CHAPTER –4

ORGANOCHLORINE PESTICIDES IN THE BODY TISSUE OF SELECTED AVIAN KEY SPECIES

1. Introduction

The possible reduction of birds due to pesticides has been of a great concern for wildlife biologist, since the beginning of organic insecticide era from 1940' s However, the publication of Rachel Carson' s Classic in 1963 book on "*Silent Spring*" did alert the mankind to the toxic side effects of the organochlorine insecticides, such as DDT, that had fuelled the green revolution The residues of these pesticides were found to persist in food chain reaching higher concentrations, and hence having more severe effects at successive trophic levels Subsequently, they were identified as the root cause of the rapid population decline in the birds of prey such as Peregrine Falcon (*Falco peregrinus*) and Sparrow Hawk (*Accipiter nisus*), through the thinning of the eggshells The offensive chemicals have now been phased out in the developed countries, but are still in use in some parts of the World

Residues of organochlorine compounds (like DDT, DDD, DDE, HCH and Lindane) are believed to be additive in assessing the degree of damage in interpreting concentration in the brain with regards to the lethality (Stickle *et al.*, 1970). Many wild birds like numerous song birds including the American Robin (*Turdus migratorius*) were reported to have died due to the use of DDT for the control of Dutch elm disease (Hickey and Hunt ,1960; Bernard, 1963).

Population of many birds in the United Sates that had declined due to the toxic effect of DDT, recovered after use of most of these compounds were banned

in 1971, but in developing countries like India, the constant use of organochlorine pesticides for one or other purpose, results in the continuous upsurge of residues in the environment Unfortunately most of our water bodies are also contaminated either by direct application to water surface drift or by run off of pesticides. The use of pesticides in the crop fields had caused an apparent decline of aquatic fauna depending on the wetland Looking on to the use of pesticides in our country, and its effect on the biotic components of ecosystem, it is necessary to minimize the organochlorine pesticides burden in the environment. How much of this should be permissible? That can be decided only after having the knowledge of, how much of pesticide from the different components of the environment is entering into avian body, in what form it is stored in body after its metabolism, how far these metabolites are toxic to the body and which organ or tissue is most affected. To fulfill some of these objectives raised, the present study on the organochlorine pesticide concentration in the body tissues of key species of avian community was conducted.

2. Material and Methods

2.1. Selection of sampling materials

The residue analysis of animal tissues is a useful tool for monitoring the exposure of species to the environmental pollution, and it reflects the severity of contamination of the given sites. The contaminant level in the tissues is influenced by the nature of the contaminant, age of the affected individual, time interval of exposure to contaminant and time elapsed after exposure. Hence proper use and selection of key species enhances the accuracy of the estimates To study the effect of organochlorine pesticides in the paddy crop agro-ecosystem, Cattle Egret and Black-throated Weaver Bird were selected (Chapter 2).

Most of the pesticides used have relatively high solubility in oils (including lipids) and organic solvents Due to this property these compounds are stored in the lipids throughout the body and is also instrumental in bioaccumulation The accumulation in different tissues will vary depending on the functioning of that tissue. Thus the residues in the brain are more diagnostic of death (due to neurotoxicity) than other tissues, such as liver, fat and kidney. Hence to assess severity of organochlorine pesticides the brain and deposited fat selected for residue analysis.

2.2. Collection of sample

The bird species (Cattle Egret and Black-throated Weaver Bird) selected for the study were collected using mist net in the evening hours from the roosting sites/nesting sites in Kheda district during July to August 2000 A total of 20 individual birds representing 2 species of different food habits were collected from different sites across the Kheda district. Body weight of individual bird was recorded using Pasola spring balance. Within 24 hours of capture, the birds were sacrificed and dissected for body tissues like brain and pectoral muscles for deposited fat. Total 40 samples were analyzed for compounds of organochlorine pesticides, namely DDT and their metabolites, HCH and lindane.

2.3. Preparation and residues analysis

Two grams of minced brain tissue was taken and homogenized with 7 ml formic acid and transferred to a 50 ml conical flask. The homogenizing tube and pastle were washed twice with 5 ml portions of n-hexane and the washings were collected in the flask. The homogenate was kept on shaker water bath at 40 °C for 1 hour and then the solvent layer was withdrawn. Minced deposited fat (0.5g) was homogenized with 3 ml formic acid and 5 ml n-hexane, transferred to a 50 ml conical flask and treated as above (Cromartie *et al.*, 1975).

Gas-Liquid Chromatography (GLC), using Electron Capture Detection (ECD, 3H), was used to determine pesticide residues by injecting a volume of 0.5 µL/L, with Hamilton micro liter syringe into injector port at following operating conditions:

Carrier gas: Pure nitrogen passed through silica gel and molecular sieve to remove moisture and oxygen, respectively.

Gas pressure	65 psi
Gas flow	40 ml/min
Detection temperature	200°C
Injection temperature	190°C
Column temperature	180°C
Column	Glass spiral column, 6 ft X 1/8 in. ID, coated with 1.5 percent OV- 17 +1.95 % OV-210.

Residue peaks were identified by thin-layer chromatography on silica gel-G coated glass plates and compared with reference standards. The residues in ppm was calculated by using following formula:

= (Concn. std. (µg/mL) X (Peak size sample/peak size std.) X (µL std. / WL sample) X diln vol/ 2g sample)

Recoveries of HCH isomers, DDT, and DDT metabolites in the fortified samples of brain and deposited fat were between 70-94%. Sensitivity of method was about 0.001 ppm for HCH isomers, aldrin and *p, p'* DDE and about 0.002 ppm for *p, p'* DDT.

All reagents and chemicals used were of high purity and were checked for interference under the experimental condition. The residue analysis was carried out at SICART, V.V.Nagar.

2.4. Data presentation

The majority of birds were collected just before or after a month of egg laying, assuming that any potential difference in contaminant levels in adult males and females due to female eliminating contaminants into eggs would have been compensated through rapid up take of contaminants (Halt *et al.,* 1979, Hebert *et al.*,1990; Gebauer and Weselon, 1993). The selected birds are from different trophic level and there is wide difference in food habits and feeding habitat, so the contaminant levels were directly compared with each other by geometric mean. Also, the data of individual contaminants were subjected to one tail pair 't' test for comparing the contaminant levels among the birds. The limit of quantitation (LQ) for organochlorine was set at 0.001 mg/kg. For the purpose of data analysis non-detected values were replaced by constant replacement value (1/2 LQ)

3. Result and discussion

The concentration of DDT and HCH residues in brain tissue and deposited fat of selected key species is presented in Table 4.1 and 4.2. All the values are expressed in terms of whole tissue wet weight. DDT, HCH and their residues are widely distributed in the ecological system. Although the HCH residues are excreted rapidly (Cramp *et al.,* 1964), but slow accumulation does occur in the body tissues and in body fats on chronic exposure. DDT and its derivatives are quite stable and are resistant to enzymatic action, thus the level of DDT residues present in the body tissue are very often taken as an index of contamination by DDT and DDT metabolites of the local environment.

Table 4.1 Range** and geometric mean* value of total DDT and DDT metabolites residues in brain tissues and deposited fat of two wild birds

Bird	Residues (ppm whole tissue wet weight)							
	Brain tissues				Deposited fat			
	p,p' - DDE	*p,p'* - TDE	*p,p'* - DDT	Total DDT	*p,p'* - DDE	*p,p'* - TDE	*p,p'* - DDT	Total DDT
CE	0.028*	0 003	ND	0 033	2.760	14 58	4.69	20.131
	0 024** – 0.031	0 002 – 0 005		0.030 – 0 040	2.131 – 3 046	2.831 – 23.47	1.416 – 7.843	8 431 – 37.241
BTW	0 023	0 003	ND	0.030	1 867	8.41	4 05	14.72
	0.020 – 0.028	0 000 – 0 004		0 028 – 0.036	1.003 – 2.098	1.71 – 17.31	1.42 – 7 31	5 47 – 21 39

CE = Cattle Egret, *Bubulcus ibis* ,
BTW = Black-throated Weaverbird, *Ploceus benghalensis*

Table 4.2: Range** and geometric mean* value of total HCH and γ□HCH (Lindane) residues in brain tissues and deposited fat of three wild birds.

Bird	Residues (ppm whole tissue wet weight)			
	Brain tissues		Deposited fat	
	Total HCH	Lindane	Total HCH	Lindane
CE				
	0.041*	0.030	5.80	4 97
	0.001 – 0 135**	0.003 – 0 057	4.70 – 7.31	3.51 – 6.41
BTW				
	0.010	0 001	4.211	2.797
	0.001 – 0 016	0.000 – 0.002	2.058 – 6.418	1.165 – 5.713

CE = Cattle Egret, *Bubulcus ibis*,
BTW = Black-throated Weaverbird, *Ploceus benghalensis*

3.1. DDT and their metabolites

The results obtained from the analysis of brain tissue and deposited fat of Cattle Egret and Black-throated Weaver Birds for DDT and their metabolites (*p,p'* DDE, *p,p'* TDE, and *p,p'* DDT) are presented in Table 4.1. In the Cattle Egret, the mean total DDT detected in brain tissue was 0.033 ppm and 20 131 ppm in the deposited fat. The residue of total DDT detected in brain tissue ranged from 0.030 – 0.040 ppm while in deposited fat it was 8.431- 37 241 ppm. The residue of pp' DDE, and pp' TDE was detected in both tested sample i.e. brain and deposited fat, while pp' DDT was detected only in deposited fat, and was not detected in the brain tissue.

In case of Black-throated Weaver Birds, the geometric mean of total DDT detected was 0. 030 ppm in brain tissue and 14.72 ppm in the deposited fat, ranging between 0.028 – 0.036 ppm in brain tissue and 5.47 – 21.29 ppm in deposited fat. The residue of DDE and TDE was detected in all the tested samples of both brain and deposited fat, except in two-brain tissue samples of Black-throated Weaver Birds, where TDE was below detectable limit.

3.2. HCH and lindane

Table 4.2 shows the residue level of HCH and lindane present in the brain tissue and deposited fats of Cattle Egret and Black-throated Weaver Bird. The geometric mean of total HCH residues detected in the Cattle Egret brain tissue was 0 041 ppm and lindane was 0.030 ppm. Whereas in deposited fats was 5 08 ppm and 4.97 ppm respectively. The highest total HCH recorded was 0.135 ppm in brain and 7.31 ppm in deposited fat. The HCH residues recovered from the samples ranged between 0.001 - 0.135 ppm in brain and 4.70 - 7.31 ppm in deposited fat. It was important to note that HCH residues were recovered from all the tested samples. The lindane residues ranged between 0.003 - 0.057 ppm in brain and 3.51 - 6.41 ppm in deposited fat. Lindane was also recovered in all the tested samples.

In case of Black-throated Weaver Birds, the geometric mean of total HCH and Lindane residues detected was 0.010 ppm and 0.001 ppm in brain tissue while 4.211 ppm and 2.797 ppm in deposited fat respectively. The highest total HCH was 6. 418 ppm and lindane 5.713 ppm in deposited fat. The HCH residues recovered from all the tested samples, ranged between 0.001 - 0.016 ppm in brain tissue and 2.058 - 6.418 in deposited fat. The lindane was also recorded in all the tested samples ranging between 0.000 - 0.002 ppm in brain tissue and 1.165 - 5.713 ppm in deposited fat.

The residue level of all the compounds was lower in Black-throated Weaver Bird compared to the residues obtained from the tissues of Cattle Egret irrespective of the tissue type. However, there was a considerable difference in the residues of HCH in deposited fat and brain tissue. While the residue level of DDT in brain tissue did not vary significantly, this might be attributed to the factor of its trace existence.

The low level of residue of all the compounds in the Black-throated Weaver Birds could be due to the food habit of the bird. The Black-throated Weaver Bird is a facultative granivore where the animal matter comprising of only 1.9 % of the total diet (Chapter 3), is comparatively low. So the intake of pesticides also will be low, which intern, is depicted in the tissues

In the earlier studies until 1962, the bird carcasses samples were analyzed at random for aldrin, dieldrin or DDT, DDE. The primary breakdown product of DDT was uniformly distributed but the frequency of residues of parent compound DDT and its metabolite TDE was less (Edwards, 1976). Kaphalia *et al.* (1981) while studying organochlorine in some Indian wild birds including Cattle Egret have shown that contaminants in Cattle Egret was as high as in Kite - the bird of higher trophic level Whereas, the studies carried out on the related species such as Little Egret (*Egretta garzetta*), Squacco Heron *(Ardeola ralloides)* and Night Heron (*Nycticorax nycticorax*) in Greece during 1996 revealed that organochlorine contaminant was below the critical level,

which could cause adverse effects (Albanis *et al.,* 1996). In case of Great Blue Heron collected from different parts of USA during 1966 to 1978, showed a wide range of variability in different tissues like DDE 0 63-130.0 μg/g in carcasses and ND- 22.0 μg/g in brain, DDT ND – 130 μg/g in carcasses (Ohlendorf *et al.*, 1981). While affected birds collected from south Dacota in 1975 showed the residue; 246.33 μg/g DDE, 98 μg/g DDD, 0.60 μg/g DDT, 0.47 μg/g Dieldrin, 0.5 μg/g Lindane, 0.35 μg/g Heptachlor epoxide in brain tissues (Call *et al.,* 1977) Dead birds collected from Washington from 1977-1981 showed organochlorine residues of DDE in brain 0.10 to 0.25 μg/g wet weight (Fitzner *et al.*, 1988) The lower residue levels found in present study compared to other related species studied by several researchers could be due to the regional difference in the contamination of the environment.

The question arises is, what is toxic level of these pesticides or what is the lethal dose and so on. Large number of scientists has put in their findings to discuss these issues. Of all the organochlorine compounds, DDT has the longest history of its use as an effective insecticide. This well-known pesticide was first synthesized in 1939. The available literature tells about toxicity of DDT and its related compounds studied in great detail. The LD50 value (Single dose calculated to kill 50 % of the test organism) of DDT for birds ranged from 595 to > 2000 mg/kg (Hudson *et al.*, 1984). The LC 50 (Dietary level calculated to kill 50 % of test organism over 5d and 3d on clean food) for birds ranged from 311 to 1869 mg/kg for DDT and 579 to 4814 mg/kg for DDD and 825 to 3572 mg/kg for DDE (Hill *et al.*, 1975). While the LD 50 for HCH and lindane for birds, ranges from 118 to >1400mg/kg and 75 to >2000 mg/kg respectively (Schafer *et al.,* 1983; Hudson *et al.*, 1984). LC 50 for lindane ranged from 400 to >5000 mg/kg in birds (Hill *et al.,* 1975). However, critical and lethal dose for tissues have not been yet established for HCH or its isomers. In spite of some experiments carried out when Rock Pigeon *Columba livia* was given daily high dose of 72 mg/kg of lindane for 5 days had accumulated up to 65 μg/g of

lindane in the liver when etherized (Turtle *et al.*,, 1963). For DDT data based on captive experimental birds, the known lethal range of residues in the brain begins at a total of 10 DDT toxic equivalents with 1 μg/g DDT or 5 μg/g DDD or 15 μg/g DDE equaling one DDT toxic equivalent (Stickle *et al.*, 1970) However ≥ 20 toxic DDT equivalents were found in the brain of most DDT killed birds. The zone of increasing hazards for DDD alone was considered to begin at 65 μg/g (Stickle *et al.*, 1970, 1984). Some of the captive American Kestrel *Falco sparverius*, died with 213 to 301 μg/g DDE in the brain (Porter and Wiemeyer 1972, Henny and Meeker 1981), suggesting that lethal level in this species may be some what lower than in other species.

The critical threshold of organochlorine pesticide in brain tissue of bird is 10 mg/kg for DDT, 50 mg/kg DDD and 150 mg/kg DDE (Stickle *et al.*, 1991) Though no critical threshold level for deposited fat has been set up, but the maximum admissible concentration in poultry birds for human consumption is 7 mg/kg for HCH and 5 mg/kg for DDT (Braune *et al.*, 1999). The critical threshold for human consumption is much lower than the level that could cause the adverse effect on the other species. In the present study, the residue levels of all the compounds were lower than the critical threshold determined for the human consumption and hence the risk to the species is much lower from the present level of contaminants. The toxic and harmful levels of two materials such as major and minor trace elements having antagonistic, additive or synergistic effects with each other and within the organic compounds is not available in the literature (Liwis *et al.*, 1992). Such studies are required to determine the composite impact of the observed residues.

Birds experiencing the greatest effect from DDE were long-lived *K* – selected species occupying top position in the food chains, they range from simple Brown Pelican to complex Peregrine Falcon. Both the species experienced the catastrophic problems from DDE; and the Falcon population was extirpated in the Eastern United States (Berger *et al.*, 1969). The Peregrine Falcon being

more tolerant to DDE and because of its top position in the food chain has a much higher potential for accumulating residues of DDE and other lipid soluble organochlorine compounds/pesticides (Blus ,1995).

In the present study, *p,p'* DDT was not detected in the brain tissue of Cattle Egret and Black-throated Weaver Bird where as it was present in the deposited fat. This being not detected in one tissue (brain) and found in deposited fat could be an indication of exposure to recently applied DDT as suggested by (Henny *et al.,* 1982a) and because of its low level as seen in deposited fat does not reach brain or if at all it reaches, is in a trace amount, which is not detected. Because of its lipid solubility it goes along with lipid, which is to be stored but does not reach the level which could be detected, it is like slow poisoning, which does not kill the animal immediately, but its presence in the body tissue does affect the population in the long run.

In the present study, the level of organochlorine residues detected in the body tissue and available literature has shown its possible impact on the population and the environment. It has covered a wide range of species by food habit, body size, and foraging tactics and by taxonomic groupings. The overall impact of organochlorine compounds in the study area is a matter of concern to the conservationist and environmentalists. The pesticide residues in the body tissue are still found in the higher range which is not causing direct mortality of the species, but may be indirectly affecting the population which includes the reproductive failure, egg shell thinning, juvenile mortality and thus ultimately decline in the reproductive success of individual population (Cooke, 1973) Loss of appetite, decrease in availability of food and habitat change is other noticeable factors, which are putting indirect impact on biodiversity. The current use of DDT in the study area is evident from the results obtained. So to save environment one needs to reduce the load of organochlorine in the environment by stopping its use. Though there is no official record of DDT being used for agricultural purpose, but illegally it is being used. For quantifying

the damage caused by these organochlorine residues accumulated in the body tissue, one requires to have detailed study on its impact on reproduction, habitat, food chain contamination and toxicological relationships between these compounds Simultaneously organochlorine compounds should be replaced by NOVEL bio-pesticides, which is least hazardous in the natural habitat