REPRODUCTIVE STUDIES
Introduction

Environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a serious problem. Due to their extensive use in agriculture and their persistence, many of these compounds are present in surface and ground waters and must be considered as a potential risk for aquatic organisms as well as for drinking water quality (Katsumata, et. al, 2005, Velisek, et. al, 2010). Worldwide contamination of surface waters has been well-documented and constitutes a major issue at local, regional, national levels (Huber, et. al, 2000, Cerejeira, et. al, 2003, Spalding, et. al, 2003).

Since Silent Spring was published in 1962, widespread public concerns have been inspired with the man-made chemicals and pollution of the environment (Carson, 1962; Gray, et. al, 2001). Because of the increasing environmental contamination caused by chemicals acting as endocrine disruptors, in July 1991, multidisciplinary group met at the Wingspread Conference Center in Racine WI, to assess what was known about chemically-induced alterations in sexual development in wildlife and humans. The researchers realized that endocrine disruption could be the cause of declines in the populations of many wildlife species which have occurred over the past years (Ankley, et. al, 2009; Carlsen, et. al, 1992). Human health could also be affected. At this session, the 21 century scientists issued a written warning, the famous Wingspread Consensus Statement and the term “endocrine disruptor” (ED) was also invented at the conference (Colborn and Clement, 1992). The current interest in this subject as a named research field dated from that time. ED was defined as an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations, or subpopulations of organisms, based on scientific principles, data,
weight-of-evidence, and the precautionary principle (EDSTAC, 1998). Current conventional agricultural practices commonly rely on the use of pesticides. Pesticides are introduced into the environment, by run off or cycling through environmental media such as air, soil and water. There may be the highest concentration of pesticides in human bodies because they are on the highest trophic levels in the food chain. Other exposures come from pesticides used in the home environment and from fruits and vegetables containing pesticide residues (Fenske, et. al, 2002; Jaga and Dharmani, 2003).

Increasing evidence demonstrated that many pesticides, suspected of estrogen mimicking or anti-androgenic substances, had become a potential threat to fertility and development of humans and animals (Anway, et. al, 2006; Gray and Ostby, 1998; Kelce, et. al, 1995; Kelce and Wilson, 1997; Vinggaard, et. al, 2006; Waters, et. al, 2001). Some pesticides have been verified to associate with certain male reproductive and developmental effects on human and animals such as underdevelopment, androgen-dependent tissues and testicular abnormalities, reduced sperm counts, sperm motility changes, sperm morphologic abnormality and genotoxic effects (Bian, et. al, 2004; Foster, et. al, 2001; Garcia, 1998; Gray, et. al, 2000, 2001; Sever, et. al, 1997; Xia, et. al, 2005; Xu, et. al, 2004). Sultan, et. al, (2001) also found that some chemical compounds with anti-androgen activity might cause degeneration of wildlife reproduction and human male reproductive system abnormalities such as the decline of sperm quality. Then the pesticides causing reproductive damages may associate with endocrine disruption, which emphasizes the need for further studies on the endocrine disrupting potential of the chemicals. One of the major mechanisms involved in pesticides endocrine disrupting is mimicking or blocking the actions of
the steroid hormone by directly binding to the hormone receptors either as agonists or antagonists (Kelce and Wilson, 1997; Waters, et. al, 2001).

Because androgen receptor (AR) is an important transcriptional regulatory factor involved in androgen signaling pathway, the anti-androgenic effects through interaction with AR have been the focus of the majority of researches. A possible mechanism responsible for the adverse effects may be consistent with the inhibition of expression of AR, the binding of androgen and AR or subsequent AR-dependent transcription (Cynthia, et. al, 2009). Some pesticides known to interfere with the androgen signaling pathway include p,p-DDT and p,p-DDE (Kelce, et. al, 1995; Wong, et. al, 1995), polybrominated diphenyl ethers (PBDEs) (Gray, et. al, 2004; Stoker, et. al, 2005), linuron (Lambright, et. al, 2000; McIntyre, et. al, 2000). Other studies have also revealed the insecticide fenitrothion and procymidone were two kinds of anti-androgenic pesticides (Lambright, et. al, 2000; Ostby, et. al, 1999; Tamura, et. al, 2001, 2003). Both p,p-DDE and the two primary vinclozolin metabolites, M1 and M2, bind the AR and act as antagonists by preventing transcription of androgen-dependent genes to inhibit the development and maintenance of male sexual functions (Kelce and Wilson, 1997).

Endocrine disrupting substances (EDSs) are a concern in aquatic toxicology, as they have the potential to alter fish reproduction through various modes/mechanisms of action (MOA) (Tyler, et. al, 1998), thereby potentially affecting population stability (Kidd, et. al, 2007). Changes in vitellogenin (VTG), sex steroid hormones, and gonad size relative to body weight (gonadal somatic index; GSI) are commonly used as biomarkers to assess the potential of contaminants to cause adverse effects on fish reproductive systems. It is been advocated that these
biomarkers can be used as signposts, to help prioritize and target detailed chemical and biological analyses of water, sediment and biota (Hutchinson, et. al, 2006).

Previous studies on reproductive biomarkers of EDC's and their effects on population levels were focused on egg production, which is intuitively related to population viability (Gurney, 2006). A population model has been developed to predict the effect of a reduction in egg production (cumulative eggs spawned per female) measured in short-term reproductive tests on population health of fathead minnow \( (Pimephales promelas) \) (Miller and Ankley, 2004). In subsequent studies, egg production in fathead minnow was linked to female VTG (Miller, et. al, 2007), 17β-estradiol (E2) and testosterone (T) levels (Ankley, et. al, 2008); these relationships were then used in the model to predict population-level responses. Using a relatively small dataset (four studies), this model has provided valuable information on how a selected group of chemicals with known mode of actions can potentially affect the reproductive capabilities of fathead minnow (Miller, et. al, 2007; Ankley, et. al, 2008).

Pyrethroid compounds are considered safe as compared to organochlorine, organophosphate and carbamate compounds owing to its less persistent in nature (Bradbury and Coats, 1989). The wide use of synthetic pyrethroids is increasing worldwide pollution risks. The synthetic pyrethroids are among the most potent and effective insecticides available, account for more than 30% in the world market (Moore and Waring, 2001). The low effect of toxicity of pyrethroid insecticides on mammals and birds and their limited soil persistence has encouraged the widespread and increasing use of pyrethroids in agriculture, as potent agents against pests (Milam, et. al, 2000; Ko”pru”cu” and Aydin, 2004). Insecticides applied to the land may be washed into surface waters and may adversely affect the life of aquatic organisms or
kill them (Datta and Kaviraj, 2003). Synthetic pyrethroids have been found to be highly toxic to zooplankton communities (Tidou, et al., 1992) and fish (Srivastava, et al., 1997; Ko’prücu” and Aydin, 2004; Yilmaz, et al., 2004).

Since the 1980s, cypermethrin a synthetic pyrethroid pesticide for its high performance, safety, broadspectrum and hypotoxicity to human and livestock, has been widely used in agricultural pest control programmes in domestic, industrial and agricultural situations because of its low environmental persistence and comprises more than 50% of the total pyrethroid market production. There is considerable human exposure to this chemical, which has been reported to have anti-androgen-like effects on male reproduction and development (Zhang, et al., 2008).

Recent review on pesticide-induced reproduction in Indian fish has been reported (Lal, 2007). Kumar, et al., (2004) have reported sperm head shape abnormality in mice after administration of cypermethrin. Recently, Joshi, et al., (2007) have reported histopathological changes in liver to vacuolization, necrosis and fibrosis of perivascular region and disruption of yellow brown grains at different times of exposure. Now it is essential to study further on the possible adverse effects and its mechanism, which could provide certain scientific basis to the safety evaluation of endocrine disruptors and risk assessment of such pesticides.

Many researchers (Kannan, et al., 1995; Ferreira, et al., 2004; Antunes, et al., 2007a, b; Johnson, et al., 2007; Singh and Singh, 2008a, b) have reported pesticide residues in wild captured fish. Though cypermethrin is very widely used in these days, little information is available on the effect of a pyrethroid insecticide cypermethrin induced changes in ovaries, testes, plasma levels of estradiol-17β, Total testosterone, 11-ketotestosterone, and Vitellogenin. This prompted us to study the effect on (1) histological changes in ovaries and testes, (2) plasma levels of estradiol-17β, total
testosterone, 11-ketotestosterone, and Vitellogenin after cypermethrin exposure (3) cytopathology of testes to understand the extent of cypermethrin effects on spermatogenic cells in detail.

Results

Histopathology of ovary

Histological sections of the ovary in control fish showed normal ovary, there were no pathological lesions with intact follicular epithelium, and atretic follicles were less visible. The observed details include ootid, oocyte, primary oogonia, secondary oogonia, yolk granules, ovulated follicle and chorion. The histophotomicrographs of control ovary are presented in plates 1 &2; fig1-4. At lethal concentration gross histoanatomy of ovarian tissue reveals degenerated oocyte, atretic follicle, degenerated epithelium, inter follicular degeneration, dilated chorion, disintegrated epithelial space, degenerated alveolar space, developing oocytes, degenerated oocytes, disintegration of epithelium, increased atretic follicle and vacuolated primary oocyte. Epithelial lesions, inflammatory responses, stromal hemorrhage, increased interstitium, and shrinkage of yolk vesicles towards periphery, degeneration of oocytes, increase in the number of atretic follicles, vascular degenerative changes in the ovarian follicles, lack of nuclei in the lining epithelial cells, disappearance of nucleolar details. The histophotomicrographs of ovary on exposure to lethal concentration are presented in plates 3, 4, 5, and 6; fig 5-12.

At sublethal concentration the changes were less when compared to lethal concentration. Prominent changes include increase in the number of atrritic follicles, degeneration of oocytes, and disintegration of intra epithelial 186
space, blebbing of epithelium, dilation and disintegration of epithelium, distortion of interfollicular space, vacuolated alveolar oocyte and nucleus. The histophotomicrographs of fish ovary on exposure to sublethal concentration are presented in plates 7, 8 and 9; fig 13-18.

**Histopathology of Testis**

The histophotomicrographs of control fish testis are presented in Plates 10 and 11; fig 19-22.

The *Labeo rohita* testis has a lobular-type cellular arrangement, characteristic of most teleosts. Following cells were found in the control fish sections; spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. The primary spermatogonia are the largest cells, have a prominent nucleolus, are basophilic and are found in cysts with varying numbers of individual cells. The cysts are usually found singly near the periphery of the testis. Primary spermatocytes are oval or spherical, they have no visible nuclear membrane and the chromatin material occupies most of the cell. Secondary spermatocytes are very small, spherical cells, unlike primary spermatocytes; the chromatin was in a clumped condition, similar to the spermatids. Spermatids are strongly basophilic spherical cells, the chromatin uniformly condensed and they remain in dense clusters. The spermatozoa have a rounded nucleus and are morphologically subdivided into head, neck piece, short midpiece and tail; as in other teleosts, there is no acrosome.
Histopathologies of testis on exposure to lethal and sublethal concentration are presented in plates 12-15; fig 23-30. Fish exposed to lethal concentration of acute cypermethrin exposure for 96 h period showed considerable degree of alteration in the histoanatomy of testis. These changes were profound and the degree of changes in histoanatomy showed variation. Changes include alteration in the normal testicular architecture, seminiferous tubules found regressed, vacuolated lumen, degeneration of tubular lumen, vacuolation, distortion and degeneration of tubular membrane, dilation of germinal epithelium (shifted off from the inner cells), degeneration of epithelium. A large number of inflammatory cells, further condensation of spermatogenic cells, presence of a large number of intertubular vacuoles and swelling of spermatids. However, the testicular changes were not much pronounced at sublethal concentration compared to lethal concentration. At sublethal concentration the observed changes in the testis include absence of interstitial cells, dilation of the germinal epithelium, distortion of spermatozoa, distorted primary and secondary spermatogonial cells, tubular vacuolation, disarray of spermatozoa and dilated tubular layer.

Ultrastructural changes in testis

Electron micrographs of control (Plates. 16-19: Fig. 31-38) and cypermethrin [lethal (Plates. 20-24: Fig. 39-47) and sublethal (Plates. 24-28: Fig. 48-55)] exposed fish testis are presented.

Testis of Control fish have lobules filled with spermatozoa and thin interlobular tissue. The electron microscope features of the testis in control
fish group showed mature spermatozoa. The sperms had rounded nucleus occupied in the head with homogeneous chromatin material. The nuclear fossa forming an invagination at the posterior end of the head followed by the elements of the axial filament, which extended posterior along the tail. The neck was short embedded in the nuclear fossa to form a short connection between the head and the flagellum. The tail consisted of the axial filament which was covered by undulating cell membrane. The proximal part of the axial filament extended in the midpiece characterized by a single layer of mitochondria extended along its length, having well developed cristae.

Lethal concentration of cypermethrin was found to affect spermatogenic cells. Some of the changes observed in the present study include degeneration of cysts in the tubular lumen, numerous vesiculations. Vacuolations were observed at many places of the ultra section. Other prominent changes include degeneration of tubules in lumen and distortion and vacuolations of primary spermatogonial cells in particular. Primary spermatogonia, secondary spermatocytes and spermatozoa were immature. Sperms had shrunken nuclei with dilated nuclear membrane and condensed chromatin material. The mitochondria had condensed with various shapes and abnormal cristae. Tails have irregular outlines and showed deteriorated oxonemal composition. The chromatin material became condensed and the mitochondria were reduced in number. In addition, only few interstitial cells were observed and we could be able to see secondary spermatocytes. Probably maturation was affected and arrested at secondary spermatocyte
stage. There is no synthetic activity since, mitochondria, rER, Golgi apparatus were found to be scarce. Other symptoms include swollen and dilated rER, reduction in the size and number of cell organelles, vacuolation and vesiculation in primary spermatogonia and sertoli cells. Finally, lethal concentration of cypermethrin affected cytoplasm. Spermatogonial cells have abruptive cytoplasm, consequently underwent cell death resulting in apoptosis.

At sublethal concentration, seminiferous tubules were regressed. Secondary spermatogonia, nucleus, heterochromatin, nucleoli, mitochondria, were condensed. Spermatozoa and interstitial cells were reduced in number. Reduction in the number of mitochondria at the mid region of spermatozoa and sperm head (L.S. and T.S) was noticed. Other symptoms include vacuoalltions in spermatozoa and degeneration of interstitium.

Changes in hormonal levels

Hormonal changes on exposure to cypermethrin at lethal and sublethal concentration of cypermethrin are presented in table 33 and figure 29-30.

Marked changes in the VTG levels were observed in male and female fish on exposure to lethal and sublethal concentrations of cypermethrin. VTG Levels were reduced, irrespective of exposure periods in the order of control 24h 48h 72h 96 h at lethal concentration. At sublethal concentration VTG levels were in the order of control 1 5 10 15 days. While, male fishes did not show the Vtg levels in control, however the vtg levels were expressed on exposure to cypermethrin at both the concentration and were in the order of
24 48 72 96 at lethal, 1 5 10 15 at sublethal concentration. Estradiol levels were decreased in male and female fish on exposure to lethal and sublethal concentrations. Responses were duration and concentration dependent. In female fish estradiol levels were in the order of 24h 48h 72h 96 h at lethal and 1 5 10 15 days sublethal concentration. 11 ketosterone levels exhibited decrease on exposure to lethal and sublethal concentration in male fish. They were in the order control 24h 48h 72h 96 h at lethal and control 1 5 10 15 days at sublethal concentration. Total free testosterone levels in male fish were continuously decreased over control at both the concentrations.

Discussion

Histopathology has received increased interest as an endpoint in EDC research in aquatic organisms, because histopathological changes are often the result of the integration of a large number of interactive physiological processes. Sub-lethal exposure to persistent organic compounds may alter key enzymatic activities and potentially cause reproductive effects, by reducing fecundity and population recruitment (Patyna et al., 1999). The hypothalamic-pituitary-gonadal (HPG) axis of teleost fishes, whose principal components are the hypothalamus and pituitary gland in the brain, the gonads and the liver, is generally similar to that of other oviparous vertebrates (Ankley and Johnson, 2004). The gonads are the primary organs of reproduction and, as such, will reflect substantive disturbances to the HPG axis (USEPA, 2006).
Histopathology helps to identify target organs of toxicity and mechanism of action (Wester, et. al, 2002). As a tool for assessing endocrine disrupting effects in fish, histopathology has also been applied in other studies such as endocrine disruption in the ovaries and testes of zebrafish (Vander Ven, et. al, 2003) and in the adult fathead minnow (Leino, et. al, 2005).

Endocrine disrupting chemicals are known to disturb the endogenous hormonal function of organisms, affecting behaviour and secondary sexual characteristics as well as the gonads. The differentiation of somatic cells in testis and ovary is both morphological and functional. The major roles of these somatic cells are to nourish developing germ tissue and to synthesize and provide the correct hormonal milieu to support either oocyte or spermatocyte development. In this study, we exposed *Labeo rohita* to cypermethrin at two different concentrations representing acute and subacute responses at lethal and sublethal concentrations.

Present study evidenced that, the cypermethrin affects the ovary at both the concentrations. At lethal concentration ovarian tissue exhibited degeneration of oocyte, atretic follicle, degenerated epithelium, inter follicular degeneration, dilated chorion, disintegrated epithelial space, degenerated alveolar space, developing oocytes, degenerated oocytes, disintegration of epithelium, increased atretic follicle and vacuolated primary oocyte. These observations suggest that, cypermethrin interferes and disturbs the normal physiology of the ovary, usually an indicative of significant inhibition of steroidogenesis. Further, Epithelial lesions, inflammatory responses, stromal
hemorrhage, increased interstitium and shrinkage of yolk vesicles towards periphery, degeneration of oocytes, increase in atretic follicles, vascular degenerative changes in the ovarian follicles, lack of nuclei in the lining epithelial cells, disappearance of nucleolar details indicate inhibition of gonadotrophic hormone secretion (Wester, et. al, 2002). However, the changes were not much pronounced at sublethal concentration, but increase in the number of atretic follicles, degeneration of oocyte and disintegration of intraepithelial space, blebbing of epithelium, dilation and disintegration of epithelium, distortion of interfollicular space, vacuolated alveolar oocyte and nucleus were observed. Similarly earlier studies documented alterations in ovary on exposure to different pesticides and chemical contaminants (Bhuiyan, et. al, 2001, Sivarajah, et. al, 1978, Jha and Jha, 1994). At the dose of 100ppm of sumithion resulted in fragmented ova with abnormal shape and arrangement in the experimental fish (Bhuiyan, et. al, 2001). Sivarajah et. al, (1978) reported fragmentation and karyolysis of ova when Salmo gairdneri and Cyprinus carpio exposed to aroclor. Jha and Jha, (1994) reported the impact of 30-days exposure to sub-lethal concentration of urea (416 ppm) and ammonium sulphate (448ppm) on the ovary of H. fossilis. Urea induced initial stimulation of vitellogenesis followed by subsequent arrest of ovarian growth. Besides, the cells of germinal epithelium developed by perplasia lead to the completed fusion of the two follicles. Contrary to urea, ammonium sulphate produced severe adverse effects as evidenced from large number of early non-vitellogenic oocytes and traces of reovulatory degenerated oocytes. The extent
of ovarian damage was such that there was complete breakage and dissolution of ovigerous lamellae.

Because of impaired steroid metabolism, particularly in the liver, pesticides alter lipid metabolism since steroids play an important role in the regulation of lipid metabolism in relation to reproduction of teleosts (Singh and Singh, 1992). Hexachlorocyclohexane, a pesticide arrests hepatic lipidogenesis, as well as translocation of lipid to the ovary by impairing gonadotrophin acting through the hypothalamo-hypophysial-ovarian axis in *H. fossilis* (Singh and Singh, 1992). Consequently, there is a reduction in the hepatic diglyceride level (precursor of vittellogenin) and consequently, interference in vittellogenesis. The liver is heavily damaged by the cypermethrin in the present study. The interference in vittellogenin synthesis may have caused the arrest of growth of oocytes. These oocytes do not have vittellogenin to undergo vittellogenesis. Rastogi and Kulashreshta, (1990) have reported the same findings in pesticide treated *Rasbora daniconius*. Furthermore, the action of pesticide is direct and selective to specific oocytes (Kumar and Pant, 1988).

Another important pathology commonly observed in female fish exposed to EDCs is ovarian atresia. Atresia is a normal process at the post-ovulatory stage of ovarian development, but not for ovaries in previtellogenic or early vitellogenic stages. Female medaka exposed to high doses of vinclozolin (2,500 µg/L) exhibit a higher incidence of ovarian atresia (Kiparissis, *et. al.*, 2003). Bhuiyan and co workers reported effects paper mill
effluents on ovarian morphology of female largemouth bass have also
detected higher rates of atresia and an overall depression of follicular
development (Bhuiyan, et. al, 2000). Similar effects have previously been seen
in guppies treated with 17β-estradiol (Venkatesh, et. al, 1991), pregnenolene
(Eversole, 1941) or methallibure (Lam, et. al, 1985), mosquitofish (Gambusia
holbrooki) treated with nonylphenol (Dre’ze, et. al, 2000) and zebrafish (Danio
rerio) treated with PCBs (O’rn, et. al, 1998) or ethynylestradiol (Van den Belt, et.
al, 2001).

The pesticides are reported to hamper reproduction both in piscine and
non-piscine species. In the present study the observed changes in the ovary of
the fish at both the concentrations could be attributed to possible depression
in the production of aromatase in the ovarian tissue. Although we have not
measured the aromatase activity in the ovary, a block of the formation
activities of aromatase by cypermethrin cannot be ruled out. Since the
reduction of 17β estradiol has been evident in the present study and can be
related to the inhibition of estrogen via the rapid degradation of the hormone
to its metabolic product catecholestrogen. There are reports that in
Oncorhynchus mykiss, estrogen levels in the serum were reduced by β-
naphthoflavone (BNF) due to an increase in glucuronyl conjugation
(Anderson, et. al, 1996). The current theory regarding the involvement of
antiestrogenic compounds in the disruption of endocrine system is that this
occurs due to their direct involvement in induction of cytochrome P-1A1
measured 7-ethoxyresorufin deethylase (EROD) activity in cultured hepatocytes of the experimental fish and found that carbofuran at nanomolar dose was able to induce EROD activity similar to that for benitrofuran treatment. EROD activity is considered as an index of CYP 1A1 induction. Therefore, the reduction in estrogen levels in the ovary of fish might be a result of CYP 1A1 induction.

The reduction in circulating serum estrogen levels affected vitellogenin content in the experimental fish. There are reports that vitellogenin levels were reduced in fish exposed to environmental pollutants (Chen, et. al, 1986; Chakraborty, et. al, 1992; Ruby, et. al, 1993). Vitellogenin synthesis in fish is a complex phenomenon and several environmental pollutants, which are estrogenic in nature, induce vitellogenin. Compounds like methoxychlor, nonylphenol, ethylene glycol and dimethyl formamide induced the vitellogenin mRNA in fish (Schlenk, et. al, 1997; Lech, 1997). Thus, reduction in serum vitellogenin level further supports the concept that estrogen levels are reduced after cypermethrin treatment in the present study.

The present study showed that cypermethrin at lethal and sublethal doses reduced hormonal levels. Our observations suggest that the cypermethrin interfere with gonadotrophs of the pituitary of the fish and probably via disruption of the pituitary-gonad axis of the fish. Other laboratories had reported that fish exposed to pollutants showed reduced plasma sex steroids, gonad size, disrupted gonadal development and delayed sexual maturation (Sivarajah, et. al, 1978a, b; Johnson, et. al, 1988; Thomas,
The mechanisms underlying these reproductive disturbances are largely unknown. Previous studies with carbofuran at sub-lethal doses to catfish, produced reproductive dysfunctions by arresting follicular maturation in the ovary (Chatterjee, et. al, 1997) reduced fertilization rates in vitro (Chatterjee and Ghosh, 1995) and reduced lipid mobilization from the liver (Chatterjee, 1996). They opine that pesticides might act by arresting the pituitary-gonad axis in fish, resulting in disrupted follicular maturation.

Recently efforts are being made to study the adverse effects of environmental contaminants with antiandrogenic activity (Gray, et. al, 2001; Sun, et. al, 2006; Xu, et. al, 2008). These so-called anti-androgens could be responsible for the increased incidence in various male sexual differentiation disorders such as hypospadias, cryptorchidism, low sperm counts and quality (Andrew, et. al, 2008). Interfering with AR has been the focus of the majority of researches (Cynthia, et. al, 2009; Kelce and Wilson, 1997; Xu, et. al, 2006, 2008; Sun, et. al, 2006). It has been shown that many chemicals such as p,p-DDE, BPA can act as antagonists by binding to AR and preventing transcription of androgen-dependent genes (Kelce, et. al, 1995; Vinggaard, et. al, 2002; Xu, et. al, 2005a, 2005b, 2008). They inhibit male sexual development and maturation, as well as the maintenance of male reproductive organs and of spermatogenesis (Roy, et. al, 1999). Previous studies have shown that several pesticides and their metabolites exerted anti-androgenic activities by
interacting with the human AR in vitro experiments (Sun, et. al, 2007; Zhang, et. al, 2008; Xu, et. al, 2008).

In the present study treatment resulted changes in the normal testicular architecture with regression of seminiferous tubules, vacuolation and degeneration of tubular lumen, distortion and degeneration of tubular membrane, dilation of germinal epithelium. In addition to this large number of inflammatory cells were witnessed. Presence of a large number of intertubular vacuoles and swelling of spermatids were observed leading to further condensation of spermatogonic cells. The nucleus exhibit degenerative changes as evident initially from the liquefaction of the perinuclear cytoplasm, which become less dense, followed by the condensation of nucleus, rendering it much smaller and the clumping together of the nucleoli. Reproductive abnormalities caused by pesticides have been observed in many vertebrates, such as inhibition of spermatogenesis in fish and mice (Dutta and Meijer, 2003; Contreras, 1999; Sobarzo, 2000). Alteration of germinal cell DNA, seminiferous epithelium and low quality of sperm have been reported previously (Recio, 2008; Oliveira, 2001; Bustos, 2005; Contreras, 1999; Contreras, 1993; Del Valle, 2004; Espinoza, 2005; Pina, 2005; Rojas, 2008; Swan, 2006). However, the results from in vivo test are in some respects contradictory to those in vitro experiments. Kunimatsu, et. al, (2002) found that none of the tested pyrethroid pesticides including esfenvalerate, fenvalerate, and permethrin caused adverse androgenic and anti-androgenic effects in vivo. Yamada, et. al, (2003) concluded that based on the results of in
in vivo assays, d-phenothrin, as a synthetic pyrethroid, did not cause adverse effects on reproductive system even at very high dose level.

However, the testicular changes were not much pronounced at sublethal concentration compared to lethal concentration. Observed changes in the testis include absence of interstitial cells, dilation of the germinal epithelium, distortion of spermatozoa distorted primary and secondary spermatogonial cells, tubular vacuolation, disarray of spermatozoa and dilated tubular layer. Similar observations were reported in male rats on intraperitoneal administration of diazonin (Dikshith, et. al, 1975). Dutta and Meijer, (2003) investigated the toxic effects of diazonin on the structure of the testis of bluegill fish. The observed changes in the present study could be related to possible increase in reactive oxygen species may decrease the effective concentration of antioxidants increasing the harmful effects of cypermethrin. Though the antioxidants are not measured in the testis, but the alterations observed in other vital organs may interfere with reproductive system. Moreover, some research has shown decreased testosterone production by interstitial cell (Leydig cells) as one of the mechanisms. In the present study also the inhibition of testosterone was observed. Thus, the altered histopathology of testis clearly infers that, cypermethrin affected steroidogenesis acutely.

Spermatogonia undergo numerous mitotic divisions to form primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa; resulting in production of cysts surrounded by sertoli cells. Each lobule of the
testes contains numerous cysts. Regularly organized cysts with all stages of spermatogenesis are an indication of testes in a healthy reproductive condition. According to Hoar (1965), spermatozoa are formed from the sperm mother cells or spermatogonia through a series of cytological stages collectively referred to as spermatogenesis. The gradual reduction in the size is carried up to spermatid formation which metamorphoses in to spermatozoa by reduction in its nuclear size. Static bio-assays conducted in the present study clearly indicate that exposure of cypermethrin is responsible for ultratructural damage of fish testis in terms of condensation of spermatogonic cells, vacuolization of tubular cells distortion of seminiferous cells, decrease in the number of spermatids, degeneration and distortion of cysts in the tubular lumen, numerous vesiculation and vacuolations, immature spermatogonia and secondary spermatocytes, affected sperm maturation and probably arrest the reproduction by affecting the synthesis of sperms in general. The observed lesions were found to be concentration and exposure dependent.

Vacuolation and vesiculation (vacuole filled with fluid) in primary spermatogonia, and sertoli cells at lethal concentration. Vacuolation of primary spermatogonia suggests impairment and arrest of mitosis leading to loss of development, maturation and spermatogenesis. Since, it is believed that, spermatogonia has the capability of producing repetitive waves of spermatogenesis in seasonally reproducing species and assumed that true spermatogonial stem cells are present in the fish testis (Billard, 1969a). Reduced number of sertoli cells indicates decrease in the volume of the
spermatocysts consequently affecting sertoli cell proliferation during spermatogenesis, leading to the decreased mitotic activity affecting developing germ cell clones. Since, the germ cell number and the spermatocyst volume increase rapidly during the mitotic phase (Billard, 1969a), it is therefore reasonable to assume that the number of sertoli cells associated with a developing germ cell clone is increasing as well. Indeed, the sertoli cell number per spermatocyst increases during normal spermatogenesis (Billard, 1969a).

The most noticeable morphological process observed (at 4.0 µg/l of cypermethrin) was the presence of massive cell death witnessing apoptosis (Programmed cell death) of primary spermatogonia. Marginal and intermediate cell layers were differentially affected showing loss of cell volume and cell junctions, having abruptive cytoplasm. In the present study we did not determine the pathophysiological and molecular mechanisms of cypermethrin induced apoptotic toxicity. However there is evidence indicating that cypermethrin activity is mediated mainly by alteration of the membrane sodium transport (Bradbury and Coats, 1989), we suggest that the effect of cypermethrin on ion transport may be a mechanism by which apoptosis of spermatogonia is induced. Moreover, this can be correlated to oxidative stress induced at lethal and sublethal concentrations of cypermethrin in the present study. Recently, Izaguire, et. al, (2000) reported dose and time- dependent cypermethrin induced apoptosis at higher than 100µg/l in neural cells of tadpoles (Physalaemus biligonigerus), they could not
distinguish any apoptotic figures. In subsequent studies, similar apoptosis produced by cypermethrin in the brain of *Bufo arenarum* tadpoles was recorded (Izaguirre, *et. al*, 2000, 2001). Further, Bortner and Cidlowski, (1996) have confirmed DNA fragmentation, a typical feature of apoptosis induced by both physiological and pathological mechanisms using the TUNEL method in thymocytes of *Bufo arenarum* on exposure to cypermethrin. The quantitative method applied to determine the DNA fragment concentration has demonstrated that the highest increase in apoptosis is produced from 156 μg/l of cypermethrin. The highest concentration of small DNA fragments (172 bp) reported at the highest dose of 2500 μg/l of cypermethrin at 96 h (Casco, *et. al*, 2006). However, there are no such studies reported apoptosis in case of fishes and the present study is the first of its kind to report apoptosis in primary spermatogonia. In contrast to the earlier reports, in the present study apoptosis was induced at much lower concentration (i.e., 4.0 μg/l) of cypermethrin indicating the sensitivity of *Labeo rohita* and vulnerability of fish testis.

The degree of testicular damages in the treated fish has been attributed to the low levels of gonadotropin (s) which in turn may be responsible for the reduced steroidogenic activity in the testes as well as for the low levels of steroid hormones in the blood plasma (Singh and Singh, 1982). In *Salvelinus fontinalis*, Freeman and Idler, (1975) have noticed regression of the testes as well as a decrease in steroidogenesis following polychlorinated biphenyl (PCB) treatment. Singh and Singh, (1982) have opined that the xenobiotics act
through the hypothalamus, where they seem to inhibit the secretion of the 
gonadotropin releasing hormone (GnRH) which, in turn, decreases the 
synthesis and release of the gonadotropin(s) from the pituitary gland 
followed by the reduced gonadal activity. On the other hand, the chlorinated 
hydrocarbon pesticides like aldrin and hexadrin act directly on the gonads to 
suppress their activity. Present study evidenced that, cypermethrin affects the 
testis. Since, serum level of testosterone is a reflection of its synthesis in the 
testis, and possible inhibition of penultimate (D^3β HSD) and ultimate (17β 
HSD) enzyme, which are involved in the biosynthetic pathway of testosterone 
and the level of testosterone is dependent on their activity. In addition, 
testosterone measured in the present study clearly supports the observation.

Androgenic sex steroids are the most important hormones regulating 
spermatogenesis, their production being stimulated in fish by both FSH and 
LH. In fish, the quantitatively dominating steroids synthesized in the testis 
are 11-oxygenated androgens, such as 11- ketotestosterone (11-KT; Borg 1994); 
testosterone is produced as well while, DHT is rarely found in fish. However, 
apparently little is known about cypermethrin-induced testicular toxicity and 
endocrine disruption in fishes, rather few available studies addressed are of 

Recently, pyrethroids have been found to have potentially adverse 
effects on male reproduction (Elbetieha, et. al, 2001, Mani, et. al, 2002, LeGac, 
et. al, 2008). An earlier study showed that fenvalerate exposure to adult rats 
by inhalation obviously induced the decrease in testicular weight, epididymal
sperm counts and sperm motility (Mani, et. al, 2002). In addition, the expression of testicular 17β-hydroxysteroid dehydrogenase (17β HSD), a testosterone (T) biosynthetic enzyme, was markedly downregulated in fenvalerate exposed rats. Correspondingly, serum T was markedly decreased in rats exposed to one-fifth LC₅₀ of fenvalerate by subchronic inhalation for 3 months (Mani, et. al, 2002). Nonetheless, relatively few studies have investigated the adverse effects of cypermethrin on male reproduction. According to an earlier report, cypermethrin significantly decreased the fertility and reduced the number of implantation sites and viable fetuses in females impregnated by males exposed to cypermethrin (Elbetieha, et. al, 2001). In addition, the number of epididymal sperm as well as daily sperm production was significantly decreased in males exposed to cypermethrin (Elbetieha, et. al, 2001).

Cypermethrin exhibited decrease in the levels of the androgens (testosterone and 11-KT) at lethal and sublethal concentration in male Labeo rohita. These changes can be correlated with observed ultrastructural changes for instance primary spermatogonia, secondary spermatocytes and sperms were immature. Abnormal sperms with shrunken nucleus, dilated nuclear envelope, and condensed chromatin material. Tails have irregular outlines exhibiting deteriorated axonemal composition. The chromatin material became condensate and the mitochondria were reduced in number. Thus it is clearly evident that, cypermethrin affects milt hydration and sperm maturation by inhibiting the whole process of spermatogenesis, or at least
some steps such as spermatogonial multiplication and spermatocyte formation or maturation. Moreover androgens are effective in supporting either the whole process of spermatogenesis, or at least some steps such as spermatogonial multiplication and spermatocyte formation or maturation (Remacle, 1976; Billard, et al., 1982; Fostier, et al., 1983; Billard, 1986; Nagahama, 1994; Borg, 1994). Possible effects of cypermethrin on testicular gene expression through inhibition of androgen receptors (α and β) can not be ruled out. Since, androgen receptor subtypes (α and β) predominantly expressed in the gonad (Takeo and Yamashita, 1999; Todo, et al., 1999; Ikeuchi, et al., 2001) and androgens strongly influence testicular gene expression (LeGac, et al., 2008).

In the present study total free testosterone levels in the blood plasma of male fish were continuously decreased over control at lethal and sublethal concentrations of cypermethrin. This can be correlated to the observed ultrastructural changes in the testis such as condensation of mitochondria, dilation of rER, absence or complete reduction of golgi apparatus indicating less or no synthetic activity, swollen and dilated rough endoplasmic reticulum, reduction in the size and number of cell organelles. It can be attributed to the possible inhibition of precursor C17–20 lyase enzyme activity, which converts progestogenic precursors into androgens. The observations in the present study are consistent with reports of earlier study in juvenile male African catfish. Where in vivo treatment of 11-KT and testosterone inhibited testicular C17–20 lyase activity (converting
progestogenic precursors into androgens) and ultrastructural changes in the
leydig cells, such as a loss of mitochondria and a reduced cell size (Cavaco, et. al, 1999).

Cypermethrin exhibited decrease in the plasma levels of 17β-estradiol and 11-KT and this can be correlated to the marked histological changes in ovary and ultrastructural changes (as discussed in above paragraphs) in testis suggesting cypermethrin may affect at pituitary level ultimately decreasing the plasma levels of estradiol and 11-KT in both female and male L. rohita. Thus they have an effect on reproductive physiology by inhibiting steroidogenesis. Singh and Singh, (2008) who reported inhibition of gonadotropins at pituitary levels in H. fossilis on exposure to cypermethrin have also supported the observed decrease in the 17β-estradiol and 11-KT. Further, they witnessed degeneration of spermatogonial cells after cypermethrin exposure. Similar studies have been reported in two different fish species Carassius auratus and H. fossilis, on exposure c-hexachlorocyclohexane (HCH), alkyl benzene sulphonate, malathion and c-BHC, HCH, DDT metabolites (Singh and Singh, 1992; Singh and Canario, 2004; Singh and Singh, 2007). Kumar, et. al, (2007) demonstrated that, the maturation was affected by benzene sulphonate. In addition, the decrease in plasma gonadotropin (s) and sex steroids hormones in Carassius auratus by γ-HCH exposure (Singh, et. al, 1994) indicates that cypermethrin acts through hypophysis at gonadal level in this species. Cypermethrin reported to cause hostological changes in gonadotropic cells, liver, gonads, plasma levels of
17β-estradiol and 11-ketotestosterone and sperm motility in *Heteropneustes fossilis* (Singh and Singh, 2008).

Vitellogenin is specifically synthesized in the liver of fish under the control of 17β-estradiol, secreted into the bloodstream and finally taken up by oocytes via receptor-mediated endocytosis. Vitellogenin gene expression is regulated by the binding of estradiol-receptor/17β-estradiol complexes to estradiol responsive elements at the promoter site of the vitellogenin gene; hence, transcription is under the control of cellular 17β-estradiol concentrations (Wahli et al, 1981).

Cypermethrin exhibited induction of vitellogenin levels at lethal and sublethal concentration in male *L. rohita*. The observed responses were time and concentration dependent. However, the vitellogenin was not detected in control male. The induction clearly suggests the estrogenicity of cypermethrin. Further, the marked testicular ultrastructural changes observed in the present study supports the induction of vitellogenin. Since, normally, vitellogenin is undetectable in male and immature female fish; however its synthesis can be induced by exogenous stimulation of estrogenic compounds. An increase in plasma vitellogenin for male *L. rohita* upon exposure to estrogens is consistent with previous studies (Panter, *et al*, 1998; Thorpe, *et al*, 2007; Sowers, *et al*, 2009; Shappell, *et al*, 2010). Previous comparisons of estrogenic effects on male fathead minnows (*Pimephales promelas*) have shown significant increases in plasma vitellogenin levels when exposed in a flow-through system to nominal concentrations (not confirmed through analytical chemistry) of 32
ng/L of estradiol-17α (Panter, et. al, 1998). Significant increases in vitellogenin were not observed until >100 ng/L estradiol-17β. In conjunction with raised plasma vitellogenin, rate of testicular growth decreased upon estradiol-17α exposure. Interestingly, an increase in male gonadosomatic index was also observed (Panter, et. al, 1998).

Cypermethrin in female fish exhibited reduction in the levels of vitellogenin and 17β-estradiol, which could be correlated to the concentration and time dependent histological changes observed in the ovary. The results of other laboratory exposures to show a decrease of the level of vitellogenin in the blood of fish (O. mykiss, S. gairdnerii, Monopterus albus) exposed to organic contaminants (PAH and PCB) (Chen et al., 1986; Singh, 1989; Thomas, 1990b; Anderson et al., 1996b). This observation was attributed to decreased levels of circulating estradiol. Furthermore, vitellogenin levels could be reduced through an Ah receptor-mediated decrease in the response to estradiol caused by blocking of ER and: or estrogen responsive gene transcription (Anderson et al., 1996b). Finally, the mechanism could involve estrogenic (and antiestrogenic) effects. The natural estrogen of vertebrates, 17β-estradiol, is a ligand of the estrogen receptor (ER) and after binding, the active transcription factor induces gene transcription through interaction with estrogen-responsive DNA elements. In fish, 17β-estradiol is associated primarily with vitellogenesis and ovarian development (Wallace, 1985; Mommsen and Walsh, 1988; Arukwe and Goksøyr, 2003). Under the direction of the brain-pituitary axis, E2 stimulates the liver to produce the egg yolk protein VTG, a calcium-
containing glycolipophosphoprotein of high molecular weight. Thus, the observed reduction in the levels of VTG and 17β-estradiol affects ovarian development and result in impaired normal physiology. This can also be correlated to the altered ions and associated ATPases, respiratory impairment, haematotoxicity, and oxidative stress induced by the cypermethrin. Moreover ultrastructural changes observed in the present study in the liver can also be correlated to the reduced levels of vitellogenin and 17β-estradiol.

The present study clearly suggests endocrine disruptive nature of cypermethrin. It is pertinent to state that cypermethrin acts as estrogenic as it induces vitellogenin, 17β-estradiol in male fish and antiestrogenic as it reduces the VTG and E2 in female fish. Considering this further studies are warranted to understand estrogenic and antiestrogenic nature of the cypermethrin to elucidate possible mechanisms mechanism. In conclusion the cypermethrin had shown its effects on testis, ovary and hormones thereby interfering in the reproduction in general.
Plate 1

Fig. 1: Control ovary from an adult female *L. rohita* showing normal histoarchitecture with different oocytes. Vitellogenic stage oocyte (O) showing numerous yolk globules in the cytoplasm. (60X)

O: Oocyte

DO: Developing oocyte

CA: Cortical alveolar oocyte

Fe: Follicular epithelium

Fig. 2: Control ovary from an adult female *L. rohita* showing normal histoarchitecture with different follicle. (60X)

O: Oocyte

CA: Cortical alveolar oocyte

SO: Secondary oocyte
Plate 2

Fig. 3: Control ovary from an adult female *L. rohita* showing details, normal arrangement of different oocytes rich in yolk globules in the cytoplasm, zona radiata and follicular epithelium. (100X)

YG: Yolk globules
Zr: Zona radiata
Fe: Follicular epithelium

Fig. 4: Control ovary from an adult female *L. rohita* showing details, normal arrangement of different oocytes rich in yolk globules in the cytoplasm, zona radiata and follicular epithelium. (40X)

O: Oocyte
OD: Ootid
SO: Secondary oocyte
PO: Primary oocyte
YG: Yolk globules
Plate 3

Fig. 5: Ovarian histoarchitecture of *Labeo rohita* on exposure to lethal concentration for 96 h showing degenerated oocytes and atretic follicles. (100X)

- DGO: Degenerated oocyte
- SO: Secondary oocyte
- AF: Atretic follicle
- PO: Primary oocyte
- N: Nucleus
- YG: Yolk granules

Fig. 6: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to lethal concentration for 96 h showing degenerated oocytes and epithelium. (100X)

- DGO: Degenerated oocyte
- DE: Degenerated epithelium
Plate 4

Fig. 7: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to lethal concentration for 96 h showing different follicular oocytes. (60X)

DIFS: Degeneration of interfollicular space

O: Oocyte

N: Nucleus

DO: Developing oocytes

DCO: Degenerated oocytes

PO: Primary oocyte

SO: Secondary oocyte

Fig. 8: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to lethal concentration for 96 h showing different follicular oocytes. (60X)

DIE: Disintegrated epithelium

AF: Atretic follicle

SO: Secondary oocyte

VPO: vacuolated primary oocyte

DAO: Degenerated alveolar oocyte

DE: Degenerated epithelium
Plate 5

Fig 9: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to lethal concentration for 96 h showing different follicular oocytes. (100X oil)

C: Chorion

N: Nucleus

CAO: Cortical alveolar oocyte

Fig. 10: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to lethal concentration for 96 h showing two different follicular oocytes. (100X oil)

DIES: Disintegrated epithelial space

CAO: Cortical alveolar oocyte

DAS: Degenerated alveolar space
Plate 6

Fig. 11: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to lethal concentration for 96 h showing two different oocytes. (100X oil)

IFG: Inter follicular degeneration
C: Chorion
CAO: Cortical Alveolar oocyte

Fig. 12: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to lethal concentration for 96 h showing changes in the portion of follicle. (100X oil)

N: Nucleus
CAO: Cortical alveolar oocyte
DE: degenerated epithelium
C: Chorion
Plate 7

Fig. 13: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to sublethal concentration for 15 days showing different follicles with lesions. (60X)

YG: Yolk granules
PO: Primary oocyte
AF: Atretic follicle
DGO: Degenerated oocyte
DIES: Disintegration of intra epithelial space
SO: Secondary oocyte

Fig. 14: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to sublethal concentration for 15 days showing different follicles with lesions. (60X)

SO: Secondary oocyte
AF: Atretic follicle
PO: Primary oocyte
N: Nucleus
YG: Yolk granules
BE: Blebbing of epithelium
DE: Dilated epithelium
DIE: Disintegrated epithelium
Fig. 15: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to sublethal concentration for 15 days showing different follicles. (100X)

YG: Yolk granules  
DIO: Disintegrated oocyte  
DIE: Disintegrated epithelium  
DO: Disintegrated oocyte  
CAO: Cortical alveolar oocyte

Fig. 16: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to sublethal concentration for 15 days showing different follicles. (100X)

DIO: Disintegrated oocyte  
DIE: Disintegrated epithelium  
DE: Dilated epithelium  
PO: Primary oocytes  
SO: Secondary oocyte  
VAO: Vacuolated alveolar oocyte  
VN: Vacuolated nucleus
Plate 9

Fig. 17: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to sublethal concentration for 15 days showing different follicles. (100X)

CAO: Cortical alveolar oocyte  
V: Vacuole  
YG: Yolk granules  
SO: Secondary oocyte  
DE: Dilated epithelium  
N: Nucleus

Fig. 18: Photomicrograph of ovarian histoarchitecture of *Labeo rohita* on exposure to sublethal concentration for 15 days showing different follicles. (100X)

DFS: Distorted interfollicular space  
PO: Primary oocytes  
SO: Secondary oocyte  
N: Nucleus  
DGO: Degenerated oocyte
Plate 10

Fig. 19: Histophotomicrograph of control testis of fish *L. rohita* showing normal histoarchitecture of testis with spermatogenic cells. (40X)

SSP: Secondary spermatocytes

SC: Sertoli cell

SZ: Spermatozoa

SP: Spermatids

PSP: Primary spermatocytes

Fig. 20: Histophotomicrograph of control testis of fish *L. rohita* showing spermatogenic cells. (40X)

SP: Spermatids

SC: Sertoli cell

PSP: Primary spermatocytes

SSP: Secondary spermatocytes

IT: Interstitial tissue lining (White arrow heads)

SZ: Spermatozoa
Plate-11

Fig. 21: Histophotomicrograph of control testis of fish *L. rohita* showing histoarchitecture of spermatogenic cells in seminiferous tubules. (100X)

- PSP: Primary spermatocytes
- SSP: Secondary spermatocytes
- SP: Spermatids
- SC: Sertoli cell
- SZ: Spermatozoa

Fig. 22: Histophotomicrograph of control testis of fish *L. rohita* showing histoarchitecture of spermatogenic cells in seminiferous tubules. (100X)

- PSP: Primary spermatocytes
- SSP: Secondary spermatocytes
- SP: Spermatids
- SC: Sertoli cell
- SZ: Spermatozoa
- IT: Interstitial tissue lining (White arrow heads)
Plate 12

Fig 23: Histoarchitecture of testis from *Labeo rohita* on exposure to lethal concentration for 96 h showing alteration in the histoarchitecture of tubular lumen, distorted limiting membrane of the tubule. (40X)

- VL: Vacuolated lumen
- SP: Spermatids
- SC: Sertoli cells

Black Arrow heads: Architectural alteration

Fig 24: Histoarchitecture of testis from *Labeo rohita* on exposure to lethal concentration for 96 h showing alteration in the histoarchitecture of tubular lumen, degenerated epithelium of the tubule. (40X)

- SP: Spermatids
- DE: Degenerated epithelium
- SZ: Spermatozoa
- VL: Vacuolated lumen
- VT: Vacuolated testis
Plate 13

Fig 25: Changes in the histoarchitecture of testis of *Labeo rohita* on exposure to lethal concentration for 96 h. (40X)

- DGE: Degenerated epithelium
- SZ: Spermatozoa
- SC: Sertoli cells

Fig 26: Photomicrograph of a section of testis showing alteration in the histoarchitecture of *Labeo rohita*. (40X)

- PSG: Primary spermatogonia
- PSP: Primary spermatocytes
- SP: Spermatids
- SZ: Spermatozoa
- SC: Sertoli cells
- IC: Interstitial cells
Plate 14

Fig 27: Histoarchitecture of testis from *Labeo rohita* on exposure to sublethal concentration for 15 days showing the histoarchitecture of seminiferous tubules, tubular lumen, multilayer of germinal epithelium. (40X)

SP: Spermatids
SZ: Spermatozoa
SC: Sertoli cells

Fig 28: Histoarchitecture of testis from *Labeo rohita* on exposure to sublethal concentration for 15 days showing the seminiferous tubules, tubular lumen, multilayer of germinal epithelium. (40X)

Sp: Spermatids
DGE: Degenerated germinal epithelium
Sz: Spermatozoa
V: Vacuolated seminiferous tubule
Plate 15

Fig 29: Histoarchitecture of testis of *Labeo rohita* on exposure to sublethal concentration for 15 days showing the seminiferous tubules, tubular lumen, multilayer of germinal epithelium. (40X)

SP: Spermatids

SSp: Secondary spermatocytes

Fig 30: Histoarchitecture of testis of *Labeo rohita* on exposure to sublethal concentration for 15 days showing the seminiferous tubules, tubular lumen, multilayer of germinal epithelium. (20X)

Ds: Disarray of spermatozoa

Sz: Spermatozoa
Plate 16

Fig. 31: Electron micrograph of control fish testis showing seminiferous tubule containing primary spermatogonia (PSG), secondary spermatogonia (SSG), and spermatocyte (SPc). (2900X Magnification)

Fig. 32: Electron micrograph control fish testis showing Leydig cells (Lg), and blood vessels adjoining seminiferous tubule which contains primary spermatogonia (PSG) and secondary spermatogonia (SSG). (2900X Magnification)
Plate 17

Fig. 33: Electron micrograph control fish testis showing seminiferous tubule containing primary spermatogonia (PSG), secondary spermatogonia (SSG), primary spermatocyte (Psc) and secondary spermatocytes (SSc). (2900X Magnification)

Fig. 34: Electron micrograph of primary spermatogonia of control fish showing large oval shaped nucleus (N) and scanty cytoplasm containing few mitochondria (m). (6800X Magnification)
Plate 18

Fig. 35: Electron micrograph of primary spermatogonia of control fish showing large oval shaped nucleus (N) and scanty cytoplasm containing few mitochondria (m). (13000X Magnification)

Fig. 36: Electron micrograph of portion of secondary spermatocyte showing condensed nucleus (N), mitochondria (M) and sER (closed arrow head). (11000X Magnification)
Plate 19

Fig. 37: Electron micrograph of part of the Leydig cells of control fish shows electron dense irregular nucleus (N), mitochondria (M), sER (Closed arrow head) and lysosomes (Ly). (11000X Magnification)

Fig. 38: Electron micrograph picture showing cross section of spermatozoa of control fish having dark irregular nucleus (N) surrounded by mitochondria (M) (M) and microtubules in the tail region. Note the presence of LS of mid and tail region in the adjoin area. (23000X Magnification)
Plate 20

Fig. 39: Electron micrograph of fish testis exposed to lethal concentration of cypermethrin showing seminiferous tubule containing primary spermatogonia (PSG) and secondary spermatogonia (SSG). Note the presence of intercellular vacuoles (V). (2900X Magnification)

Fig. 40: Electron micrograph of primary spermatogonia of fish testis exposed to lethal concentration of cypermethrin showing vacuolations (V) and presence of few cell organelle like mitochondria (M), rER (Closed arrow head), lysosomes (Ly) and golgi bodies (G). (11000X Magnification)
Plate 21

Fig. 41: Electron micrograph of fish testis exposed to lethal concentration of cypermethrin showing secondary spermatocytes containing cytoplasmic vacuoles (V), and less number of cytoplasmic organelles. (4800X Magnification)

Fig. 42: Electron micrograph of fish testis exposed to lethal concentration of cypermethrin showing the details of secondary spermatogonia with oval shaped nucleus (N), condensed mitochondria (M), dilated rER (closed arrow head). (11000X Magnification)
Plate 22

Fig. 43: Electron micrograph of fish testis exposed to lethal concentration of cypermethrin showing the portion of secondary spermatogonia. Note the presence of dilated outer nuclear membrane, dilated rER (Closed arrow head), condensed mitochondria (m). (13000X Magnification)

Fig. 44: Electron micrograph of fish testis exposed to lethal concentration of cypermethrin showing portion of spermatocytes. Note the cytoplasmic vacuoles (V). (2900X Magnification)
Plate 23

Fig. 45: Electron micrograph of showing immature spermatozoa in the testis of cypermethrin exposed (lethal) fish. Note the presence of condensed nucleus (N) and absence of microtubules. (6800X Magnification)

Fig. 46: Electron micrograph of portion of sertoli cell of cypermethrin treated (lethal) fish showing elongated nucleus (N) and a few mitochondria (M). Note the presence of vacuoles (V) and few cell organelles in the adjoining spermatogonia. (11000X Magnification)
Plate 24

Fig. 47: Electron micrograph of apoptic spermatogonia from the cypermethrin treated (lethal) fish showing highly disrupted nucleus (N). (6800X Magnification)

Fig. 48: Electron micrograph of cypermethrin treated (sublethal) fish showing primary spermatogonia (PSG) and secondary spermatogonia (SSG) have many cytoplasmic vacuoles (V). (4800X Magnification)
Plate 25

Fig. 49: High magnification of the above portion of primary spermatogonia (PSG), and secondary spermatogonia (SSG) showing many vacuoles (V). (11000X Magnification)

Fig. 50: Electron micrograph of sublethal treated fish showing spermatogonia having many vacuoles (V) occupied majority of the cell cytoplasm. Note the presence of condensed mitochondria (M) and vesicles (Ve). (11000X Magnification)
Plate 26

Figure 51: Electron micrograph of sublethal treated fish showing spermatids containing condensed nucleus (N) and many vacuoles (V) in the cytoplasm. (6800X Magnification)

Figure 52: Electron micrograph of leydig cell of sublethal treated fish shows irregular nucleus (N) and a few condensed mitochondria (M). Note the presence of vacuoles (V) and condensed mitochondria in the adjoining spermatogonia. (6800X Magnification)
Plate 27

Fig. 53: Electron micrograph of sublethal treated fish showing spermatids (SP) and spermatozoa (Sz). Note the presence of conspicuous vacuoles (V) and condensed mitochondria (M) in the cytoplasm. (4800X Magnification)

Figure 54: Electron micrograph of spermatozoa of sublethal treated fish showing condensed & distorted nucleus (N) and presence of condensed mitochondria (M). (23000X Magnification)
Plate 28

Figure 55: Electron micrograph of head region of spermatozoa of sublethal treated fish showing the reduced number of cell organelles, condensed and vacuolated mitochondria (M) and distorted nucleus (N). (30000X Magnification).
Table 33: Changes in the hormonal levels in the blood plasma of the fish *Labeo rohita* on exposure to cypermethrin

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Control</th>
<th>Exposure Periods</th>
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<tbody>
<tr>
<td>VTG (pg/ml) in</td>
<td>Not detected</td>
<td></td>
<td>Lethal</td>
<td>Sub lethal</td>
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<td>96</td>
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<td>Female</td>
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<td>11 KT (pg/ml) in</td>
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<td>Male</td>
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<tr>
<td>Testosterone</td>
<td>7.23±0.28A</td>
<td>5.37±0.18B</td>
<td>4.72±0.13C</td>
<td>3.16±0.18D</td>
<td>3.19±0.29E</td>
<td>5.71±0.12B</td>
<td>4.13±0.42C</td>
<td>4.52±0.19D</td>
<td>3.53±0.32E</td>
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<tr>
<td>in Male</td>
<td></td>
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<tr>
<td>E&lt;sub&gt;2&lt;/sub&gt; (pg/ml) in Male</td>
<td>2.18±0.15D</td>
<td>1.99±0.27E</td>
<td>2.64±0.13B</td>
<td>3.26±0.14A</td>
<td>0.93±0.23F</td>
<td>2.03±0.19E</td>
<td>2.19±0.12D</td>
<td>2.45±0.14C</td>
<td>2.71±0.16B</td>
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<tr>
<td>in Female</td>
<td>27.84±0.31A</td>
<td>14.58±0.21C</td>
<td>6.86±0.19F</td>
<td>4.43±0.16G</td>
<td>3.22±0.23H</td>
<td>22.37±0.17B</td>
<td>18.59±0.26B</td>
<td>13.96±0.22D</td>
<td>9.18±0.17E</td>
</tr>
</tbody>
</table>
Fig 29: Changes in the hormonal levels in the blood plasma of the fish *Labeo rohita* on exposure to cypermethrin

Note: C- Control; Asterisk: VTG in male was not detected; h-hour (lethal); d-days (sublethal)
Fig 30: Changes in the hormonal levels in the blood plasma of the fish *Labeo rohita* on exposure to cypermethrin

Note: C- Control; h-hour (lethal); d-days (sublethal)