Chapter 9 Summary and Conclusions

In habitats like the temperate and tropical/sub-tropical zones, where local environmental factors are quite variable and less predictable, seasonal cyclicality in reproduction is a highly adopted phenomenon by vertebrates, including birds (Wikelski et al., 1999; Hau, 2001). Environmental factors, such as changes in day length, food availability, predator arrival, temperature, rain and humidity etc., provide the information to which birds respond in order to regulate reproductive processes, from the onset to the termination of breeding (Stevenson and Ball, 2011). Seasonal changes in the reproduction are one of the most dramatic examples of naturally occurring neuroendocrine plasticity that have been described in many vertebrates, particularly birds.

The HPG axis is the principal neuroendocrine system that has evolved in order to frame the time of reproduction which is regulated by GnRH and GnIH (Smith et al., 2008; Messager et al., 2005). **GnRH** is a highly conserved dodecapeptide, synthesized by GnRH neurons in the POA and PVN of DMH (Ubuka and Bentley, 2009; Roth et al., 2004). GnRH neuron terminals generally project to the ME and connect brain with the peripheral endocrine system by regulating the release of pituitary gonadotropins LH and FSH (Boehm et al., 2005; Ubuka and Bentley, 2009). **GnIH** is an antagonist of GnRH (Tsutsui et al. 2000) that inhibits gonadotropin synthesis and reproductive behaviors in birds and mammals (Bentley et al., 2006; Johnson et al., 2007; Ubuka et al., 2013). GnIH is synthesized by GnIH neurons in the PeVN as well as PVN of DMH where it contacts to GnRH neurons (Bentley et al., 2006; Kriegsfeld et al., 2006). GnIH also inhibits the GnRH neurons directly as mRNA transcripts of GnIHR has been expressed in GnRH neurons (Ubuka et al., 2008). Gonadotropins regulate the synthesis/release of sex steroids that in turn regulate GnRH secretion through an indirect action on sex steroid specific...
signals to GnRH neurons (Ubuka et al., 2013). In avian species, environmental factors are integrated at multiple levels ultimately converging on and regulating GnRH neurons and/or their terminals. Sex steroids regulate the neuropeptides, particularly GnRH; however GnRH neurons do not express the ARs and ERs themselves (Herbison, 2006) and are believed to mediate the GnRH/LH secretion through negative feedback of androgen and estrogen.

Prohormone T4 is synthesized from follicular cells of thyroid gland and is converted into biochemically more active form T3 in peripheral tissues by DIO2 which catalyzes the removal of one iodine group from outer ring of the T4 (Yasuo et al., 2005). DIO3 catalyzes the removal of inner ring iodine from T3 and converts into inactive T2 (Yasuo et al., 2005). Both these enzymes are essential for the gonadal control of the T4 and T3. Plasma levels of T4 and T3 are regulated by the negative feedback of the HPT axis. Low plasma T4 level triggers the pituitary thyrotrophs for overproduction of TSH under the negative feedback of HPT axis. In vertebrates, various neuroendocrine axes are usually interlinked, and thus, the HPT axis has potential to interact with the HPG axis and affect the seasonality of reproduction. Hypothalamic activation of THs and TSH further suggests that HPT axis can directly interact and modulate the HPG axis.

Display of normal secondary sexual characteristics/SSCs and sexual behaviors coordinate fertilization and reproduction. Sexual behaviors are modulated by multiple factors including seasonal changes during reproductive development/maintenance. These changes use to influence sexual differentiations and results in profound sex differences in SSCs as well as sexual courtship, territoriality and copulation. SSCs and sexual behaviors are under the homeostatic regulation of the HPG axis. Development of SSCs and sexual
behaviors largely depend on androgens and estrogens (Balthazart and Ball, 1995; Canoine et al., 2007). Sex steroids affect the brain by binding to intracellularly located ARs and ERs. Testosterone undergoes rapid modulation in hypothalamus and affects courtship behavior whereas E2 induces the specific brain regions for song production as well as facilitates mating behaviors (Ball et al., 2002). Being a positive regulator of synthesis/release of gonadotropins as well as sex steroids; GnRH also affects sexual behaviors. GnIH down regulates the gonadotropin synthesis/release form pituitary and has been reported to inhibit female sexual behaviors in female white-crowned sparrows (Bentley et al., 2006).

Hypothalamic metabolism of THs may sdirectly modulates the HPG axis therefore the HPT axis can also regulate the seasonal development and maintenance of secondary sexual characters and sexual behaviors in birds. Gonadal metabolism of THs can also be an important regulator of sexual behaviors because testicular activation of THs directly regulate the Sertoli cell proliferation and maturation as well as Leydig cell differentiation and steroidogenesis (Mendis-Handagama and Ariyaratne, 2005; Svingen and Koopman; 2013). THs also have been suggested to regulate the seasonal expression of ARs and ERs (Gahr, 2001). In mammals, T4 interacts with E2 during the modulation of estrogen-sensitive gene expression (Zhu et al., 1996) and therefore might affect the ERs expression (Fujimoto et al., 1997).

Many environmental chemicals have potential to interact with endocrine systems of various vertebrates and disrupt it (McLachlan, 2001; Diamanti-Kandarakis et al., 2009). These endocrine disrupting chemicals/EDCs are extremely heterogeneous and includes synthetic chemicals used as agro-industrial chemicals as well as some phytoestrogens.
EDCs act through diverse mechanisms by binding with nuclear receptors, nonnuclear steroid hormone receptors (membrane ERs), nonsteroid receptors (serotonin receptor, dopamine receptor, and norepinephrine receptor), orphan receptors (aryl hydrocarbon receptor), enzymatic pathways involved in steroid biosynthesis and/or metabolism, and numerous other mechanisms of endocrine systems. Metabolic and reproductive systems are the major targets of EDCs who are mostly regulated by THs, sex steroids and adrenal steroids (Diamanti-Kandarakis et al., 2009), and therefore, thyroid, gonads and adrenal are the major targets of EDCs.

Pesticides have wide distribution in agroecosystems because of their indiscriminate applications for pest management (Verma and Mohanty, 2009). Many pesticides have potential to interact with endocrine systems and constitute a potential group of EDCs who can impair the endocrine physiology of vertebrates. These EDPs (and their metabolites) are wide spread in agroecosystems (Köhler and Triebskorn, 2013; Caserta et al., 2008; Brucker-Davis, 1998; De Angelis et al., 2009). Thyroids and gonads are particularly sensitive to environmental exposures to pesticides resulting in impaired metabolic and reproductive performances. Long term exposure of environmental pesticides to avian populations often becomes deleterious to their reproductive health and survival. Pesticide exposures have been associated with reproductive dysfunction and slower growth rate of offspring (Fry, 1995; Caslin and Wolf, 1999). Neuroendocrine regulation of reproductive system is particularly sensitive to pesticides exposures. Pesticides-induced disruption of HPG axis has been reported in different groups of animals, including birds (Thangavel et al., 2005; Verma and Mohanty, 2009; Ottinger et al., 2005). THs are particularly sensitive
to environmental contamination by EDPs. Disruption of THs can disrupt both neural as well as gonadal mechanisms of reproduction, and therefore, disruption of HPT axis may also lead to impairment of HPG axis, altered reproductive performances and infertility. Since, regulation of reproductive behavior and reproductive success in seasonal breeders are also linked to THs (Nakao et al., 2008), therefore, EDPs-induced disruption of THs can also be related to disruption of reproduction-related behaviors. However, studies on impact on pesticides-induced disruption of HPT axis on seasonal reproductive behaviors is lacking in both mammals as well as birds.

As pesticides are mostly persistent and not easily biodegradable, non-target organisms are susceptible to exposures of their combinations/mixtures in environment. Field level concentration of individual pesticide may have subtle toxic effects but can be deleterious as a cumulative action of pesticides mixtures. Recent laboratory studies on rodents have shown that combinatorial exposures to pesticides, even though the dose of each single pesticide was below its no observed adverse effect level/NOAEL, can affect neuroendocrine systems (Jacobsen et al., 2012; Bhaskar and Mohanty, 2014).

Dithiocarbamates (fungicides) and neonicotinoids (insecticides) are groups of contemporarily used pesticides. Members of dithiocarbamate group (zineb, maneb, thiram and mancozeb) have been demonstrated as thyroid disruptors in laboratory rodents. MCZ, a dithiocarbamate, is a potent thyroid and gonadal disruptor in mammals (Cox, 2001; Ksheerasagar and Kaliwal, 2003). MCZ exposure induced oxidative stress was reported to cause neurotoxicity and disrupt the neuroendocrine mechanisms (Domico et al., 2006; Overgaard et al., 2013). However, detailed studies on MCZ-induced disruption of avian neuroendocrine systems and reproductive cyclicity are lacking. IMI, a
neonicotinoid, is used as an insecticide having affinity to bind with nAChR and is toxic to mammals (Kimura-Kuroda et al., 2012). Thyroid lesions on acute high dose exposure in laboratory rodents also has been demonstrated (Cox, 2001); however studies with environmentally realistic exposure dose of this neonicotinoid is lacking for both laboratory models as well as wild species. IMI disrupts normal testicular functions in rodents (Bal et al., 2012); however studies on avian reproductive systems are lacking.

Specific action of MCZ and IMI, as fungicide and insecticide respectively, demands their simultaneous use in agricultural fields making the wild birds vulnerable to their combinatorial exposures as well. Both these pesticides (and/or their metabolites) may exert additive/synergistic effects due to combinatorial action, modulate the metabolic & neuroendocrine homeostasis and affect their fertility and survival. However, studies on combinatorial exposures to MCZ and IMI on avian species are lacking.

The major objective of the present investigation was to investigate the interrelationship of pituitary-thyroid and pituitary-testicular axes during reproductive cycle of a seasonally breeding tropical/sub-tropical zone bird, Red Munia (Amandava amandava). To achieve the objective, the present study was planned to investigate this interrelationship of pituitary-thyroid and pituitary-testicular axes through the process of neuroendocrine disruption caused by EDPs.

Study was carried in two stages:

(i) For the first, interrelationship of pituitary-thyroid and pituitary-testicular axes were studied during four major stages of reproductive cycle: quiescent, preparatory, breeding and regressive.
(ii) Second, EDPs-induced disruption of HPT and HPG axes was studied using MCZ and IMI, both individually as well as combinatorially. This investigation was conducted during two important stages of breeding phase of reproductive cycle: preparatory and breeding.

To achieve the objectives following parameters were investigated:

- Study of secondary sexual characteristics and sex-related behaviors.
- Histomorphology of target endocrine organs: thyroid and testis.
- Study of GnRH and GnIH in hypothalamus and testis through immunohistochemistry.
- Study of ARs in testis through immunohistochemistry.
- Study of hormones of pituitary-thyroid axis (TSH, T4 and T3) and pituitary-testicular axis (LH, FSH, PRL, Testosterone and E2) through ELISA.

**Experimental design to evaluate the neuroendocrine regulation of reproeduction**

To evaluate the neuroendocrine regulation during reproductive cycle and interrelationship of HPT and HPG axes, the investigation was carried in two parts.

i. First, we evaluated the seasonality of HPT and HPG neuroendocrine axes as well as their potential interrelationship during quiescent, preparatory, breeding and regressive stages of the reproductive cycle of *A. amandava*.

ii. Second, we examined the impact of neuroendocrine disruption on seasonality of reproduction as well as interrelationship of HPT and HPG axes using endocrine disrupting pesticides/EDPs.

In second part of investigation, to evaluate the EDPs-induced neuroendocrine disruption, two experimental set ups were maintained:
Experiment I: Evaluation of MCZ and IMI as Neuroendocrine Disruptors of Pituitary-Thyroid Axis

Two experimental set ups were maintained (one in each stage preparatory and breeding) to evaluate the neuroendocrine disruptive potential both the pesticides using environmentally equivalent dose.

In both preparatory (stage of testicular recrudescence; mid July-mid August) and breeding (stage of active mating and breeding with fully grown testes; mid September-mid October) stages, acclimatized male birds (bw 8.5 ± 0.5 gm) were divided randomly and maintained in three groups (n = 8/group): MCZ-exposed group, IMI-exposed-group and control. Birds were exposed to environmentally equivalent low dose of commercial pesticides MCZ and IMI through diet using soy oil as vehicle. Control birds were given food with vehicle. The doses were selected taking reference of established chronic no observable adverse effect level (NOAEL) of respective technical compound (MCZ and IMI) in birds.

**Mancozeb/Uthane M45 (75% wt/wt MCZ; United Phosphorous Ltd., India)**

Dietary route LD$_{50}$ of technical compound in birds: **860mg/kg BW** (HCPDG, EC, 2009).

Dose Selected: **0.5% LD$_{50}$** (0.028 mg: 5.5 ml soy oil/3 gm food)

**Imidaclorpid/Confidor (17.8% wt/wt IMI; Beyer Crop Science Ltd.)**

Dietary route LD$_{50}$ of technical compound in Japanese quail: **31mg/kg BW** (Lopez-Antia, 2012).
Dose Selected: **0.5% LD$_{50}$** (5.5 µl:5.497 ml soy oil/3gm food)

Food was mixed/coated with pesticides using vehicle and kept overnight and exposed for 30 d in both sets. All the birds (pesticides-exposed as well as control) in each set of experiment were euthanized at the end of experiment. Body weight was recorded every alternate day. Precision of pesticide-dose intake by each bird was maintained by exposing them to the decided dose through calculated amount of food taken by birds during first 2 h of feeding (7:00–9:00 A.M.) each day.

**Experiment II: Experimental Manipulation of Interrelationship between Pituitary-Thyroid and Pituitary-Testicular Axes using EDPs**

Two experimental set ups were maintained (one in each stage) to evaluate the disruption of interrelationship between pituitary-thyroid and pituitary-testicular axes using low dose individual as well as their combinatorial doses.

In both the preparatory and breeding stages, acclimatized male birds (bw 8.5±0.5 gm) were maintained in five groups (n=8/group): **MCZ**-exposed group, **IMI**-exposed group, **MIX-I** exposed group, **MIX-II** exposed group and **control**. Commercial formulations of pesticides were given through food using soy oil as vehicle in every morning 07:00-09:00 A.M. The doses and precision of dose-intake were maintained as in Experiment-I.

**Mancozeb/Uthane M45**

Dose Selected: **0.25% LD$_{50}$** (0.014mg: 2.5ml soy oil/3gm food)

**Imidacloprid/Confidor**

Dose Selected: **0.25% LD$_{50}$** (2.75µl:5.495ml soy oil/3gm food)
Effects of combinatorial exposures were assessed using two different doses of each pesticide in mixture through same diet and vehicle. In one group (MIX-I), the doses of combinatorial exposure were decided keeping in view the dose of individual exposure of each of MCZ and IMI (0.25% LD$_{50}$). In another group (MIX-II), the individual pesticide dose was increased (0.5% LD$_{50}$ of each) to make a mixture dose double to the individual dose. Birds were exposed for 30 days; BW recorded every alternate day and terminated by decapitation.

**MIX-I (1:1LD$_{50}$ of MCZ and IMI)**

Dose Selected: **0.25% LD$_{50}$ of both MCZ and IMI** (0.014mg MCZ+2.75µl IMI) in 5.495ml soy oil/6gm food

**MIX-II (1:1LD$_{50}$ of MCZ and IMI)**

Dose Selected: **0.5% LD$_{50}$ of both MCZ and IMI** (0.028mg MCZ+5.5µl IMI in 5.495ml soy oil/6gm food)

GraphPad Prism 5 (GraphPad Software Inc., USA) statistical software was used for all analyses. Results with a p-value of less than 0.05, 0.01 and 0.001 were considered significant. Data with normal distribution and homogeneity of variance were analyzed using one way analyses of variance (ANOVA), represented as mean ± S. D. (standard deviation) followed by Tukey’s posthoc test. Statistical analyses of data were done using Fisher’s exact test.

Substantial thyrotoxicity was induced on exposure to both MCZ and IMI as evident by damage to thyroid follicles and lesions in stroma, more prominent in breeding phase.
MCZ is reported to cause thyroid lesions on acute high dose exposures (Axelstad et al., 2011). IMI and their metabolites are reported to cause thyroid lesions in rodents on acute high dose exposures (Cox, 2001). Hypertrophy and hyperplasia of epithelial and stromal cells, as observed in MCZ and IMI exposed groups, might have contributed to changes in the thyroid gland, specifically weight and volume. High dose exposures to MCZ have been reported to cause hypertrophy and hyperplasia in follicular cells in rodents (Axelstad et al., 2011; Baligar and Kaliwal, 2001). Decreased N/C in epithelial and stromal cells might be due to pesticides-induced toxicity. Genotoxicity and DNA damaging effects of MCZ (Calviello et al., 2006) and IMI (Costa et al., 2009) have been shown in mammalian in vitro systems. Thyroid disruption also reflected from impaired plasma levels of the hormones of pituitary-thyroid axis i.e. TSH, T4 and T3. Plasma T4 level was reduced in both the phases from exposure to both MCZ and IMI. MCZ and ETU are reported to reduce the synthesis and release of T4 and their storage in the colloids (Axelstad et al., 2011; Maranghi et al., 2013) by inhibiting the iodide uptake and thyroid peroxidase activity in the epithelial cells (Miller et al., 2009; Doerge et al., 1990). Increased plasma T3 levels in preparatory phase in both MCZ and IMI exposed groups might be due to the increased conversion of T4 to T3 in epithelial cells as well as increased T3 synthesis from non-thyroidal sources, such as brain and pituitary in a compensatory response to the decrease in T4 concentrations. Hypothyroidism can induce mineralization of colloids which was evident in IMI-exposed thyroids in breeding phase; neonicotinoids have been reported to induce mineralization of colloids as a response of hypothyroidism (Rose, 2012). Plasma TSH level was increased in preparatory phase of MCZ exposed birds indicating the normal negative feedback response of the HPT axis to
low plasma T4 concentration. However in breeding phase, plasma TSH level did not increase in response to decreased T4 and T3, indicating impairment of HPT axis. Plasma TSH level was decreased in IMI exposed birds in both preparatory and breeding phases indicating lack of responsiveness of HPT axis to the negative feedback against decreased plasma T4 level. IMI can directly induce neurotoxicity due to cholinergic receptor inhibition in neurons (Kimura-Kuroda et al., 2012) and result in disruption of HPT axis.

Disruption of THs synthesis/release in seasonally breeding birds may disturb neuroendocrine regulation of gonadal functions and impair the reproductive cycle. THs imbalance and reproductive abnormalities have been reported in some wildlife birds (Mayne et al., 2005; Cesh et al., 2010). Impaired plasma THs levels are associated with the impairment of reproduction-related behavior and decreased reproductive success because THs activation in the hypothalamus plays a critical role in the regulation of the neuroendocrine axis involved in seasonal reproduction in both birds and mammals (Nakao et al., 2008; Yoshimura et al., 2013). Impaired plasma T4 levels and changes in reproductive success and behavior have also been observed in birds exposed to flame retardants (Marteinson et al., 2011; Marteinson et al., 2012).

Growth inhibiting effects of the pesticides, during preparatory stage of reproductive cycle, was evident from impairment of weight and volume as well as histopathology of testis. Increased width of testicular capsule, largely contributed by the tunica albuginea, suggests the inhibition of testicular development in exposure groups (Aire and Ozegbe, 2007). Decreased diameter of seminiferous tubules with reduced stages of seminiferous cycle and increased interstitium were exhibiting the characteristics of testicular regression. Both MCZ and IMI have been reported to decrease testis weight in rodents.
IMI and its metabolites cause oxidative stress and cellular death in male reproductive organs of rat (Bal et al., 2012b). Fibroid cysts, observed in exposure groups, might be a result of impaired development or degeneration/fibrillization of seminiferous tubules. Testicular regression further reflected in impaired plasma levels of reproduction related hormones and hypothalamic expression of GnRH and GnIH. Reduced plasma level of LH, FSH and testosterone, as observed in this bird, have been reported on exposure to MCZ (Joshi et al., 2005) and IMI (Najafi et al., 2010) in rodents. Both LH and testosterone regulate the size of seminiferous tubules and volume of testis and their suppression causes the regression of testis (Bentley et al., 2000). MCZ has been reported to induce oxidative stress in hypothalamic neurons (Domico et al., 2006; Overgaard et al., 2013), and therefore, it can disrupt the GnRH synthesis. IMI and its metabolites are reported to be estrogenic (Kojima et al., 2004), and therefore, it can modulate the ligand-ER binding in GnRH neurons and impair its synthesis/release. Pesticides-induced disruption of GnIH has not been studied so far. Increased GnIH expression in zona externa and zona interna of ME and PeVN of exposed groups might be due to suppression of GnRH in PVN. High plasma PRL level is antigonadal because it suppresses the plasma LH and testosterone level (Grattan et al., 2007). Pesticides-induced disruption of testicular expression of GnRH and GnIH suggest impaired local action of gonadotropins by autocrine/paracrine signaling. GnRH has been localized in testis of variety of animals from fish to mammals (Anjum et al., 2007; Singh et al., 2007); it is believed to facilitate the local action of gonadotropins by binding with their receptors (LHR and FSHR) and also modulate the local action of sex steroids. Local expression of GnIH can suppress the gonadotropin action and modulate synthesis of
testosterone/E2 by autocrine/paracrine mode of action. In combinatorially exposed groups, enhanced toxic effects were observed with marked effects suggesting the additive/synergistic action of pesticides.

Thyroid hormones are important homeostatic regulators of seasonal testicular growth and development in vertebrates, including birds (Yoshimura, 2013). Role of thyroid hormones during seasonal testicular development and spermatogenesis is controversial; both up regulation (Follett and Nicholls, 1988) and down regulation (Bentley et al., 1997) of seasonal gonadal growth and development has been reported. In Red Munia, thyroid hormones are not linked to attainment of breeding phase of reproductive cycle (Thapliyal and Gupta, 1984). As thyroid hormones play crucial roles in cellular remodeling and differentiation (Holsberger and Cooke, 2005), pesticides-induced impairment of thyroid hormones might disrupt testicular tissue remodeling and cellular differentiation in preparatory phase and inhibit its progression into breeding phase. Chronic hypothyroidism is reported to be associated with impaired spermatogenesis, germ cells degeneration and reduced seminiferous tubule diameter in mammals (Simorangkir et al., 1997; Maran & Aruldhas, 2002). In Red Munia, we have reported that both these EDPs impair the thyroid physiology and cause hypothyroidism (Pandey and Mohanty, 2015). Decreased Leydig cell density in interstitium and Sertoli cells in seminiferous tubules may be due to hypothyroidism. Studies in rodents have demonstrated that hypothyroidism arrests mesenchymal cell differentiation into steroidogenic progenitor Leydig cells (Wagner et al., 2008) as well as proliferation and maturation of Sertoli cells (Holsberger & Cooke, 2005). Disruption of thyroid hormone can also cause the inhibition of testosterone because thyroid hormones have been reported to mediate the metabolism of
sex steroid hormones as well as gonadal development in mammals (Jannini et al., 1995). Along with direct neuronal toxicity, hypothyroidism could also cause the disruption of GnRH. Thyroid hormones mediate synthesis of nerve growth factors which controls microtubule and axonal growth of neurons in the central nervous system, and therefore, this may affect GnRH secretion as well as synthesis/release of gonadotropins from pituitary (Bentley et al., 2000). Decreased plasma gonadotropins level has been reported in thyroidectomized birds as a result of decreased T4 (Dawson et al., 1985). In case of combinatorial exposures, effects of pesticides can vary from those of their individual exposures which result in cumulative toxicity that may be a result of additive/synergistic or negative/antagonistic interactions of pesticides and/or their metabolites. In present investigation, enhanced disruption of hypothalamic-pituitary-testicular axis was evident on exposure to pesticides combinations suggesting the additive/synergistic interaction of MCZ and IMI. Being chemically different, both the pesticides have different mechanisms and can initiate series of reaction cascades to induce cumulative toxic effects.

Pesticides-induced neuroendocrine disruption has been suggested to disrupt the sexual behaviors (Ottinger et al., 2001). GnRH has been reported to have some direct effects on social behavior, such as breeding solicitation, however its mechanisms are not understood well (Maney et. al., 1997). GnIH has been reported to suppress sexual behaviors and reproductive performances (Bentley et al., 2006). Gonadotropins influence the development and maintenance of secondary sexual characters, sexual behavior and reproductive performances; however they are believed not to directly affect the sexual behavior but act primarily through their effects on sex steroids production. Recently it has been reported that LH can directly act in brain LH receptors/LHRs expressed in defined
neural circuit to influence reproductive behavior (Yang et al., 2007). Pesticides-induced neurotoxicity can cause impairment in these neural circuits. Development and maintenance of secondary sexual characteristics are principally regulated by circulating plasma levels of sex steroids and their receptors (androgen receptors/ARs and estrogen receptors/ERs). Reduced synthesis and release of gonadotropins as well as sex steroids have been reported on exposure to MCZ (Joshi et al., 2005) and IMI (Najafi et al., 2010) in rodents which reduce the bioavailable plasma level of TST. Sex steroids modulate the underlying neural factors including structure and electrophysiology of brain regions that control secondary sexual characters and courtship behaviors during reproduction (Balthazart et al., 2009). In the hypothalamus of birds, high levels of aromatase activity has been found that is involved in steroidogenesis similar to gonads where estrogens are made from androgens such as testosterone (Forlano et al., 2006). MCZ has been reported to antagonize the AR actions \textit{in vitro} (Kjeldsen et al., 2013), and therefore, can inhibit E2 synthesis in hypothalamus. In mammals, androgens affect the development and maintenance of secondary sexual characters & sexual behaviors in both males and females whereas in birds they are principally regulated by estrogens (McCarthy, 2008; Balthazart et. al., 2009). Hypothalamic steroidogenesis of E2 contributes to male sexual behavior (Trainor and Marler, 2002; Ball and Balthazart, 2004). Circulating PRL concentration is closely related to sexual behaviors including sexual desire and libido. High plasma PRL level causes decrease in sexual desire, libido and regression of secondary sexual characters. High plasma PRL level is supposed to be antigonadal because it suppresses the plasma LH and TST level (Mishra and Mohanty, 2010). Low TST levels in males are often insufficient to stimulate the brain enough to initiate sexual
desire (Krüger, et al., 2003). During high PRL level, low plasma TST level changes the Leydig cell sensitivity to LH stimulation (Grattan et al., 2007).

Following are the important conclusions of present study:

- The interplay of pituitary-thyroid and pituitary-testicular axes is a key factor towards the maintenance of the seasonal reproductive homeostasis, particularly in birds.

- Though contrary to earlier reports on thyroid-testicular interrelation of Amandava amandava (Thapliyal and Gupta, 1984; Dawson and Thapliyal; 2001), increased plasma THs level in preparatory and breeding stages, as compared to non-breeding/quiescent stage, suggest that THs may up regulate the mechanisms of reproductive development/growth during preparatory stage and help towards the attainment of fully active breeding status during courtship stage of this bird.

- However plasma THs levels in regressive/non-breeding stages remained comparable to breeding stage, similar to earlier investigations, which suggests that THs have no roles in the reproductive maintenance but they can play roles in regulation of post-breeding physiological processes, including molting. This might be a reason that could have lead to conclude earlier about the negative regulation of reproductive homeostasis by THs.

Such interrelation between pituitary-thyroid and pituitary-testicular axes were further supported by the experimental manipulation of neuroendocrine system by using EDPs:

- Both MCZ and IMI are potent thyroid disruptors which act through HPT axis. Even low dose exposures to EDPs can disrupt the HPT axis.
• EDPs could also cause the impairment of the HPG axis and affect the reproductive cycle by acting on both hypothalamic and testicular levels. However, in addition to pesticides-induced toxicity, disruption of HPG axis an also be a result of EDPs-induced modulation of HPT axis because the HPG axis is highly sensitive to the impairment of HPT axis.

• EDPs-induced disruption of HPT axis might caused impaired thyroidal regulation of testicular development/growth during preparatory stage and caused premature onset of regression.

Besides the thyroid-testicular interrelation, some important conclusions on EDPs exposures are:

• EDPs can modulate the neuroendocrine physiology even at very low concentrations and can become deleterious to reproductive health of non-target organisms. Wildlife species, particularly birds, are more susceptible to environmental exposure to EDPs.

• On combinatorial exposures, effects of pesticides can vary from those of their individual exposures which result in cumulative toxicity. Enhanced disruption of HPT and HPG axes was evident on exposure to pesticides combinations suggesting the additive/synergistic interaction of MCZ and IMI.

• Being chemically different, both the pesticides have different mechanisms and can initiate series of reaction cascades to induce cumulative toxic effects. Cumulative effect may be a result of additive/synergistic or negative/antagonistic (Pape-Lindstrom and Lydy, 1997; Hertzberg and
MacDonell, 2002; Laetz et al., 2009) interactions of pesticides on combinatorial exposures.

• Dose-dependent and/or pesticide-specific responses could not be predicted on combinatorial exposures.