CHAPTER - 5

Impact of organochlorine pesticides on reproduction

1. Introduction

There is a mandate testing impact of new pesticide on reproductive efficiency of one or more avian species before pesticide registration (U. S. Environmental Protection Agency 1978, Indian Pesticide Act, 1992) Unfortunately, the results of such tests are rarely published as the data are considered to be the rights of an individual

The available data for wildlife species is limited due to practical difficulties in rearing of the birds, testing the wildlife species for toxicity, dose response studies and also the cost Thus most of the available figures are from existing wildlife death rate and poultry data (use of model-generated values) for estimating the quality of habitat (Nebeker *et al.*, 1994) Under such circumstances the pesticides have open access into the environment with little or no restriction resulting into serious problems. These effects of pesticides on wildlife being sublethal were not taken seriously, until its first documentation affecting reproduction due to inhibitory action of DDE observed by Broely in Bald Eagles in Western Florida over the period of 1939-1958. Later several studies have shown sublethal effect of pesticides on population decline in birds like Ospreys, Bald Eagles and Peregrine Falcon (Hickey and Anderson, 1968; Risebrough, 1986) and even on many of the wetland species like Brown Pelican, Double Crested Cormorant (Gress *et al.,* 1973, Anderson and Gress, 1983).

Theoretical assessment of pesticide effect on avian population have shown that the adult mortality, reduced fecundity and partial sterility induced by the pesticides could differentially reduce reproductive potential according to the rate of population turn over (Young, 1968). Reproductive impairment by 0.6 mg/kg of Dieldrin as a sub-lethal dose has been demonstrated in several studies (Dewitt 1955, Borg *et al.*, 1969, Spann *et al.*, 1972). For birds, DDT is well known for its sub-lethal effects which induce failure in adults to breed, egg shell thinning, decrease hatchability, reduction in survival rate and ultimately hindering breeding success (Cooke 1973). In India, such studies on the avian community and related risk to ecosystem are very limited. Even though, 40 % of the total globally threatened animals of India are form the avian community. Thus it becomes very important to document the impact of organochlorine pesticide residues on the reproductive potential of birds. This will also serve the purpose to evaluate the hazard caused by pesticides to the environment and draw proper management strategies.

2. Material and methods

2.1. Selection of sampling material and method for evaluating the impact

The eggs served a very useful entity for residue analysis of oraganochlorines because the concentrations in the eggs reflects, the level of contaminants in the female at the time of egg laying, which in turn may influence the reproductive success. In addition, the eggs decompose slowly and are easily handled (Ohlendrof *et al.*, 1978).

The "sample egg technique" (Custer *et al.*, 1984) used to study the impact of organochlorine on breeding performance, involves contemporary collection of one egg from each nest in a series within a population. The nests were marked for the study and checked regularly at an interval of 2-

3 days. Residues of organochlorine in the eggs were analysed. The effect of residues on nest success is indicated when rate of success decreases with an increase in residues content. In birds the significant difference in the egg shell characteristic and organochlorine concentration in eggs within clutch have sometimes been found in relation to the sequence in which the eggs were laid (Nisbet 1982) but, this sequential effect has not been found always true (Cluster *et al.*, 1983). Thus to avoid this, the first egg of the clutch was used for the organochlorine residue analysis.

2.2. Nesting survey and monitoring

The study area was intensively surveyed for nesting colonies of Cattle Egret during June-July 1999 (Plate III). Of the 85 heronries studied (Chapter 3) one pure Cattle Egret heronry was selected for detailed study on breeding performance and impact of organochlorine residues. The monitoring of particular heronry was done from the stage of nest building (last week of June 2000). Total ten nests of Cattle Egret selected and marked for the study were visited regularly every 2-3 days to determine its success. The parameters like number of eggs laid, number of egg hatched and number of chicks fledged was noted, and on these basis mean clutch size, hatching success and fledging success were calculated.

First egg was removed from individual nests of the colony during laying period for organochlorine analysis but as an egg removal appeared to affect nest success, these eggs were not included for the analysis of reproductive success.

2.3. Sample collection, preparation and analysis

First laid egg was collected from all ten marked nests of Cattle Egret heronry and was replaced with the hen' s infertile egg. The collected eggs were individually wrapped in aluminum foil, and placed in plastic

Plate III. Cattle Egret nesting Colony.

containers to minimize the water loss, and refrigerated till they were processed. Eggs diametric (length, breadth, weight, volume, Egg shape Index) were measured. Content of the egg was placed in a chemically cleaned jar previously washed with acid and rinsed with acetone for residue analysis by method of Cromartie *et al.* (1975).

The samples were homogenized and a 10 g portion was mixed with anhydrous Sodium Sulfate and extracted in Hexane in soxhlet apparatus for 7 hrs, the lipids were removed by florisil column chromatography. Each sample extracted was then analyzed for a series of organochlorine contaminants including *p,p'* DDT, *p,p'* DDE, *p,p'* DDD, total DDT, HCH and lindane. All the concentrations of residue obtained is presented on wet weight basis.

3. Results and Discussion

3.1. Reproductive success

The study on the reproductive success of Cattle Egret was carried out in Kheda district, Gujarat and detail results are presented in Table 5.1. The determined mean clutch size was 4.3 eggs. The eggs diametric were as follows; mean length 43.9 mm, mean width 32.4 mm. The mean egg shape index was 75.08. The mean egg weight was 25.6 g.

Of the 43 eggs laid in ten nests one egg from each nest was removed for pesticide residue analysis and remaining 33 eggs of the clutch were monitored for breeding success. Out of total 33 eggs monitored, 2 eggs were destroyed by predators or physical factors and 1 failed to hatch. Thus the average hatching success was 92.17%. One egg that failed to hatch accounts 3.03 % hatching failure. Rest 4.8 % loss was due to unidentified mortality factors. However, wind velocity or predation could be assumed in this case.

Table 5 1. Cattle Egret nesting success and egg diametric.

Sr. No.	1st Egg Diametric				Clutch size	Clutch for calculation	Hatching success	Fledging success	Breeding success
	L	W	ESI	Wt.					
1	44 2	33 1	74.80	24.2	4	3	100.00	66.66	66.66
2	46 3	34.0	73.40	27.0	5	4	100.00	50.00	50.00
3	41.9	32.2	76.85	24.8	3	2	100.00	50.00	50.00
4	44 5	31.7	71.24	25.2	4	3	100.00	66.66	66.66
5	45.2	33.1	74.38	24.0	4	3	100.00	66.66	66.66
6	46 1	34.3	74.40	27.0	4	3	66.66	100.00	66.66
7	42.4	31.1	73.35	26.5	5	4	75.00	100.00	75.00
8	45.0	32.0	71.11	26.9	6	5	80.00	75.00	60.00
9	41 0	31.0	75.61	24.0	3	2	100.00	50.00	50.00
10	42.3	32.0	75.65	26.1	5	4	50.00	100.00	50.00
Mean	43 9	32.4	75.1	25.6	4.3	3.3			

Note: L: Length (mm), W: Width (mm), ESI· Egg Shape Index, Wt.: Weight (g)

Fledgling success was 72.50 % as eight chicks failed to reach the fledgling stage. Thus the overall breeding success was 60.16 % in this representative population of Cattle Egret.

3.2. Residues analysis in eggs

The organochlorine pesticides DDE, TDE, DDT, Total DDT, HCH and Lindane obtained in analyzed eggs are presented in Table 5.2. The concentration of DDE in the Cattle Egret eggs ranged from 0 07 to 2 12 ppm. The mean (geometric) concentration of DDE was 0.92 ppm It was important to note that all the eggs had significant concentration of TDE residues mean 2 39 ppm. The mean (geometric) total DDT residues obtained was 5.79 ppm and ranged between 2.45 to 10.98 ppm. The other organochlorine like HCH and lindane were detected at the range of 1.52 to 3.49 ppm and 1.02 to 3.10 ppm respectively. The mean concentration of DDE residues in egg was 33 times higher than it was detected in brain tissue and 3 times lower than observed in deposited fat.

3.3. Impact of organochlorine on reproductive success

Results indicates that the residue level of DDE obtained (Table 5.2) was lower in all the eggs analyzed than critical level 12–15 ppm of DDE in the eggs of Black-crowned Night herons (Blus, 1984) and > 5 ppm for Great Egret (Cornely *et al.*, 1993)), however, which has an adverse affect on reproduction. The correlation derived for egg length, breadth, weight, egg shape index and clutch size in relation to each contaminant was not statistically significant. But, it was negatively correlated with DDT and their metabolite and lindane, which indicate that organochlorine contaminants definitely alter the egg diametric While DDE, and TDE residues also were negatively correlated with breeding success.

Table 5.2: HCH, Lindane and Total DDT residues in Cattle Egret eggs.

Sr. No.	Residues (ppm whole egg wet weight)					
	HCH and its isomer		DDT and Its Metabolites			
	HCH	Lindane	P,P DDE	P,P TDE	P, P DDT	Total DDT
1	1.82	1.02	0.43	2.52	0.92	6.16
2	1.52	2.31	0.07	2.58	1.05	6.88
3	2.52	3.10	1.32	1.98	0.82	4.75
4	2.73	2.73	1.54	1.72	0.52	3.42
5	2.22	1.16	2.01	5.02	3.12	10.98
6	3.49	2.15	2.12	1.03	0.61	2.45
7	2.93	3.00	0.91	3.37	1.83	4.78
8	3.38	2.17	1.98	2.82	1.02	6 92
9	2.23	1.92	1.65	2.55	1.08	8.79
10	3.12	2.18	0.57	2.19	1.28	7.92
Geometric Mean	2 52	2 06	0.92	2.39	1.07	5.79

Similar studies carried out on other bird species have shown the following trend: The Black Skimmer population in Texas declined by 24 % between 1974 and 1980 (Texas colonial waterbirds society, 1982). Several studies conducted in Texas during 1970 and early 1980 had reported a very high organochlorine contamination in the Black skimmers eggs at the level (0.5-86 ppm of DDE) that was within the range, which could lower the reproductive success (King *et al.,* 1976; White *et al.,* 1984; Custer and Mitchell, 1987).

In Black-crowned Night Herons some exceptionally high level of DDE (> 8 ppm) found in the eggs was shown to be responsible for low productivity and high incidence of cracked eggs (Henny and Blus, 1986). Even declined in the population of Fulvous Whistling Ducks in Texas was attributed to mortality from Dieldrin and Aldrin (Flickinger and King, 1972)

The Peregrine Falcon population declined when DDE concentration had reached 15 - 20 ppm wet weight in egg (Peakall *et al.*, 1975; Fyfe *et al.*, 1976; Newton, 1979). Even the amount of DDE 1.00 ppm wet weight in prey consumed during the breeding season was considered to be sufficient to reduce the productivity in peregrine population due to egg shell thinning (Enderson *et al.,* 1982). However, it was lower than 3.0 ppm suggested for this effect by DeWeese *et al.* (1986). Since the pesticide used are for targeting different pest, the cases of secondary poisoning can occur when predators (birds) consume the prey (insects) contaminated with pesticides Generally, these insectivorous and carnivorous feeders exhibit higher DDE levels due to biomagnification than the omnivorous feeders (DeWeese *et al.,* 1986; Fyfe *et al.,* 1990). However all the species which did not show higher levels of DDE, it varies with the species, which depends up on the amount of contaminant ingested along with the diet like DDE level found in both insectivorous and carnivorous feeders, like Southern Lapwings (*Vanellus* sp) 2.31 ppm and Aplamado Falcon 1.34

ppm (Hector, 1985) and the omnivorous Great-tailed Grackle *Cassidix mexicannas* 1.56 ppm pose no known threat to the reproductive success (Banasch *et al.,* 1992).

The occurrence of parent compound of DDT in the samples has been an indicator of exposure to recently applied DDT (Henny *et al.,* 1982a). The residues levels even at > 15 ppm dose not impair the breeding success of Peregrine Falcon (Ambrose *et al.*, 1988). There are no reports regarding HCH and Lindane having any adverse effect on reproductive success of birds (Blus et al., 1995) The hatchability of Ring-necked Pheasants eggs were unaffected when lindane residues in eggs was about 10 ℓg/g (Ash and Tylor, 1964). This show that neither HCH nor its isomer (Lindane) cause significant adverse effects on birds, if the levels are lower than the effective value.

The organochlorine in Grey Heron (*Ardea cinerea* L.) in France indicated that alpha HCH, delta HCH, Dieldrien, *p,p*' DDD and *p,p'* DDT were below the detectable limit while *p,p'* DDE, lindane and HCH were detected Thus concluded that the residues level were lower than those associated with effects on reproduction known from the field and laboratory studies in other bird species (Cruz *et al.*, 1997). Longcore *et al.* (1971) reported that the DDE/DDT residues level in egg at 46 ppm to 144 ppm cause shell thinning, increased shell cracking, and reduced duckling survival in Black Duck. Same effects were observed by Fox *et al* (1980) in Loons at 5 8 ppm organochlorine residues in eggs. The zone of increasing hazard for DDD alone was considered to begin 65 μg/g, whereas this zone for DDE alone was considered to begin at 300 μg/g (stickel *et al.,* 1970, 1984).

The available literature tells us intake of DDT and its break down products DDD and DDE by birds results in thin eggshells that break easily, so birds cannot reproduce successfully and this problem in severe in birds that are at the top of the food chain- predators The organochlorine residues obtain from Cattle Egret are within the range of the organochlorine residues recorded in other or related birds species from different location. Also, it is below the level, which can impair the breeding performance Short and long-term toxicity in birds is often observed when the organochlorine pesticides concentrations in eggs are above 0.5 ppm (Fox, 1976, Nisbet and Raynolds, 1984; Hall *et al.*, 1989).

The results obtained and literature available indicates there are changes due to presence of pesticide residues, which are not specific and significant. Of the organochlorine residues DDE is given more importance, though DDE is less toxic to birds compared to the majority of other organochlorine but is the main root cause for shell thinning and in turn affecting the breeding success due to its:

i. Significantly negative correlation between DDE residue in the eggs and eggshell thickness.

ii Experimentally shell thinning can be induced by DDE at the levels encountered in the environment.

iii. Affected population recovers after DDT use stopped (Blus *et al.*, 1995).

The physiological mechanism of affecting shell thinning is quite complex, where DDE controls at various levels like, Ca^{+2} from mucosa to shell levels through enzyme, 2nd messengers and even hormones (Lundholm 1987). The levels obtained are far below the critical level as suggested by number of workers (Fox *et al.*, 1976; King *et al.*, 1976; Nisbet and Reynolds, 1984; White *et al.*, 1984; Custer and Mitchell, 1987; Hall *et al.*, 1989). Thus the results indicate that the DDT and its metabolites have impaired the breeding success to some extent, but not significantly. This might be due to having organochlorine contaminants leveling the study area below the level suggested for hazardous zones. These sublethal levels they do have the impact on breeding success but not remarkable, which if not controlled now may show its action on generations to come like slow poisoning.