SUMMARY

Summary & Conclusion:

Different oraganophosphate pesticides are widely used in the agricultural fields for controlling insects and disease-borne vectors. Very often, the wild birds that live on agricultural products are exposed to various pesticides through ingestion of contaminated foods. Moreover, reproduction in majority of wild birds is discontinuous or seasonal and their seasonal attainment of sexual maturity often coincides with the time of application of OP pesticides in agricultural field. Although reports exist on the organophosphate-induced wide range of mortality of the non-target organisms specifically birds, very few information is available on the reproductive performances of wild birds that survive even after exposure to low concentration of OP compounds. A series of study on the effects of three organophosphate pesticides namely phosphamidon, methyl parathion, and quinalphos in male wild birds *Psittacula krameri* and *Lonchura malabarica* revealed that very low concentrations (5 μg, 10 μg, or 20 μg/100g body weight) of each organophosphate pesticide can inhibit spermatogenesis and induce degenerative changes in the testes of the concerned birds (Maitra and Sarkar, 1991, 1993, 1994,1995,1996). However, the nature of influences of different OPs on the reproductive physiology in any female bird remains almost unknown. Accordingly, an attempt has been made in the present investigation to study the influences of organophosphate pesticides at very low sub-lethal concentrations on the reproductive performances of female wild birds.

The present investigation has been carried out involving three commonly used OP pesticides, namely phosphamidon (PD), methyl parathion (MP), and quinalphos (QP),

and two species of wild birds, like roseringed parakeet (*Psittacula krameri borealis)* and white throated munia (*Lonchura malabarica malabarica*). Only adult females of each species have been considered for experimental studies. Each pesticide has been orally administered in three graded doses i.e., 10 μg, or 20 μg, or 50 μg/100g body weight/day, to different groups of each avian species, and the effects of each dose have been studied after 24 hrs of application for 1-day, 5-day and 10- day. Responsiveness of the birds in general to ingested OP pesticide has been studied at the level of body weight, feeding and locomotory behaviour as well as on the activity of acetylcholinesterase (AChE) in brain, while reproductive responsiveness to each OP pesticide has been evaluated following different analytical techniques including the gravimetric, gametometric and histometric study of the ovary at light microscopic levels, and gravimetric, morphometric and histological studies of the oviduct at light microscopic as well as scanning electron microscopic levels. Moreover, the study included quantitative estimations of the activities of AChE and of two steroidogenic enzymes like *3β-hydroxysteroid dehydrogenase* (3β-HSD) and *17β- hydroxysteroid dehydrogenase* (17β-HSD) in the ovary of considered avian species to gather basic information on the probable mechanism of action of concerned OP pesticides.

Consequently, the basic information emerged from the current investigation include:

1. None of the currently adopted experimental schedules resulted in mortality of any of the considered species of birds. However, some signs of intoxication such as salivation, abnormal sitting posture were found only in munias treated daily with phosphamidon 50 μg/100 g body wt. for 5 or 10 days, while remaining birds showed friskiness and normal feeding behaviour throughout the experiment.

2. Variable responsiveness to each pesticide was noted in the acetylcholinesterase (AChE) activity of brain and in several studied features of the ovary and the oviduct in each avian species in relation to the dose and duration of treatment.

3. The degree of inhibition of AChE activity in both the brain and the ovary was shown to vary in relation to the chemical nature of the given pesticide, dose and duration of exposure as well as to the investigative avian species.

4. Following exposure to PD, brain AChE activity in munias and parakeets became significantly decreased at each dose of pesticide and at each time point. Identical changes were noted with the study of the effects of MP in munia. In parakeets, such changes were noted following treatment of MP at the dosage of 10 μg or 20 μg for 5 or 10 days and of 50 μg at each level of study. In either species of birds, QP treatment induced significant reductions in brain AChE activity after 10 days at 10 μg dose, after 5 and 10 days at 20 μg dose, and after 1-, 5-, and 10 days of treatment at 50 μg dose level.

5. None of the used OP pesticides at the dose of 10 μg/100g body weight, irrespective of the duration of treatment, could alter the AChE activity in ovary in any avian species. At a dosage regimen of 20 μg for 10 days, only PD, but not MP or QP, induced reductions in ovarian AChE activity in both munias and parakeets. MP or QP caused similar effect only at 50 μg dose after 5 or 10 daily treatment in munia, and after 10 daily administration in parakeet.

6. Among the currently considered three OP pesticides, PD appeared to be most potent neurotoxic agent in each avian species.

7. In consideration to the changes noted in AChE activity in brain and in the ovary to the given OP pesticide, white throated munias appeared to be more sensitive than the roseringed parakeets.

8. None of the used pesticides at the dose of 10 μg or 20 μg, irrespective of the duration of treatment, could influence the ovarian conditions in any bird species.

9. The steroidogenic activity of the ovary as assessed through the measurements of 3β-HSD and 17β-HSD appeared to be influenced in relation to the pesticide, dose and duration of treatment, as well as to the species of studied birds. A significant inhibition of the activity of both 3β-HSD and 17β-HSD was found in munias following daily administration of 50 μg of PD (for 5 or 10 days), or MP (for 10 days), QP (for 10 days). In case of parakeets, the activity of 3β-HSD became significantly decreased following treatment of 50 μg of PD or MP (for 10 days), but not QP. The ovarian activity of 17β-HSD in parakeets, on the other hand, was found to be reduced following the treatment of 50 μg of PD (for 5, or 10 days), or MP (for 10 Days), or QP (for 10 days)

10. Each of the currently used OP pesticide caused significant decrease in the values of gono-somatic index in either species of birds when administered at the dosage regimen of 50 μg/100 g body wt. for 10 days. Similar changes were also noted in munias and parakeets following ingestion of 50 μg PD for 5 days.

11. Daily ingestion of each of the currently used pesticides at the dose of 50 μg for 10 days resulted in significant reductions in the relative number of undifferentiated follicles and fully differentiated follicles with concomitant increase in the number

of atretic follicles in the ovary of both munias and parakeets. Similar changes were also observed in birds which received 50 μg PD even for 5 days.

12. The size of the fully differentiated follicle became decreased significantly in each species of bird after treatment of 50 μg PD for 5 or 10 days, or 50 μg MP or QP for 10 days.

13. At histological level, degenerative changes of ovary were found in 50 μg PD and 50 μg MP fed munias for 10 days. The changes included exfoliation of the cells separately or in groups from the granulosa layer, vacuolation of the granulosa layer, reduction in the thickness of the granulosa layer, and decrease in the number of thecal gland cells around the mature follicle. However, in similarly treated parakeets the noted features included reduction in the thickness of granulosa layer and decrease in the number of thecal gland cells in the mature ovarian follicles. Quinalphos failed to elicit any changes in the cellular organization of the ovary in parakeets irrespective of the dose and duration of treatment whereas QP fed munias showed reduction in the thickness in the granulosa layer of the mature follicle after 10 days.

14. Significant reductions in the weight and length of oviduct were found only in 50 μg for 10 days dosed PD treated munias and parakeets.

15. Out of the five distinct regions of the oviduct, only magnum and uterus had shown remarkable changes at microscopic levels in both munias and parakeets which were fed with 50 μg PD or 50 μg MP for 10 days. Scanning electron microscopic study of oviduct was complimentary to the findings at light microscopic level. The major changes included reduced proprial glandular tissue

in the mucosal folds, reduced epithelial cell height, loss of cilia, distorted ciliary arrangement, and decrease in the number of tubular glands in both magnum and uterus parts of the oviduct. QP, irrespective of the dose and duration of treatment, failed to elicit any detectable changes in any part of the oviduct in any species of studied birds.

16. Taken together, it appeared possible that the given OP pesticides had more suppressive influences on the functions of ovary in munias than in parakeets.

17. The present study showing (i) a lack of a direct correlation between the rate of inhibition of ovarian AChE activity and the degree of inhibition of ovarian functions in different OP pesticide treated birds, and (ii) a significant decrease in the brain AChE activity in all the birds which responded to the given pesticides at gonadal level, it appears reasonable to suggest that the anti-gonadal actions of the concerned OP pesticides may be due to impaired functions of the brain (hypothalamo-hypophyseal system?) in the concerned birds (Goldman *et al.*, 1997; Baligar and Kaliwal, 2002; Hiremath and Kaliwal, 2002; Chattopadhyay *et al.*, 2003; Recio *et al.*, 2005), rather than pharmacological actions of the OPs at gonadal level.

18. It deserves special mention here that earlier studies on testicular functions in munias and parakeets employing identical experimental regimens of the same OP pesticides indicated that quinalphos (Maitra and Sarkar, 1991, 1994), compared to methyl parathion (Maitra and Sarkar,1993, 1996) and phosphamidon (Maitra and Sarkar, 1994, 1995), had the most potent gonado-toxic effects in the concerned birds. However, the results of the current study on the female reproductive organs

in the same avian species collectively suggest that at a given dose phosphamidon has more anti-gonadal effects than generally induced by methyl parathion and quinalphos. Accordingly, present study indicates for the first time that influence of any OP pesticide on gonadal functions of the bird varies not only with the dose and duration of treatment (Rattner *et al.,* 1982 a), chemical nature of the pesticide (Hall and Clark, 1982 ; Pla and Johnson, 1989) species of the animal (Maitra and Sarkar, 1991, 1994), but also with the sex of a particular avian species.

In essence, the information embodied in this thesis significantly contributes to the existing knowledge that OP pesticides, even in very low sub-lethal doses and for short period of application, may induce ovarian dysfunctions in wild birds. Though the mechanism of antigonadal actions of OP compounds remains speculative, the results of present investigation provide the basis of a belief that impaired cholinergic activities in the brain may play a role in impairing the hypothalamic and/or pituitary functions involved in the regulation of reproduction or may cause direct inhibition of steroidogenesis in the ovary. The precise physiological mechanism of OP-induced reproductive dysfunctions in wild birds, however, remains as an important topic of future research.