EFFECTS OF DIFFERENT TOXICANTS ON REPRODUCTION OF DIFFERENT FISH

Exposure to environmental estrogenic alkyl phenolic chemicals in rainbow trout resulted in a decreased fertility and egg production in females, reduction of gonadal size (Jobling et al., 1996; Ashfield et al., 1998), reduced sperm production and demasculinisation of genetic male Japanese medaka, intersex gonads (Nimrod and Benson, 1998; Gimeno et al., 1998) etc. Another frequently observed effect of exposure to environmental estrogens is the induction of the yolk precursor protein vitellogenin (VTG) in male and sexually immature fish (Jobling et al., 1996; Folmar et al., 1996).

The VTG is also known as a biomarker in toxicology, which represents intersex or testicular dysfunction such as alternations in steroidogenesis, sperm production and quality (Denslow and Sepulveda, 2008; Gregory et al., 2008). Impacts on VTG synthesis by androgens have been reported and exposure may result in either increases or reductions in the production of VTG (Hornung et al., 2004; Andersen et al., 2003; Orn et al., 2003; Zerulla et al., 2002). An increase in VTG levels has often been found in fish exposed to high levels of androgens (μg/L range) and is probably due to an increased rate of conversion of the androgens to estrogens by the enzyme aromatase (Ankley et al., 2001; Hornung et al., 2004; Zerulla et al., 2002).

Different studies show VTG induction in a dose - and time- dependent manner. For example, significant increase in VTG has been reported in adult common carp (Mandich et al., 2007) and medaka (Kang et al., 2002). Ishibashi et
al., (2005) at 1000 mg/L BPA after 14 and 21 days exposure, respectively. Lindholst et al., (2000) observed vtg induction in rainbow trout exposed to 500mg /L BPA for 12 days through a continuous flow system. In Atlantic cod, BPA at 59mg/L induced VTG production after 21 days exposure (Larsen et al., 2006). In fathead minnow, significant induction in VTG production was observed at 43 or 71 days after exposure at 460 or 160mg/L BPA (Sohoni et al., 2001).

Increase in vitellogenin levels were observed in male mummichogs (*Fundulus heteroclitus*) exposed to 4-nonylphenol, 4-tert- octyl-phenol, bisphenol-A, and 17β–estradiol (Pait, 2003) and in male Japanese medaka (*Oryzias latipes*) exposed to estradiol 200ng/L (Sun et al., 2009). Male Japanese medaka (*Oryzias latipes*) fed with methyl testosterone (MT) contaminated diet, (0.02 and 0.2mg MT/g diet) exhibited increased VTG plasma levels (Chikae et al., 2004) also induced VTG synthesis in male fathead minnow (Ankley et al., 2001; Hornung et al., 2004). The induction of vitellogenesis (i.e. oestrogenic response) was reported following intraperitoneal administration of ethyl-, propyl-, and butylparaben to juvenile rainbow trout (*Oncorhynchus mykiss*) (Pedersen et al., 2000) and medaka (*Oryzias latipes*) (Inui et al., 2003). Studies show VTG induction in fish exposed to high BPA concentrations (100– 1000mg/L or more) for about 2–4 weeks (Lindholst et al., 2000; Kang et al., 2002; Yamaguchi et al., 2005; Mandich et al., 2007).

The hatching time and fry development of the Japanese medaka (*Oryzias latipes*) were also affected by endosulfan (Gormley and Teather, 2003). Common carp (*Cyprinus carpio*) embryos treated with diazinon at levels of 0.25mg/L or
greater exhibited significant depression in their hatching rate (Aydn and Koprucu, 2005).

Hassanin et al., (2002) and Patino et al., (2003) observed the adverse effects of endocrine disrupting chemicals on GSI in common carp captured from highly polluted rivers, mostly due to combination of BPA with other EDCs, mostly estrogenic chemicals such as E$_2$, nonylphenol or octylphenol. GSI alterations in females have been reported in sheepshead minnows (Cyprinodon variegatus) exposed to 5.0mg/L 17- b-trenbolone (Hemmer et al., 2008) and in female Chinese rare minnows (Gobiocypris rarus) exposed to 16ng/L 17β-ethinylestradiol (EE$_2$) (Zha et al., 2008). Sun et al., (2009) reported that HSI was significantly increased in male Japanese medaka exposed to 17β-estradiol (E$_2$) (200ng/L).

Adverse effects of BPA on sperm quality in brown trout (Salmo trutta f. fario) shows delay in spermiation and decrease in sperm density, motility and velocity in males exposed to BPA at low dose (Lahnsteiner et al., 2005). In vitro study on the effects of HgCl$_2$ on sperm of Perca fluviatilis showed its effects through the damage of sperm cells (plasma membrane, mitochondria and axoneme) and ATP content of sperm (Hatef et al., 2011). Ova-testes were detected in male rare minnows during waterborne EE$_2$ exposure, which may result in decreased fertilization and would be expected to coincide with vitellogenin synthesis and accumulation in the livers of malefish (Zha et al., 2008).
In Japanese flounder (*Paralichthys olivaceus*), Nile tilapia (*Oreochromis niloticus*) and chinook salmon (*Onchorhynchus tshawytscha*) treatments with fadrozole during sexual differentiation caused genetic females to develop into phenotypic males (Kitano et al., 2000; Kwon et al., 2000). Exposure of zebrafish (*Danio rerio*) or fathead minnow (*Pimephales promelas*) to estrogens or androgens during early development has been shown to change the sex ratio towards more females or more males, respectively (Andersen et al., 2003; Orn et al., 2003; Panter et al., 2006; Holbech et al., 2006). The freshwater fish *Heteropneustes fossilis* (Bloch.) exposed to low concentrations of E2 (5, 25 and 100ng E2/L) demonstrated early life stages of the fish are sensitive to low concentrations of E2 leading to partial feminization of the population and to vitellogenin induction and highlight the effects on vulnerable developmental stages (Velu and Ramanathan, 2011). Exposure to 9–70μg/L prochloraz for 2 weeks induced an inhibition of the process of spermatogenesis in rainbow trout (*Onchorynchus mykiss*) (Le Gac et al., 2001). Jobling et al., (1996) reported a significant reduction in testicular growth in sexually maturing male rain-bow trout following a 3-week exposure to 30μg/L Octonyl phenol (OP).

Decrease of androgens has also been observed in juvenile turbot (Labadie and Budzinski, 2006) and adult common carp (Mandich et al., 2007). In fathead minnows, prochloraz reduces plasma androgen concentrations, suggesting inhibition of cytochrome P450-dependent enzymes, in addition to aromatase, involved in steroidogenesis and may also act as an androgen receptor antagonist (Ankley et al., 2005). Female and male fathead minnows exposed for 21 days to
the aromatase inhibitor fadrozole and prochloraz showed a decrease in mature oocytes and an increase in preovulatory atretic follicles and in males resulted in a marked accumulation of sperm in the testes and decreased plasma vitellogenin concentrations in fathead minnow females (Ankley et al., 2002; Ankley et al., 2005). Tributyltin (Cooke, 2002), and androgens such as methyltestosterone (Hornung et al., 2004) are known to inhibit aromatase activity in fathead minnow.

Accumulation of sperm in testes has been caused by aromatase inhibitors in fathead minnow (Pimephales promelas), which may be referred to increased levels of androgen steroids in plasma (Ankley et al., 2002). By Kinnberg et al., (2003) in guppies (Poecilia reticulata). In male Japanese medaka, brain aromatase activity increased due to 10 day exposure to E\(_2\) (Melo and Ramsdell, 2001). Exposure to 6:2 FTOH and 8:2 FTOH in Japanese medaka caused the activation of hepatic ER\(\alpha\), ER\(\alpha\) gene transcription was up-regulated and induced vitellogenin (VTG) (Ishibashi et al., 2008). Up-regulation of the liver’s ER\(\alpha\) response to ethinylestradiol (EE\(_2\)) or E\(_2\) has been reported in Japanese medaka (Zhang et al., 2008b), zebrafish (Martyniuk et al., 2007), fathead minnow (Filby et al., 2007) and in goldfish (Marlatt et al., 2008). Afonso et al. (2000) showed the acceleration of maturation and spawning in Coho salmon with the use of fadrozole. In black porgy, aromatase inhibitors increased LH secretion (Lee et al., 2001) but it also blocked the natural sex change (Lee et al., 2002). On the contrary, in tilapia it was possible to achieve sex reversal after treatment with aromatase inhibitors (Afonso et al., 2001). Short-term exposure to high
concentrations of MT (µg/L range) decreased the aromatase activity in male Japanese medaka (Melo et al., 1999) and fathead minnow (Hornung et al., 2004).

Decrease of Testosterone has been reported in mature male common carp exposed to 1000mg/L BPA for 14 days (Mandich et al., 2007), in juvenile turbot (*Psetta maxima*) exposed to 59mg/L BPA for 21 days (Labadie and Budzinski, 2006) and in larvae of brown trout (*Salmo trutta*) exposed to 50mg/L BPA for 63 days (Bjerregaard et al., 2008). Iwamatsu et al., (2006) reported that aromatizable T in high concentrations may induce a significant increase in E<sub>2</sub> content in embryos of medaka (*Oryzias latipes*) and also a paradoxical sex reversal. Similar observations were made in zebrafish (Orn et al., 2003). In the African catfish (*Clarias gariepinus*), in vivo treatments with androgens inhibited C17–20 lyase activity in the testis (Cavaco et al., 2001; Schulz and Miura, 2002), and MT depressed the in vitro production of T in the testis of the mummichog (Sharpe et al., 2004).

A number of studies have reported androgenic effects such as masculinization of female fish and occurrence of male-biased sex ratios in fish sampled in the vicinity of a pulp and paper mill (Parks et al., 2001; Ellis et al., 2003; Larsson and Forlin, 2002). Stimulation of growth has been reported in whitefish (*Coregonus lavaretus*) and rainbow trout (*Oncorhyncus mykiss*) exposed to pulp mill effluents (Mattson et al., 2001), and E<sub>2</sub> has been reported to have a number of metabolic and endocrine effects. Changes in external sex characteristics have been reported to be the most sensitive endocrine-disruption endpoint in fathead minnows exposed for 4 months to pulp mill effluents (Parrott
et al., 2003). Stimulatory response pattern in terms of an increased egg production has been reported in fathead minnow exposed to 1% pulp and paper mill effluent (Rickwood et al., 2006). Higher ovarian and brain aromatase activity has been reported in female mosquitofish exposed to paper mill effluents (Orlando et al., 2002), and phytosterols present in these effluents, mainly β-sitosterol, have been shown to affect the first steps of steroidogenesis in fish gonads resulting in reduced levels of steroids along the biosynthetic pathway (Leusch and MacLatchy, 2003; Sharpe et al., 2007).

Exposure to pp-DDE decreased estradiol in largemouth bass (Micropterus salmoides) females, but increased 11-ketotestosterone, the expression of vitellogenin and estrogen receptor in the liver of both sexes. Dieldrin on the other hand, decreased estradiol, 11-ketotestosterone in both sexes, altered the expression of vitellogenin and genes involved in hormone synthesis and metabolism, which dramatically lowers plasma hormone levels (Garcia-Reyero, 2006).

Mimeault et al., (2006) also reported decreased transcript levels in the hepatic pparb mRNA expression in goldfish after 14 days exposure to 1.5mg/L gemfibrozil and at 1.5µg/L or even higher concentrations of gemfibrozil (1.5mg/L) exhibited significant decreases in the levels of plasma testosterone associated with a down-regulation in the levels of star mRNA in testis (Mimeault et al., 2005). The high incidence of germ cell syncytia in the tubular lumen and the trend of increased number of spermatocytes in fish exposed to the high concentration of bezafibrate correspond to some of the criteria defined by the
OECD to determine gonadal alterations after exposure to potential endocrine disruptors (OECD, 2010). E₂ decreased mRNA levels of metabolic enzyme for steroidogenic synthesis, such as 3β-HSD, 17α-hydroxylase/lyase, and 11β-hydroxylase, in the testis of male rainbow trout (Govoroun et al., 2001).

Exposure of fathead minnows (Pimephales promelas) to ketoconazole, another imidazole compound, resulted in a doubling in ovary and testis size. The latter was associated with a compensatory proliferation of interstitial Leydig cells, which in turn was associated with maintained testis androgen concentrations.

Abnormal gonadal development, such as delayed maturation, high levels of atresia or intersexuality may also be detected histologically. Such parameters are frequently investigated in fish from contaminated environments or those exposed to anthropogenic chemicals (Jobling and Tyler, 2003; Bateman et al., 2004). Follicular atresia has been reported as a response to plant sterol exposures, in Japanese medaka (Oryzias latipes) exposed to the phytoestrogens genistein and the bioflavonoid quercetin (Kiparissis et al., 2003). Female fish from polluted areas have shown a higher incidence of oocyte atresia compared to control females (Jobling et al., 2002ab).

Male red sea bream implanted with silicone capsules containing estradiol-17β (E₂), testosterone (T) or 11-ketotestosterone (11-KT) in immature and early spermatogenic stages revealed decreased gonadosomatic index, with testicular regression in both stages, decreased circulating 11-KT levels, inhibited meiotic division of spermatogonia and progress from spermatogonia to spermatid.
Testosterone decreased serum 11-KT and LH levels, and FSHβ and LHβ mRNA levels in the early spermatogenic stage, decreased serum 11-KT levels through the suppression of FSH and LH secretion, resulting to inhibition of testicular development in the early spermatogenic stage whereas 11-KT increased LHβ and αGSU mRNA levels in immature, and decreased FSHβ mRNA levels in the early spermatogenic stage (Yamaguchi et al., 2006).

Hatef et al., (2011) reported disruption of male reproductive physiology in goldfish exposed to environmentally relevant concentrations of BPA via alternations of androgens (T and 11-KT) and VTG synthesis and sperm motility and velocity. When medaka were exposed to the environmental estrogen, o,p'-DDT, for two or for 8 weeks, fertility and hatching success were reduced (Cheek et al., 2001). Males produced the yolk precursor, Vitellogenin and when exposed male cohorts were subsequently paired with control females, a 50% reduction of fecundity was seen.

Sewage exposed adult male and female common carp (Cyprinus carpio) at four sites in Lake Mead and two reference locations in the lake have shown slightly elevated levels of Vitellogenin in males (Snyder et al., 2004).

Performing Fish Sexual Development Test (FSDT) (fertilized eggs exposed until 60 days post hatch) compounds with estrogenic (Orn et al., 2003; Holbech et al., 2006), androgenic (Orn et al., 2003; Holbech et al., 2006), anti-estrogenic (Andersen et al., 2004) and aromatase inhibiting (Andersen et al., 2004) effects were detected. The period of sexual differentiation and development
is generally considered to be the most susceptible to endocrine disruption in fish (Andersen et al., 2003; Brion et al., 2004).

Several studies in zebrafish (*Danio rerio*) have shown that estrogens such as the natural E\textsubscript{2} or the synthetic pharmaceutical 17α-ethinylestradiol (EE\textsubscript{2}) affect gonad development and have an impact on egg viability and production, fertilization success, sexual differentiation and sex ratios (Andersen et al., 2003; Brion et al., 2004; Hill and Janz, 2003; Maack and Segner, 2004; Nash et al., 2004; Orn et al., 2003; Van der Ven et al., 2007).

Exposure of zebrafish for 60 days from 24h post fertilization to 202μg/L prochloraz showed a significant (p< 0.05) bias towards male fish (77%) in the group with 12% incidence of intersex, (ranging from a few sporadic oocytes in the testicular tissue to gonads separated into larger testicular and ovarian parts) significantly differing from the 0% incidence of intersex gonads in the control group and the altered stages of the gonads at a concentration of 16 and 202μg/L, such as 17% and 25% of the females, respectively with ovaries in primary growth stage (stage I) and males at 16, 64 or 202μg/L, 88%, 79% or 80% respectively had testes categorised as stage III, with abundant spermatozoa, could be due to enhanced sperm production related to an increased androgen concentration owing to the aromatase inhibiting effect (Kinnberg et al., 2007). They have also studied significant decrease of vitellogenin at an exposure concentration of 202μg/L prochloraz in female is consistent with the aromatase inhibiting mode of action of prochloraz causing reduced endogenous 17β-estradiol levels and there by reducing
vitellogenin synthesis and in male fish at 16 or 64μg/L expressed significantly increased vitellogenin concentrations.

Dietary administration of the aromatase inhibitor fadrozole, to zebrafish caused gonadal masculinization of genetic female zebrafish when exposed between 15 and 40 days post hatch (Uchida et al., 2004), 35 and 71 days post fertilization (Fenske and Segner, 2004), 20 to 60 days post hatch thereby causing a decreased number of females and an increased number of undifferentiated fish (Andersen et al., 2004).

Velasco-Santamaria et al., (2011) studied exposure of Bezafibrate, a lipid-lowering pharmaceutical to male zebrafish at a concentration of 70mg/g for 21 days and showed significantly decreased plasma 11-KT, down-regulation in ppara and pparg mRNA levels in testis after 48h and increased expression of pparg after 21 days. They have also stated increase in the star and cyp17a1 mRNA expression in the same fish and gonadal histology revealed the presence of germ cell syncytia in the tubular lumen of fish and increased number of cysts containing spermatocytes, indicating testicular degeneration whereas zebrafish when injected with 50 and 5000mg/kg of di (2-ethylhexyl)phthalate (DEHP) exhibited alterations in the proportion of germ cells like increased proportion of spermatocytes as a consequence of disruption in the spermatogenesis probably via PPAR signaling pathways (Uren-Webster et al., 2010).

Paired adult male and female zebrafish (Danio rerio) were exposed to various concentrations of butachlor (0, 25, 50 and 100μg/L) for 30 days. Results by Chang et al., (2011) reported significant reduction in cumulative eggs
produced in the 50 and 100µg/L treatments, this effect was apparently associated with the reduced number of eggs per spawn and the reduced number of spawning events. Plasma T and E₂ levels were significantly decreased in females at 100µg/L butachlor and a significantly elevated level of VTG in males was seen in 30 days of exposure. Plasma T₄ and T₃ concentrations in males were significantly increased in the 50 and 100µg/L butachlor-treated groups and in females at 100µg/L only. Similar results were reported for polybrominated diphenyl ethers (PBDEs) which produced a significant dose-dependent increase in circulating T₃ and T₄ levels in zebrafish (Kuiper et al., 2008).

Alterations in plasma sex steroid concentrations may have resulted from several different mechanisms of action, including direct effects on steroidogenic enzymes such as aromatase, or indirect modifications associated with altered feedback loops (Mills and Chichester, 2005). Sex steroids are transported to sex steroid-binding proteins in plasma resulting in a decreased rate of steroid degradation and regulation of free sex steroids available for receptor binding in different tissues (Borg, 1994). VTG is synthesized in the liver of female fish in response to estrogens, through binding to specific estrogen receptors (Kime, 1998), however, males do have the VTG gene, and exposure of male fish to environmental estrogens or estrogen mimics can trigger expression of the gene, leading to VTG accumulation in the blood (Ankley and Johnson, 2004). Measurement of VTG levels in males or juvenile fish is one of the most commonly used biomarkers for exposure to estrogenic chemicals in the aquatic environment (Jin et al., 2008).
Exposure of 18-week-old zebrafish (Danio rerio) to 0, 0.03, 0.3 and 3.0mg/L concentrations of 6:2 fluorotelomer alcohol for 7 days showed significantly increased plasma estradiol (E\textsubscript{2}) and testosterone (T) levels in both males and females. Further, the ratio of T/E\textsubscript{2} was reduced in females while increased in males. In females, the increase of E\textsubscript{2} was accompanied by up-regulated hepatic estrogenic receptor α (ER\textalpha) and vitellogenin (VTG1 and VTG3) expression. In males, the elevation of the T level is consistent with the up-regulation of cytochrome P450 17α-hydroxylase, 17, 20-lase (CYP17) and the down-regulation of cytochrome P450 aromatase A (CYP19A). GSI and HSI was also significantly increased in both male and females at higher concentrations (Liu et al., 2009). Up-regulation of the liver’s ER\textalpha response to ethinylestradiol (EE\textsubscript{2}) or E\textsubscript{2} has been reported in zebrafish (Martyniuk et al., 2007), zebrafish exposed to waterborne E\textsubscript{2} resulted in a strong stimulation of liver ER\textalpha, while ER\textbeta was markedly reduced (Menuet et al., 2004).

Christianson-Heiska et al., (2008) reported that adult zebrafish (F0) exposed to two wood extractives, dehydroabietic acid, DHAA(50µg/l), betulinol, BET(5µg/l) and (1nM) 17β-estradiol, E\textsubscript{2} (0.27µg/L) for 3 months affected growth in terms of increased condition factor, stimulation of spawning, lowered plasma VTG concentrations in females, increased VTG in males and also the histological study revealing alterations in spermatogenic stages of F0 males exposed to DHAA and BET and in F1 females, the percentage of vitellogenic oocytes were decreased. The ovaries of these non spawning females were regressed and mean ovary somatic index (OSI) was significantly below the reference OSI determined.
in non-exposed females prior to spawning and adverse impact of EE\textsubscript{2} on male fertilization capacity and demonstrate a significant reduction in testis somatic index after exposure to 10 and 25ng/L EE\textsubscript{2} (Van den Belt et al., 2001).

EE\textsubscript{2}-exposure blunted the cortisol response of male zebrafish \textit{in vivo} and \textit{in vitro} and as well as corticotropin-releasing factor (crf) expression in the pre-optic area (POA) of the brain. While the expression of some interrenal genes was suppressed by E\textsubscript{2}-exposure in both male and female zebrafish and 11KT-exposure increased whole-body cortisol of males at rest and vortex-exposed females (Fuzzen et al., 2011).

Ecotoxicogenomics is a field that focuses on using genomic techniques (e.g. polymerase chain reaction (PCR), microarrays) to understand the molecular and cellular effects of chemicals via changes in gene expression in aquatic organisms (Snape et al., 2004). The use of genomic techniques in ecotoxicology has increased greatly in recent years, largely due to the fairly recent sequencing efforts and gene annotation for several fish species, including zebrafish and medaka. Chemically induced changes in gene expression have the potential to be used in the evaluation and hazard identification of EDCs with various modes of action (e.g. estrogens, androgens, aromatase inhibitors). EE\textsubscript{2} exposure to adult female zebrafish exhibited significantly affected gene expression, GSI, E2, T, and VTG and observed significantly affected 1622 genes in the liver (p ≤ 0.001) at either 24 or 168h. Gene ontology (GO) analysis also revealed that EE2 exposure affected genes involved in hormone metabolism, vitamin A metabolism, steroid binding, sterol metabolism, and cell growth. Plasma VTG was significantly
increased at 24, 48, and 168h (p ≤ 0.05) at 40 and 100ng/L and at 15ng/L at 168h. 
E2 and T were significantly reduced following EE2 exposure at 48 and 168h and 
GSI was decreased in a concentration-dependent manner at 168h. (Hoffmann et 
al., 2006).

EE2 functions as an oral contraceptive by suppressing the release of the 
gonadotropin, luteinizing hormone, from the pituitary (Van den Belt et al., 2002). 
Therefore, exposure to EE2 may have resulted in a reduction in gonadotropin 
release from the pituitary providing one possible mechanism that led to the 
decrease in plasma T and E2. An increase in the pro-apoptotic genes, cdkn1b and 
gadd45b, genes involved in regulating growth and proliferation including insulin-
like growth factor pre-cursor (ilgf2), insulin like growth factor binding protein I 
(igfbp1), and insulin-like growth factor 1(ilgf1) were altered on exposure to EE2. 
Genes involved in vitamin A biosynthesis, metabolism and transport were affected 
by exposure to EE2, including retinoic acid receptor alpha 2a (rara2a), several 
retinol dehydrogenases (rdh), and retinol binding protein (rbp2a).

Lei et al., (2010) demonstrated that male and female zebrafish exposed to 
20 and 50nmol/L E2 for 2 days had significantly upregulated vtg1 expression by 
34 and 41 fold and 1.2 and 1.4 fold, respectively, compared with the male and 
female controls. Short-term exposure of male zebrafish to the lowest 
concentration of methyltestosterone (4.5ng MT/L) observed increased VTG 
synthesis (Andersen et al., 2006). MT can be aromatized into methylestradiol 
(ME2) (Hornung et al., 2004) and the estrogenic E2 could presumably mediate the 
increase in the VTG synthesis via the estrogen receptor (ER) and decreased levels
of T and KT might be due to a direct inhibition of MT on gonadal steroidogenesis or through negative feedback at the hypothalamus–pituitary axis decreasing synthesis of gonadotropins in turn diminishing the synthesis of androgens in the testis or due to decreased cholesterol levels for steroidogenesis. Nash et al., (2004) have also described EE2 to increase VTG and decrease KT in adult male zebrafish. Juvenile zebrafish exposed to 26–500ng MT/L from 20 to 60 dph, VTG concentrations in whole-body homogenates decreased whereas in juvenile fish exposed to 1000ng MT/L, VTG levels returned to control level (Orn et al., 2003).

It has been shown that aromatase activity and CYP19 expression can be modulated by a variety of endocrine disrupters (Monod et al., 1993; Scholz and Gutzeit, 2000; Andersen et al., 2003; Hornung et al., 2004; Kazeto et al., 2004). Exposure to EE2 from hatch to 6 days post hatch up-regulated the expression of CYP19A2 in juvenile zebrafish (Trant et al., 2001). Trant et al., (2001) have reported that gene expression of CYP19A2 can be up-regulated in juvenile zebrafish exposed to MT (51.1 µg/L) for 6 days.

Lower fecundity in the higher endosulfan concentration (200ng/L) was seen compared with fish of the control group and lower endosulfan (10 and 50ng/L) concentrations. Additionally, a significant increase of the GSI was seen in males under the 10-ng/L treatment (p < 0.05), and the GSI value in the female fish treated with 200ng/L β–endosulfan was significantly lower than that of the control fish (p < 0.05). The HSI value of male fish in the 200-ng/L treatment group was significantly higher than that of the control group (p < 0.05) and no significant change in the HSI value was observed in the female fish. The plasma
vitellogenin levels of exposed male zebrafish were significantly higher than in the control male fish. In the 50 and 200ng/L β-endosulfan–treated fish, oocytes were detected in the testes and sperm necrosis were seen. The atretic follicles detected in the 200ng/L β-endosulfan–treated female group exhibited characteristic degenerative changes, including decreased vitellogenesis, oocyte membrane folding, and an increase in the number and size of follicular cells (Han et al., 2011). In a similar study conducted in zebrafish, no significant effect on the HSI in males or females was found, but the GSI value was significantly higher in males exposed to 0.3mg/L tribromophenol than in females (Andersen et al., 2001).

Brown et al., (2011) studied exposure of both inbred and outbred zebrafish to 43.7μg clotrimazole/L led to male-biased sex ratios compared with controls (87% versus 55% and 92% vs 64%, for inbred and outbred males, respectively), advanced germ cell development, and reduced plasma 11-ketotestosterone concentrations in males. In outbred male zebrafish following 96 days exposure to 43.7μg clotrimazole/L, proliferation of Leydig cells and plasma androgen (11-KT) concentrations were maintained at levels equivalent to controls until sexual maturity (exposure day 48), and subsequently declined. The direct effect of clotrimazole on sexual differentiation of zebrafish was indicated by the inhibitory responses seen on the steroidogenic enzyme transcripts cyp 51, hsd17b3, and cyp19a1a. The latter gene encodes aromatase, responsible for the conversion of testosterone to estradiol, which prevents oocyte apoptosis during a juvenile hermaphrodytic stage in zebrafish, thus leading to ovarian (rather than testis) development (Uchida et al., 2002).
Exposure of zebrafish to propylparaben did not affect vitellogenesis after 20 days exposure but seemed to influence the sex differentiation processes, as evidenced by a sex ratio significantly skewed towards females in the group fed 500mg/kg of propylparaben following 45 days of exposure (Mikula et al., 2009). Changes in the proportions of males and females were observed in populations of juvenile zebrafish exposed during the critical period of gonadal development, to 17α-ethinyloestradiol and methyltestosterone (Orn et al., 2003), trenbolone (Orn et al., 2006), anti-oestrogen ZM 189, 156 (Andersen et al., 2004), the fungicide prochloraz (Kinnberg et al., 2007), and bisphenol A (Drastichová et al., 2005).

Exposure of adult male and female zebrafish to 5, 10, 25 and 50ng/L EE₂ and to 12.5, 25, 50 and 100µg/L 4t-octylphenol (OP) demonstrated reduction in the number of spawning females at 10ng/L EE₂ with a complete inhibition of spawning at levels of 25ng/L EE₂, regressed ovaries, reduced ovary somatic index (OSI), adverse impact on male fertilization capacity and a significant reduction in testis somatic index (TSI) (Van den Belt, et al., 2001). Orn et al., (2000) demonstrated a reduced egg production in female zebrafish exposed to 10 and 25ng/L EE₂ from the embryo stage to the age of 4 months. Exposure of adult zebradanios (Danio rerio) to 5ng/L ethylestradiol leads to arrest in the development of eggs produced at the early blastula stages and induces vitellogenesis in exposed males (Kime and Nash, 1999).

A study on zebrafish (Danio rerio) that were exposed to the synthetic estrogen 17α-ethinylestradiol (25ng/L) found increased phagocytosis of sperm
cells by Sertoli cells in the testes as a result of this exposure (Islinger et al., 2003). Dietary exposure to another imidazole compound, fadrozole (10mg/g of food) has similarly been shown to result in oocyte apoptosis and male-biased zebrafish populations.

When zebrafish were exposed to musks (nitrated benzenes), hepatosomatic index eas increased and gonadosomatic index decreased. Exposure of parents resulted in decreased survival of offspring during early life-stage (Carlsson et al., 2000; Carlsson and Norrgren, 2004). Zebrafish exposed to xenoestrogens showed vitellogenin induction, ovotest formation and a cohort showed altered sex ratios and impaired reproductive capacity (Andersen et al., 2003; Hill and Janz, 2003).

Phytosterols disrupted sex ratios and reproduction in zebrafish (Nakari and Erkomaa, 2003). The E2 led to the disappearance of vitellogenic oocytes in the ovary and an increased area of relatively large, eosinophilic cells in the testis, identified as spermatogonia in zebrafish (Danio rerio) model systems (Van der Ven et al., 2003). Negative effect on number of sperms released, the activity of sperm as well as life span of the trails was reported in zebrafish exposed to DDT for one month (Njiwa and Muller, 2002). Schmitz et al., (2001) studied the effect of a neem based pesticide, Neemazal on the life cycle of zebrafish and found a significant reduction in fecundity after exposure of 0.63mg/L of Neemazal.

Wide range of xenobiotics, used for various purposes have interfered with development as well as reproduction effort on adult zebrafish. Significant decrease in fecundity and hatchability as a consequence of Nimbecidine and
Ultineem treatment in fishes were observed (Singh and Ansari, 2010). Influence of another neem based pesticide Azacel at a concentration of 0.10μg/L on embryo and fingerlings of zebrafish studied by Ahmad and Ansari (2011) revealed decrease in hatching success. Domoic acid, analog of excitatory amino acid glutamate has reduced hatching success by 40% at 0.4mg/kg and by more than 50% at doses of 1.2mg/kg and higher (Tiedeken et al., 2005).

Lin and Janz (2006) determined the effects of nonylphenol (NP) on zebrafish and observed suppressed gametogenesis in females at 60dpf. Significant reductions in egg viability, egg hatchability and/or F1 swim-up success, irreversible effects on egg quality and progeny even after periods of depuration. Binary mixture of a weak estrogen receptor agonist, NP and potent estrogen receptor agonist (11α ethinylestradiol) on sex ratio, gamatogenesis, Vitellogenin induction and reproductive capacity in the same fish when exposed from 2-60dph.

There was an increase (p<0.05) in the mortality of embryos and decrease in the number of larvae that were hatched compared to control, extension of hatching up to day 6 and decrease in heart beat from day 3-6 larvae exposed to 1μg/L and 5μg/L of Ibuprofen (David and Pancharatna, 2009). The same drug at 10μg/L increased mortality rate of embryos further with a corresponding decrease in the rate of hatching, bodymass and body length of the hatched larvae compared to control.

Dumitrescu et al., (2010) has studied histological changes in gonads, liver and kidney of zebrafish (Danio rerio) exposed to octylphenol from 21-115 days.
and within 21-75 days. They observed female gonads consisting only of small ovocytes, with cytoplasm loaded with primary vitellus. Disruption processes of the nephrocytes epithelium, large number of cells becoming hypertrophic, vacuolar-type cytoplasm was also observed.

Powers et al., (2010) examined the effects on survival, morphological and behavioral parameters in zebrafish embryos and larvae when exposed from 0 to 5 days post-fertilization to concentrations of silver ranging from 10nM to 100μM. They also observed delay in hatching was accompanied by persistent dysmorphology with continued formation of aggregates in the chorion and decreased embryo size, effects that were pronounced at 10μM silver and higher.

**EFFECTS OF PYRETHROID ON FISH:**

A growing number of environmental toxicants like synthetic pyrethroids are believed to have deleterious effects on the development of non-target organisms by disrupting hormone sensitive processes. LC$_{50}$ values of different forms of cypermethrin were evaluated by different research groups under laboratory conditions. The 1, 24, 48, and 96h LC$_{50}$ values (with 95% confidence limits) of cypermethrin for common carp larvae were estimated as 7.813 (2.829–33. 652), 6.196 (2.4 81–22.89 7), 2.940 (1.327–8. 125), 1.304 (0.6 12–3.3 89) and 0.809 (0.5 30–1.3 08) 1g/L 1, respectively. The 96h LC$_{50}$ value for Nile tilapia was estimated as 5.99μg/L and cited 96h LC$_{50}$ cypermethrin toxicity as 2.2μg/L for *Tilapia nilotica*, 0.9-1.1μg/L for carp (*Cyprinus carpio*), 1.2μg/L for brown trout (*Salmo trutta*), 0.5μg/L for rainbow trout(*Salmo gairdneri*), and 0.4μg/L for *Scardinius erythropthalmus*. 
Stephenson (1983) has compiled 96h LC$_{50}$ of cypermethrin on different species of fish as 2.8μg/L for rainbow trout (Oncorhynchus mykiss), 1.2μg/L for fathead minnow and 0.93μg/L for Pimephales promelas (juvenile). The LC$_{50}$ value of alpha-cypermethrin for guppies as 17.94μg/L.

Smith and Stratton (1986) reported the toxic effects (LC$_{50}$) of cis-cypermethrin on various fish species as 2.0μg/L (96h) for Atlantic salmon (Salmo salar), 6.0μg/L (96h) for rainbow trout (Salmo gairdneri), 9.0μg/L (24h) and 8.0μg/L (48-h) for mosquito fish (Gambusia affinis) and 10.0μg/L (24h) and 6.0μg/L (48h) for desert pupfish (Cyprinodon macularius). 72h LC$_{50}$ values of aqueous cypermethrin and acetone-solubilized cypermethrin to Heteropneustes fossilis were 0.67 and 1.27μg/L.

The two pyrethroids, cypermethrin and fenvalerate have been studied to know what extent they caused behavioral abnormalities in adult fish and larvae. Polat et al., (2002) observed in guppies when exposed to beta-cypermethrin as loss of equilibrium, hanging vertically in the water, rapid gill movement, erratic swimming, swimming at the water surface, staying vertically and gulping for air, prolonged and motionless laying down at the aquarium bottom, color changes to yellow in the abdominal area, turning around its axis, all fish gathering at one corner of the surface of the aquarium, keeping the gills in open position for prolonged periods were also noticed. Further defects in backbone such as bending and enlargement of the eyes.
Sarıkaya (2009) described peculiar behavior in adult Nile Tilapia (*Oreochromis niloticus* L.) as a consequence of Alpha-Cypermethrin. When the aquaria walls were tapped fish left water currents and made movements such as somersaulting around their own axis. Floyd et al., (2008) observed swimming abnormalities such as Twitching, erratic swimming or lying on one side in larvae of Fathead minnow (*Pimephales promelas*) when exposed to esfenvalerate. Pyrethroids also caused changes in hematological parameters in different fish. Cakmak and Girgin (2003) described decreased levels of haematocrit, hemoglobin, leukocytes, red blood cells, mean corpuscular hemoglobin concentration with increased levels of mean cell volume in rainbow trout exposed to cypermethrin The same pyrethroid was shown to cause significant decrease of erythrocyte count (RBC), haemoglobin content (Hb), hematocrit and increased leukocyte count (TLC), mean cell volume (MCV) and mean cell haemoglobin in *Labeo rohita* at sublethal levels (Adhikari et al., 2004). Sopinska and Guz (1998) conducted experiments with another pyrethroid permethrin and observed a decrease in total leukocyte count and neutrophil granulocyte count in carp. Further cypermethrin was reported to decrease total erythrocyte (RBC), hemoglobin (Hb), hematocrit (Ht) and mean corpuscular hemoglobin concentration (MCHC) values and increased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values in *Prochilodus lineatus* (Parma et al., 2007).

Impairment of liver integrity by cypermethrin, fenvalarate, bifenthrin have also been observed in fishes. Histopathological examination of liver in rainbow trout revealed teleagioectasiae in the secondary lamellae with the rupture of pillar cells, degeneration of hepatocytes in periportal zones with affected hepatocytes
showing pycnotic nuclei and many small or one big vacuole in the cytoplasm as well as fatty degeneration on exposure of bifenthrin (Velisek et al., 2009). In the same fish, the same research group observed cypermethrin induced teleangioectsiae of secondary gill lamellae and degeneration of hepatocytes in the liver (velisek et al., 2006). In the liver of catfish (Clarias gariepinus) histopathological changes induced by fenvalerate were cytoplasmic vacuolization of the hepatocytes, blood vessel congestion, inflammatory leucocytic infiltration, necrosis and fatty infiltrations (Sakr and lail, 2005).

Various biochemical parameters were affected by different pyrethroids in different fishes. Significant decrease in plasma ammonia, increased glucose, creatine kinase, alkaline phosphatase and lactate dehydrogenase, decreased mean erythrocyte volume, erythrocyte haemoglobin, and band neutrophil granulocytes and degeneration of hepatocytes were observed in rainbow trout fish exposed to Talstar 10 EC pesticide preparation (active substance 100g/L bifenthrin) at different concentrations (Velisek et al., 2009). The same chemical changed the respiratory rhythm and inhibited the energy metabolism of Prussian carp, bleak and perch at all concentrations (Ponepal, 2010).

Sub-lethal doses of cypermethrin significantly altered the levels of total protein, total free amino acid, in muscle and liver tissues, nucleicacids (DNA and RNA) in gonadal tissues and the activity of enzyme acetylcholinesterase (AChE), lactic dehydrogenase (LDH) and succinic dehydrogenase (SDH) in nervous tissue of the freshwater teleost fish Colis fasciatus in time and dose dependent manner (Singh et al., 2010). Ceyhun et al., (2010) demonstrated decreased activities of
enzymes glutathione reductase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase in gill, muscle, liver and kidney and increased expression of the stress-related protein Hsp70 with increasing deltamethrin concentrations and exposure time in rainbow trout (Oncorhynchus mykiss).

Various reproductive parameters were affected by different pyrethroids in other fishes. 100% mortality reached in medaka after 4 days exposure to 9.4mg/L esfenvalerate and downward trend in fecundity and fertilization success with increasing esfenvalerate concentration (Werner et al., 2002). Exposure of male mature parr Atlantic salmon (Salmo salar L.) to 0.04μg/L of cypermethrin significantly reduced or inhibited the olfactory response to PGF2α and also reduced the ability to respond to the priming effect of the pheromone (Moore and Waring, 2001).

Cypermethrin exposure caused decrease in size of gonadotrophic cells, presence of immature oocytes and atretic follicles in the ovaries and gross condensation of spermatogenic cells in testes in Heteropneustes fossilis (Bloch), Singh and Singh (2008). They have also demonstrated decrease in the gonadosomatic index (GSI), plasma levels of estradiol-17β (E2) and 11-ketotestosterone (11-KT) and sperm motility at 1 ppm concentration. On the whole cypermethrin causes inhibition of reproduction by acting at the hypothalamo-hypophyseal-gonadal axis. Jaensson et al., (2007) observed disturbance in the reproductive behaviour of the male parr of brown trout (Salmo trutta L.) on exposure to 0.1μg/L of cypermethrin. Fish displayed fewer courting events, spent less time
near the nesting females and had lower volumes of strippable milt with significantly lower amounts of 11-ketotestosterone (11-KT) in the blood plasma.

In another fish *Channa punctauts* significant changes were noticed when exposed to commercial chemical devicyprin (cypermethrin 25%) (Srivastava et al., 2008). In male fish after 15 days of initial exposure period, reduction in number and condensation of spermatogonic cells and appearance of a large number of inter tubular vacuoles were observed. A large number of inflammatory cells, further condensation of spermatogonic cells, presence of a large number of intertubular vacuoles and swelling of spermatids can also seen after 30 days exposure periods and 45 days exposure caused reduction in the number of spermatogonic cells, starry sky appearance, contraction and vacuolization of tubules, and necrosis of seminiferous tubules.

In females exposed to sub-lethal concentration of the same chemical (devicyprin) for 15 days showed oocyte with de-shaped, disrupted follicular epithelial cells, condensation nucleolus into crescent shaped dark granules at one side and degeneration of epithelial cells causing vacuolation. After 30 days of exposure periods, vacuolated follicular epithelium and more degenerative cytoplasm was reported and after 45 days of exposure the cytoplasm begins to show vacuolization at the periphery of oocyte which gradually extends towards the centre. De-shaped Yolk vesicles, stromal hemorrhage, damage in the architecture of tunica albuginea and inner germinal epithelium was reported.

Giri et al., (2000) reported the effects of insecticide basathrin induced histoanatomical defects in ovarian tissue of cat fish, *Heteropneustes fossilis*. They
reported marked damage in germinal epithelium, atresia of oocyte, stromal hemorrhage, vacuolization of oocytes and general inflammation. Anita et al., (2010) reported a decrease in protein content in the liver, muscle, brain and gill of the two carps, *Labeo rohita* and *Cirrhinus mrigala* exposed to sub-lethal concentration of fenvalerate.

Larval zebrafish exposed to 150μg/L of bifenthrin for 72h induced vitellogenin1 with approximately 5.7-fold relative to the control and with the lengthening of exposure time to 96h a significant increasing vtg1 expression was observed (Jin et al., 2009). They have also assessed that the developmental toxicity of bifenthrin has been shown to cause significant sublethal toxic effect in early life stages of zebrafish like accelerated the hatching process after 50h exposure, the percentage of hatched embryos significantly increased to 72.9%, 86.2% and 93.3% for the 50, 100 and 200μg/L group respectively. At 72h 10.9% of the 50μg/L exposed organisms presented curved body axis and a significant increase of the proportion to 35.1% was observed at concentration of 200μg/L BF and larvae staged at 96h from both concentrations had significantly higher frequencies of curved body axis compared to the larvae of 72h. At 72h, few larvae exhibited pericardial edema except for the highest concentration.

Another pyrethroid, lambda-cyhalothrin (LCT) caused curved body axis and edema in zebrafish (Xu et al., 2008). Coats (1990); Benli et al., (2009) reported highest concentration (200μg/L) of BF larvae of zebrafish as abnormal way of swimming observed under the microscope, hyper activity, fine tremors, intermittent twist and convulsions.
Jin et al., (2011) reported significant increase in mRNA level of p53 and puma in zebrafish when exposed to 3 and 10μg/L cypermethrin for three days during the embryo development, with increases of 1.96 and 1.55 fold and 2.09 and 2.16 fold respectively compared with the control group. Similarly Apaf1mRNA levels also increased significantly when exposed to 10μg/L cypermethrin for 3 days showing a level of 1.58 fold higher compared to the control and also induces apoptosis via caspase pathway. Up regulation of mRNA levels of Cas9 and Cas3 by 2.09 and 2.61 and 1.84 and 1.62 fold respectively at the concentration of 3 and 10μg/L of cypermethrin for 3 days was also shown. It also altered the mRNA levels of cytokines including IFN, CXCL-CN and CC-Chem in a dose-dependent manner, suggesting that cypermethrin exposure induced the immune response in zebrafish.

Fingerlings exposed to Lambda-cyhalothrin exhibited erratic swimming, inconsistent jumping, loss of balance and decrease in opercular beat has been reported by Ahmad et al., (2011). Ansari and Ahmad (2010) observed the aggregation of zebrafish at one corner of the aquarium, swimming erratically and loss of equilibrium, darkened body colour and expansion of pectoral and pelvic fins and fishes rolled vertically prior to death.

Fenvalerate inducing oxidative stress might be one of the most important eurotoxic mechanisms of pyrethroids (Shafer et al., 2005). Among the antioxidant enzymes, SOD is the first defensive barrier in the antioxidant system (Jin et al., 2009; Lin et al., 2009). Jin et al., (2009) studied that 7 d exposure of 250ngL\(^{-1}\) of permethin (PM) enantiomers stimulated vtg1, esrα and cyp19b expression in
embryo-larval zebrafish. Significant differences were also detected between the enantiomers in the induction of estrogen-responsive gene expression.

Fenvalerate in zebrafish embryos and larvae induced notable signs of apoptosis accumulated in the brain, alterations in SOD activity, down-regulated the expression of ogg1 and dlx2 genes indicating the oxidative-DNA repair system as well as neurogenesis were impaired and decreased expression of dlx2 gene (Gu et al., 2010).

*In vivo* study by Jin et al., (2009) in embryo larval zebrafish found that a 7 day exposure to 250ng/L permethrin racemate and its enantiomers was sufficient to stimulate vtg1, esrα and cyp19b expression and at level of 1000ng/L, the vtg1, esrα and cyp19b responses to the enantiomers were about 3.2, 1.8 and 1.5 fold higher respectively. Christinesen et al., (2005) reported that cypermethrin affected the swimming performance of zebrafish larvae.

Toxicity of deltamethrin to different fishes during larval/embryonic/adult stages at different time periods have been determined by different laboratories across the globe. (Mittal et al.,1994) reported deltamethrin to be more toxic to *Poecilia reticulata* of all the pyrethroids studied (LC$_{50}$= 0.016ppm). The calculated 48h LC$_{50}$ value (95% confidence limit) for adult guppies, *Poecilia reticulata* in a static bioassay was estimated to be 5.13µg/L (Viran et al., 2003). Mestres and Mestres (1992) determined 96h LC$_{50}$ value for *Salmo gairdneri* (0.39µg/L), *Cyprinus carpio* (1.84µg/L) and *Sarotherodon mossambica* (3.50µg/L).
The calculated 1, 12, 24, 48, 72, and 96h LC$_{50}$ values (95% confidence limits) of water-soluble deltamethrin, using a static bioassay system for fry rainbow trouts were 15.8708 (10.8550–24.2067), 7.0014 (4.3854–11.51772), 1856 (1.8438–5.1897), 1.6568 (0.7287–3.1600), 0.9800 (0.3060–1.8760), and 0.6961 (0.3184–1.6575) lgL$^{-1}$ respectively (Ural and Saglam, 2005). The 24, 48, 72 and 96h LC$_{50}$ values (95% confidence limit) for young mirror carp were 9.41 (7.13-13.70), 4.47 (3.40-6.14), 2.37 (1.84-3.06) and 1.65 (1.32-2.07) respectively (Calta et al., 2004). Golow and Godzi (1994) reported 96h LC$_{50}$ value for Oreochromis niloticus fingerlings as 14.50µg/L. In static system the 48h LC$_{50}$ value for Nile tilapia fingerlings was estimated as 4.85µg/L.

Significant increase in number of dead larvae in Cyprinus carpio from a dose of 0.005µg/L of deltamethrin and the decrease in hatching success of eggs of the same fish was studied by Kuprucu and Aydin (2004). Abnormal behavioral responses in fishes was investigated as a consequence of deltamethrin exposure. Fish hanging in water, rapid gill movement, erratic swimming, swimming at the water surface and gulping for air and prolonged and motionless laying down on the aquarium bottom were observed in guppies by Viran et al., (2003). Hughes (1993) reported that exposure of Rainbow trout to 40µL/20L of deltamethrin for 50mins showed almost incapability of swimming. Behavioural changes were also noticed in larvae of Nile tilapia, such as swimming sideways, shaking their head, spiral twist in the tail region and the fry of same fish showed turning around their vertical axis and tried to gulp air from the water surface with increased deltamethrin concentration (Benli, 2009).
Toxicant induced changes in hematological parameters are one of the early effects that could be noticed. For this reason studies have been carried out on the effects of deltamethrin on these parameters. Deltamethrin caused a significant increase in erythrocyte counts, but a small decrease in hemoglobin, mean cell volume, mean cell heamoglobin, and hematocrit of *Heteropneustes fossilis* (Kumar et al., 1999). Pimpao et al., (2007) have reported that intoxication with deltamethrin (0.1 or 0.3mg kg/L) in *Ancistrus multispinis* showed induced leukocytosis and increase in number of erythrocytes and haemoglobin after 96h exposure. Deltamethrin significantly induced Micronucleus and nuclear abnormalities such as blubbed, lobed, notched nuclei and binucleated erthrocytes with increased lipid peroxidation in *Channa punctata* after exposure to three different concentrations of technical grade deltamethrin (0.4, 0.8, 1.2, µg/L) for 48 and 72h (Ansari et al., 2009).

In another fish, *Cyprinus carpio* (Cengiz, 2006) desquamation and necrosis were very common in gill. Besides he has also shown aneurism in secondary lamellae, lifting of the lamellar epithelium, edema, epithelial hyperplasia and fusion of secondary lamella in the same tissue. Lesions observed in kidney tissue of the same fish were degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the haemopoetic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen. Hyperemia, fusion of secondary lamella, proliferation of chloride cells, rupture of branchial epithelium in gill were observed after 14 days of exposure to deltamethrin by Diana et.al., (2007) in *Carrsius auratus gibelio*. 
Srivastav et al., (2011) have shown decrease in calcium level from day 7 to day 28 and decrease of nuclear volume of aldehyde fuchsin (AF) cells from day 14 to day 28 at a concentration of 0.37µg/L in fresh water cat fish, *Heteropneustes fossilis*. Also these AF positive cells of Corpuscles of Stannius (CS) of deltamethrin treated fishes exhibited increased granules in the same fish. Studies with *Carpinus carpio* exposed to different concentrations of deltamethrin (0.5, 1.0, 2.0 and 4.0 ppm) led to decrease of phosphatidyethanolamine (PE), phosphoglyceride (PG) and phosphatidic acids (PA) in erythrocyte plasma membrane of carp. At higher concentrations of deltamethrin, elimination of phosphatidic acids, cardiolipin and marked difference in the fatty acid patterns of the phospholipids was also reported. Increased levels of saturated fatty acids, primary palmitic (16:0) and stearic acid (18:0) as well as polyunsaturated fatty acids (PUFA), especially arachidonic acid (20:4 n-6) was observed in deltamethrin exposure in carp erythrocyte plasma membrane (Kotkat et al., 1999).

A single exposure of deltamethrin (0.75µg/L) for 48h caused induced various antioxidant enzymes and non enzymatic antioxidants in kidney, liver and gill of *Channa Punctatata*. Induction of lipid peroxidation Glutathione levels and ascorbic acid content in all of these tissues were observed (Sayeed et al., 2003). *Carassius auratus gibelio* exposed to 2µg/L for 1, 2, 3, 7, 14 days exhibited decreased activity of catalases in kidney. Activities of glutathione reductase and glutathione peroxidase was shown to decrease in gill tissue with increase in Glutathione-S- transferase activity (Diana, 2007). The mRNA levels of IGF-I, IGF-II and GH - I significantly decreased with increasing concentration of deltamethrin (0.25µg/L 1µg/L and 2.5µg/L) in rainbow trout (Aksakal, 2010).
Amplification of HSP70 mRNA was shown in rainbow trout exposed to two different doses (0.3 and 0.6µg/L) of deltamethrin for 28 days (Atamanalp and Erdogan, 2010).

Studies from our laboratory have also shown that lethal and sub lethal concentrations of deltamethrin has effects on carbohydrate metabolism in *Labeo rohita*. Depletion of pyruvate level was observed on 4th day of lethal and sub lethal exposure in liver (-75.5%) (-56.8%) followed by muscle (-71.9%), (-37%) and gill (-38.5%), (-23.2%). Lactate levels were found to increase on 4th day of lethal and sub lethal exposure. Maximum elevation was observed on 4th day of lethal and sub lethal exposure in gill (+70.9%), (+33.2%) followed by liver (+66.3%), (+27.0%) and muscle (+58.1%) (+24.4%). Decreased activity levels of isocitrate, succinate and malate dehydrogenase enzymes were noticed in gill liver and muscle tissue (Rathnamma et al., 2009).

Experiments conducted by Tramujas et al., (2006) with a concentration of 6µg/L and 10µg/L of deltamethrin did not cause any significant changes in the number of eggs laid, hatching and gonadal histology. But reduction in fecundity and hatchability has been observed when in normal water after the fish were exposed to 0.016µg dm⁻³ of deltamethrin for three months (Sharma and Ansari, 2010). In another study they have shown that different concentrations of deltamethrin viz. LC₅, LC₁₀, and LC₁₅ caused significant alterations in DNA, RNA, total protein and free amino acid contents in liver, ovary and muscle (Sharma and Ansari, 2011).
They further determined toxicity tests using four concentrations of deltamethrin (0.10.0.30, 0.50 and 0.70µg/L and showed behavioral changes like fish aggregating to one corner of the aquarium, erratic swimming increased gill movement, loss of equilibrium with darkening of body colour, expansion of pectoral and pelvic fins muscle (Ansari and Sharma, 2009). Another group working with this pyrethroid have just reported LC$_{50}$ values to be 50µg/L for embryos with confidence interval value between 19-100 (DeMicco et al., 2010).

From the literature it is evident that there are no studies on the interference of Cypermethrin during the find the carps are in reproductive phase. Hence an attempt has been made to fill this gap by studying the effect on cypermethrin an steroidogenesis in the edible fish, *Labeo rohita*. 