fleming, 1982). Brain AChE activity is used as a specific indicator of exposure and death from cholinesterase inhibiting pesticides (Shellenberger *et al.,* 1970). In addition, brain AChE inhibition is dose-dependent and certain levels of depression can be correlated with death (Bunyan *et al.,* 1968; Ludke *et al.,* 1975). Because this is a quantitative diagnosis, rather precise knowledge of the baseline norm is required and previously thought only obtainable from collection of concurrent controls (Hill and Fleming, 1982).

Brain AChE activity was the highest in Asian Openbill followed by House Crow, Painted Stork, Indian White-backed Vulture and Crow Pheasant, while Bank Myna, White Ibis and Barn Owl had comparatively less AChE activity. As reported by Wang and Murphy (1982), brain AChE activity in smaller birds is higher than the bigger birds except Indian White-backed Vulture. Brain AChE levels measured in Painted Stork, Blue Rock Pigeon and Crow Pheasant were almost equal to the activity reported in two species of birds, namely Rose-ringed Parakeet (3.75 $\mu$moles/min/g) and White-throated Munia (3 $\mu$moles/min/g) before they were exposed to quinalphos and methyl parathion respectively (Anam and Maitra, 1995; Maitra and Sarkar, 2006). The brain AChE activity varied among species significantly (ANOVA, $P < 0.05$) as reported by Hill (1988).

Comparatively low BChE activity was recorded in all the species of birds and it did not vary significantly among species (Table 7.2). The highest BChE activity was recorded in House Crow and the lowest was in Asian Openbill. In general the low BChE activity observed in the brain, supports the hypothesis that butyrylthiocholine is hydrolyzed by an atypical cholinesterase (Toutant *et al.*, 1989) and acetylthiocholine by both a true cholinesterase and a pseudocholinesterase. BChE activity was very much similar in all the species included in the study.

In the present investigation number of individuals received per species was adequate only in five species of birds. Hence, only five species, namely Cattle Egret, Pariah Kite, Blue Rock Pigeon, Indian White-backed Vulture and Asian Koel were selected to demonstrate the consistency in brain AChE and BChE activity over a period of three years (Figure 7.1a). However, there was no significant difference observed among years ($P > 0.05$) with in each species. Hence, these values can be treated as baseline values.

AChE activity was comparatively low in plasma samples of birds than brain. Many studies have reported the plasma AChE activity to be erratic when pesticide expose studies were conducted (Bunyan *et al.*, 1968; Fleming, 1981). It is also reported that plasma AChE activity is inferior to brain activity for environmental monitoring, because of its rapid recovery and large degree of variation among individuals (Dieter and Ludke, 1978; Fleming, 1981). Sex wise variation in plasma samples was not possible to be assessed because of the inadequate sample size. Since plasma samples were collected from live birds, it became difficult to identify sex differences unless cut open the birds.

The present study found the brain AChE and BChE activity to be unaffected by the sex of the birds as reported by Westlake *et al.*, (1983). Although, the present study did not look at seasonal variation, it is understood from the literature that the ChE activity generally does not vary among seasons. However, there are a few exceptions where extreme temperature conditions had influenced the ChE activity (Mohamed *et al.*, 1983; Hill and Murray, 1987). In Indian context as numerous incidents of wildbird mortality keep happening due to indiscriminate use of pesticides, efforts must be made to enhance our ability to accurately diagnose such poisoning. As reported by Hill and Murray (1987), development of database for wildlife is necessary and it requires a standardization of methods and careful documentation of factors that may influence results.

Due to lack of published information on the ChE activities in birds in India, the data generated in the present study do not allow any comparison or show any possible chemical influences. In species, namely Asian Koel, Blue Rock Pigeon, Pariah Kite, Cattle Egret and Indian White-backed Vulture as the number of individuals is comparatively more and the activity did not vary among the three years significantly, the levels recorded could be considered as reference values if not normal values. Further, considering the fact that all the birds included in the study were victims of kite flying, the recorded levels could to some extent be called as normal till such time disproving data sets are available.

The species specific variation in AChE was very much perceivable in the present study. This is in line with what was reported from 83 sets of control specimens submitted to the Patuxent Wildlife Research Centre between 1978 and 1985 for use as reference samples in the evaluation of possible cases of wildlife poisoning by anticholinesterase pesticides (Hill, 1988). AChE activity recorded in brain and plasma samples of Blue Rock Pigeon, House Crow, Pariah Kite and Indian White-backed Vulture can be considered as normal. However, in the case of White Ibis, Black Ibis, Barn Owl, Crow Pheasant, Asian Openbill and Painted Stork bigger sample size needed. Although sub-lethal anticholinesterase activity exposure could affect the utility of free-ranging birds as control, the source of error was not proved to be a serious problem (Hill, 1988).

### 7.7. Conclusion

AChE and BChE activity should be routinely evaluated in diagnosing anticholinesterase poisoning in wildlife. Since timely acquisition of concurrent controls is not always feasible, development of a reference data of normal ChE activities for various wildlife species particularly for birds is suggested. The estimated normal whole brain ChE activities presented in Table 7.2 and 7.4 are offered as the 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 of the referred region in India and constitutes a starting point for future studies that evaluate the 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 birds, and creation of a much larger database.

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**Annexure**

| | |
|---|---|
| Aldrin | 1,2,3,4,10,10-hexachloro -1,4,4a,5,8,8a-hexahydro-1,4-eno-exo-5,8-dimenthanon-naphthalene. |
| Heptachlor | 1,4,5,6,7,8,8-heptachloro -3a,4,7,7a- tetrahydro -4,7-methano indene. |
| Heptachlor epoxide | 1,4,5,6,7,8,8-heptachloro -2,3 epoxy -4,7 – endomethano 3a, 4,7,7a- tetrahyydroindene. |
| Endosulfan | (Alpha and beta). I,4,5,6,7,7- hexa-chlorobicyclo (2.2.1) -5-heptene -2,2,3-bis-(methylene)-sulfite. |
| Endosulfan sulfate | 6,7,8,9,10,10-hexachloro- 1,5,5a,6,9,9a-hexahydro- 6,9-methano-2,3,4-benzodioxythiepin-3,3-dioxide. |
| Dieldrin | 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro -1,4-endo-5,8-exo-dimethanonaphthalene. |
| HCH | 1,2,3,4,5,6-hexachlorocyclohexane (mixed isomers) |
| *p,p'*-DDT | 1,1,1-trichloro-2,2-di(p-chlorophenyl)-ethane |
| *p,p'*-DDE | 1,1-dichloro-2,2-bis(p-chlorophenyl)- ethylene |
| *p,p'*-DDD | 1,1-dichloro-2,2-bis(p-chlorophenyl)- ethane |
| EPA | Environmental Protection Agency |
| WHO | World Health Organization |
| FAO | Food and Agriculture Organization |
| FDA | Food and Drug Administration |
| PCBs | Polychlorinated Biphenyls |
| PAHs | Polycyclic Aromatic Hydrocarbons |
| ChE | Cholinesterase |
| AChE | Acetylcholinesterase |
| BChE | Butyrylcholinesterase |
| CbE | Carboxylesterase |
| POPs | Persistent Organic Pollutants |
| OCPs | Organochlorine Pesticides |
| OCs | Organochlorines |
| SACON | Sálim Centre for Ornithology and Natural History |
| ICMR | Indian Council for Medical Research |
| ICAR | Indian Council of Agricultural Research |
| TNAU | Tamil Nadu Agricultural University |
| NIOH | National Institute of Occupational Health |

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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<br>05.05.2003 | Ahmedabad | 23°03.00′ N to 72°58.00′ E | 2 |
| 4 | 22.02.2003 | Mudumalai | 11°37.00′ N to 76°34.00′ E | 1 |

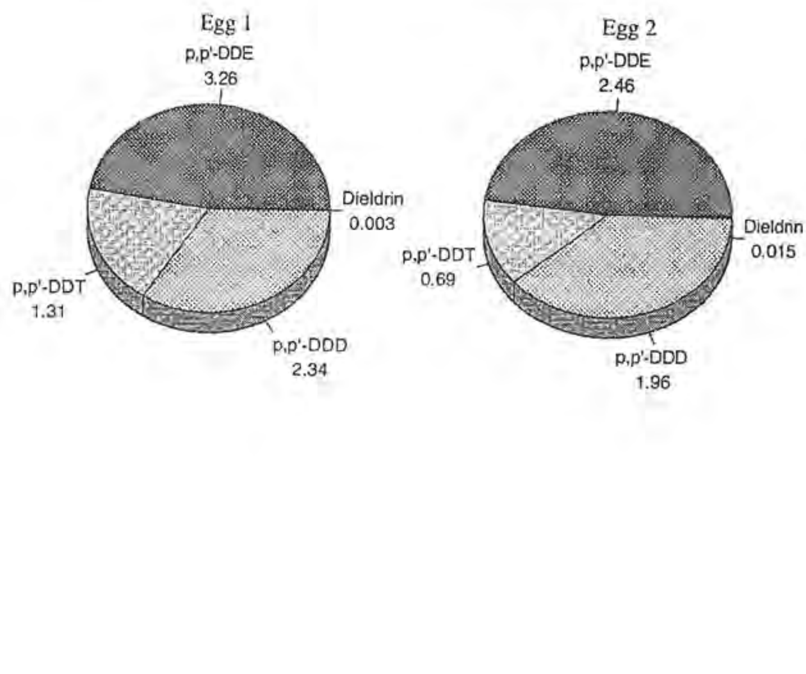


**Fig. 1** Organochlorine residues (µg/g, wet weight) in eggs of white-backed vulture received from the vicinity of Bharatpur, Rajasthan (December 1999)

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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