General Consideration

Over the past two decades, there has been an increasing concern regarding the impact of man-made chemicals released in the environment that are able to interfere with the endocrine system and alter physiological functions in organisms (Vos *et al*., 2000). A large number of chemicals enter aquatic systems through a variety of direct and indirect sources; it is therefore not surprising that aquatic animal, and in particular fish are directly affected by chemicals with endocrine disrupting potential (Matthiessen, 2003). Varied pollutants from industries and agricultural processes have contributed to contamination of freshwater systems, making adverse impact on aquatic biota. Among freshwater inhabitants fishes are frequently prone and economically the most important that suffer from exposure to different toxicants in various ways, leading to major decline in their population (Wang, 2002, Kalita *et al*., 2003; Deutremepuits *et al*., 2004; Shukla *et al*., 2007; Agtas *et al*., 2007; Yoon *et al*., 2008; Despande *et al*., 2011).

Increased industrialization, urbanization, population growth and overall man's greed to over exploit Mother Nature has created a serious threat to all kinds of life in the form of pollution, which has now become a global problem. Agrochemicals and chemical fertilizers are widely used under Green Revolution to protect the crops from pests and enhance yield, thereby increasing the productivity and economical gain of the crop yield to meet the high demand for food for the fast growing population. Aquatic ecosystems that run through agricultural areas have high probability of being contaminated by runoff and ground water leaching by a variety of chemicals used in agricultural operations. Among all types of pollution, aquatic pollution is of greater concern as each and every kind of life depends on water. Application of chemical agrochemicals and fertilizers in agricultural operations and indiscriminate letting out of highly toxic industrial effluents cause severe pollution of major freshwater bodies such as ponds, lakes and rivers. Presence of agrochemicals in aquatic systems may be by different ways, but is assessed by identifying three major routes, due to which it leads to water bodies (Kosygin *et al*., 2007; Sarkar *et al*., 2008). These routes are water column, organic substrates such as mosses, algae, leaf litter, vascular hydrophytes and inorganic substrate including materials from sediments varying in size (Murthy *et al*., 2013). The toxic effects of such agrochemicals on reaching the aquatic bodies deteriorate the life sustaining quality of water and cause damage to both flora and fauna. Nature has provided only a limited magnitude of effective control

mechanisms for these chemicals. When the quantity of such chemicals reaches beyond a threshold level, the problem increases many fold and is of great concern due to the persistent toxic nature of these non biodegradable pollutants because of their bioaccumulation and biomagnifications in the food chain.

The deterioration of aquatic ecosystem by pollution has created a hazardous situation for commercially most important non-target species such as the fish and for the people who consume fish for their protein supplementation. Among the denizens of freshwater lakes, fish is the commercially most important non-target species that are adversely affected by severe aquatic pollution (Alam and Sadhu, 2001; Rao, 2004; Saraswathi *et al*., 2004; Aydin *et al*., 2005; Begum, 2005 and Pandey *et al*., 2005). Increasing awareness of the adverse effects of anthropogenic activities and pollution on aquatic environment has focused interest on health of fish populations and possibilities to utilize these health parameters for assessment of the quality of aquatic environment.

The usage of inorganic agrochemicals in controlling agricultural pests in India dates back to 1944 with the introduction of DDT by the British and American armies in Orissa and Karnataka (Sharma, 2003). With the growing awareness of the deleterious effects of indiscriminate usage of vast quantities of agrochemicals by the farmers, the usage of such agrochemicals is now dispensed with, by the farmers in India and abroad. Instead, application of bio-agrochemicals (mainly from plant products) is now on the increasing usage by the Indian farmers. However, application of inorganic agrochemicals and fertilizers is still practiced by the farmers in most of the remote villages to enhance crop production without due consideration of their ill effects environmentally. Besides, the persistent toxic effects of the already applied chemicals and agrochemicals accumulated in various ecosystems cannot be totally ruled out. The toxic effect of either newly applied pollutants together with the already available persistent toxic residues are known to affect the different fish species reared in major lakes under inland fisheries program. An understanding of the effects of pollutants on different fish species reared in the ponds and lakes is felt essential for a commercially successful inland fish breeding. The fish living in close contact with the surrounding water are separated by only a few microns of delicate gill epithelium, making it susceptible to aquatic pollutants. The scientific experiments on the toxic effects of agrochemicals substances on fish were carried out as early as in 1863 (Penny and

Adams, 1863 and Jones, 1964). With growing knowledge of physiology and biochemical informations with reference to fish, a number of researchers started investigations on the effects of agrochemicals on the different aspects of fish body.

Agrochemicals greatly reduce food organisms' abundance in aquatic bodies and ecosystem which is necessary for fish survival (Helfrich, 2009). By this mean it indirectly interrupts the fish food supply and change the habitat of water bodies (Maskaoui *et al*., 2005; Chau, 2005). Besides this it can also make the fish more susceptible to predators by decreasing habitat suitability and changing their behaviour as well, which is a direct effect as a consequence of indirect effect (Holden, 1973; Prashanth *et al*., 2011; Gill and Raine, 2014). Results have shown that these indirect effects can be much more vital than the direct ones (Murthy *et al*., 2013).

Fish is directly affected by different agrochemicals (Rao and Pillala, 2001). Agrochemicals induce different types of toxicity in fish, which these agrochemicals lead to, such as changes in fish behaviour (Scott and Sloman, 2004; Krian and Jha, 2009; Satyavardhan, 2013; Marigoudar *et al*., 2009; Ilavazhahan *et al*., 2010; Nwani *et al*., 2010; Nagaraju *et al*., 2011; Prashanth *et al*., 2011; David *et al*., 2012; Desai and Parikh 2013; Ullah *et al*., 2014; Rani and Kumaraguru, 2014), haematological changes (Bhatkar and Dhande, 2000; Svoboda *et al*., 2001; Joshi *et al*., 2002; Johal and Grewal, 2004; Gautam and Kumar, 2008; Devi *et al*., 2008; Saeedi *et al*., 2012; Ullah *et al*., 2014; Upadhyay and Parikh, 2014; Upadhyay *et al*., 2014; Sadrkarpawar *et al*., 2015; Patel and Parikh 2015), histopathological disturbances (Das and Mukerjee, 2000; Mohan, 2000; Cengiz and Unlu, 2002; Parashar and Banerjee, 2002; Dutta *et al*., 2003; Johal *et al*., 2007; Kunjamma *et al*., 2008; Velmurugan *et al*., 2009; Ba-Omar *et al*., 2011; Desai *et al*., 2011; Rani and Venkataramana, 2012; Deka and Mahanta, 2012; Sadekarpawar and Parikh, 2013; David and Kartheek, 2014; Patel *et al*., 2016), biochemical alteration, genotoxicity (Gartiser *et al*., 2001; Vargas *et al*., 2001; Hoeger *et al*., 2005; Maskaoui *et al*., 2005; Çavas and Könen, 2007; Murthy *et al*., 2013; Desai and parikh, 2013; Dey and Saha, 2014; Upadhyay and Parikh, 2014;), endocrine system disruption , nutrient profile disturbance (Palanisamy and Bhaskaran, 1995; Jha and Verma, 2002; Aguigwo, 2002; Jenkins *et al*., 2003; Borah, 2005; Zorriehzahra, 2008; Singh *et al*., 2010; Thenmozhi *et al*., 2011; Bose *et al*., 2011; Muthukumaravel *et al*., 2013; Bibi *et al*., 2014), variation in feeding biology (Chandra *et al*., 2008; Bhandare *et al*., 2011; Ravindran *et al*., 2012), changes in antioxidant defence system (Kamble and Muley, 2000; Jiraungkoorskul *et al*.,

2003; Shanker *et al*., 2005; Milaeva, 2006; Neto *et al*., 2008; Nwani *et al*., 2010; Muthukumaravel *et al*., 2013; Sadekarpawar *et al*.,2015; Patel *et al*., 2016) and alteration of acetylcholinesterase activity (Minier *et al*., 2000; Lionetto *et al*., 2003; Chandrasekara and Pathiratne, 2005; Glusczak *et al*., 2006; Rao, 2006; Glusczak *et al*., 2007; Rao *et al*., 2007; Marigoudar *et al*., 2009; Singh *et al*., 2010; Marigoudar *et al*., 2010; Joseph and Raj, 2011; Bibi *et al*., 2014). Different fish species are susceptible to these agrochemicals at different concentration. The changes in different body parts have been observed to be different than each other as well as in response to different agrochemicals. These effects have been observed in almost all parts of the fish body and systems. Table XX summarizes a list of some of the common agrochemicals along with their lethal concentrations for different fish species.

Sr. No.	Name of the agrochemicals	Test organism	Duration of exposure	LC_{50}	Reference
1.	Diazinon	*Channa punctatus*	96 hrs	3.09 ppm	Rahman *et al*., 2002
2.	Diazinon	*Anabas testudineus*	96 hrs	6.55 ppm	Rahman *et al*., 2002
3.	Dimethoate	*Heteropneustes fossilis*	96 hrs	2.98mg/L	Pandey *et al*., 2009
4.	λCyhalothrin	*Danio rerio*	96 hrs	0.119µ/L	Ansari and Ahmad, 2010
5.	Cypermethrin	*Colisa fasciatus*	96 hrs	0.02mg/L	Singh *et al*., 2010
6.	Metasystox	*Nemacheilus botia*	96 hrs	7.018 ppm	Nikam *et al*., 2011
7.	Malathion	*Labeo rohita*	96 hrs	15mg/L	Thenmozhi *et al*., 2011
8.	Rogor	*Puntius stigma*	96 hrs	7.1mg/L	Bhandare *et al*., 2011
9.	Endosulfan	*Channa striatus*	96 hrs	0.003mg/L	Ganeshwade *et al*., 2012
10.	Malathion	*Heteropneustes fossilis*	96 hrs	0.98mg/L	Deka and Mahanta, 2012
11.	Termifos	*Clarias gariepinus*	96 hrs	0.86 mg/L	Nwani *et al*., 2013
12.	Endosulfan	*Catla catla*	96 hrs	0.98 µg/L	Ilyas and Javed, 2013
13.	Endosulfan	*Cirrhinus mrigala*	96 hrs	1.06 µg/L	Ilyas and Javed, 2013
14.	Endosulfan	*Labeo rohita*	96 hrs	2.15 µg/L	Ilyas and Javed, 2013
15.	Dimethoate	*Labeo rohita*	96 hrs	24.55 µg/L	Dey and Saha, 2014
16.	λ Cyhalothrin	*Labeo rohita*	96 hrs	0.7 µg/L	Dey and Saha, 2014
17.	Karate	*Cyprinus carpio*	96 hrs	0.160 µg/L	Bibi *et al*., 2014

18.	Dimethoate	*O. mossambicus*	96 hrs	5.59mg/L	Parikh *et al*., 2013
19.	Curzate	*O. mossambicus*	96 hrs	49.6 mg/L	Desai *et al*., 2014
20.	Curzate	*Labeo rohita*	96 hrs	49.6 mg/L	Desai and Parikh 2014
21.	Imidaclorpid	*O.mossambicus*	96 hrs	0.74mg/L	Desai *et al*., 2013
22.	Imidaclorpid	*Labeo rohita*	96 hrs	0.80mg/L	Desai *et al*., 2014
23.	Micronutrient	*O. mossambicus*	96 hrs	5000mg/L	Sadekarpawar *et al*.,2015
24.	Micronutrient	*Labeo rohita*	96 hrs	4000mg/L	Sadekarpawar *et al*.,2105
25.	Pyrazonsulfuron etlyl	*O.mossambicus*	96 hrs	500mg/L	Upadhyay *et al*., 2014

Table XX: LC_{50} value of some agrochemicals for different species

Fish is considered as an sentinel organism for bio-monitoring studies and various species have been used for the same, where comet assay (Sumathi *et al*., 2001; Kushwaha *et al*., 2012), chromosomal aberration test and micronuclei assay (Saotome and Hayashi, 2003; Pantaleao *et al*., 2006) as well as DNA repair synthesis (Grummt, 2000) are being well studied (Scott and Sloman, 2004; Rani and Kumaraguru, 2014). Further in fish, DNA repairing takes place at a much lower speed than in mammals which render fish as sentinel organism for bio-monitoring studies (Gernhofer *et al*., 2001). Various studies conducted till date have shown that pollutants cause carcinogenesis (Erickson and Larsson, 2000), teratogenesis, clastogenesis and mutation in fish (Obiakor *et al*., 2012) which ultimately lead to reduced growth, malignancies, reduction in survival of fish in early life stages as well as adult stage, abnormal development and different deformities of body organs (Akpoilih, 2012).Chromosomal aberrations have been observed after exposure of fish species to different chemicals. Genotoxicity of fenvalerate resulted into various structural abnormalities in chromosomes such as chromatid break, fragment, gap, chromatid separation, deletion and ring type chromosomes *Channa punctatus* (Saxena and Chaudhari, 2010). The toxicity of different agrochemicals has been associated with changes in replication of DNA and DNA aberration that leads to mutation, and hyper-proliferation of cells due to local irritation. Ali *et al*., (2009) and Chaoudhry and Saxena (2015) assessed the genotoxic and mutagenic effects of chlorpyrifos and fenvalerate in freshwater fish *Channa punctatus* (Bloch) and have opined that micronuclei test and comet assays are the most sensitive and rapid methods for detecting mutagenicity and genotoxicity of agrochemicals. Interestingly longer exposure was associated with lower frequency of DNA aberrations. Cypermethrin caused changes in nucleic

acids (RNA and DNA) in gonadal tissue of *Colisa fasciatus* (Singh *et al*., 2010). Agrochemicals have shown disruption of immune system in fish (Bols *et al*., 2001; Maskaoui *et al*., 2005).

Agrochemicals in the form of EDCs have been reported to act as sex hormones' blockers, which leads to anomalous and atypical sexual development, irregular sex ratios, males' feminization and disturbed mating behaviour. Susceptible fish reproductive behaviour depicts its vulnerability by different pollutants such as pesticides (Hoeger *et al*., 2005). It can also alter other hormonal processes of fish like development of bones and proper thyroid functioning (Murthy *et al*., 2013). Dimethoate and Lambda-cyhalothrin showed lethal effect on Thyroid hormone of *Labeo rohita* (Dey and Saha, 2014). Agrochemicals that mimic naturally occurring hormones or are antagonistic to their modes of action are also well studied and some of the effects attributed to these agrochemicals include reduced fertility, hatchability and viability of offspring, as well as impaired hormone activity, altered sexual behavior and trigger reproductive problems (Makynen *et al*., 2000; Baatrup and Junge, 2001; Bayley *et al*., 2002; Ankley *et al*., 2003).It is likely that these agrochemicals in the form of endocrine disrupting chemicals (EDCs) affect reproduction either by disrupting the synthesis or degradation of endogenous hormones or by directly activating steroid hormone receptor-mediated gene activation pathways. For example, it is now well recognized that exposure to estradiol (E_2) results in the synthesis of specific proteins required for reproduction. Several genes that encode proteins induced by this process are the estrogen receptor (ER), vitellogenins (Vtg), the egg yolk precursor proteins, and choriogenins, which are required for making the egg membrane; Denslow *et al*., 2001; Bowman *et al*., 2000; Celius *et al*., 2000; Folmar *et al*., 2000; Funkenstein *et al*., 2000; Arukwe *et al*., 2001; Hemmer *et al*., 2001; Lattier *et al*., 2001; Lee *et al*., 2002). As reported, the estrogen mimicking chemicals increases the number of estrogen receptors, as well as induces the synthesis of Vtg which is normally present at high levels only in females undergoing oogenesis. In males the gene for Vtg is normally suppressed; however, Vtg can be induced in males by exposure to estrogen mimics. In addition to affecting the expression of Vtg and ER, they also induces an array of genes, some by direct interaction with the ER and others by alternative routes. The cascade of genes that are induced is tissue specific. For example, Vtg is produced only in the liver, yet we know that E2 targets other tissues besides the liver, such as the gonads and the brain. While the gene for Vtg is present in these other tissues, it is not induced by E_2. Other hormones such as androgens and thyroid hormones activate their own tissue specific cascades of genes. The

identity of many of those genes is limited. The two (and perhaps three) ERs, a and b (and possibly g) in fish (Hawkins *et al*., 2000; Sabo- Attwood *et al*., in press), alone or in combination may control different subsets of genes. New research indicates that E_2 mimics may bind differently to these receptors, acting as agonists in one case and antagonists in another. This complicates the issue of determining the risks of environmental exposure (Larkin *et al*., 2003). It is likely that EDCs will have their own specific gene expression profiles because they may bind with low affinity to more than one steroid receptor resulting in a complex gene activation pattern. It is clear that competition for ligands and trans-acting factors plays a large role in the molecular events that take place. If a compound can bind to both the ER and androgen receptor (AR), are both pathways induced? Or, does one pathway predominate? What happens with mixtures? Do the specific compounds in mixtures interact with each other or compete?

A way to begin to unravel this complicated system and to understand the mechanisms that might be involved is to use global, open-ended gene expression profiling experiments to determine the pathways that are affected, which give a clear view of the molecular mechanisms deployed by a particular agrochemical exposure and can help in clearly understanding the risk of reproductive health. A plethora of information is available on the toxic effects of the chemicals in fish, however very less and scanty information is available for the gene expression studies (Gartiser *et al*., 2001; Vargas *et al*., 2001; Çavas and Könen, 2007). ***Thus, understanding how specific hormones as well as the expression of the related genes are altered on exposure of the agrochemicals was a central theme of the present work.***

The genomic action of hormones is mediated by their binding to specific hormone receptors expressed in the target tissues. Such interactions lead to an increase in the production of intracellular second messengers, which are more directly responsible for the activation of the cell and the regulation of physiological processes (Nikolenko and Krasnov, 2007). The use of hormone cascades to regulate physiological processes is a strategy that has been conserved during evolution. These cascades provide a communication link between the nervous system and endocrine system in animal phyla. Thus, the role of hormones to stimulate or inhibit the activity of target cells directly or indirectly through modulation of the actions of other chemical messengers is a common strategy across animals. Teleost fish appear to posses all the nuclear receptor types fond in mammals. Due to an apparent whole-genome duplication that causes the

expansion of a large number of gene families in the teleosts lineage, followed by the retention of some duplicates and the loss of others, teleost have more nuclear receptors than most mammals (Thornton, 2003).

A review of literature and investigations on IMI toxicity on fish shows that a considerable work has been carried out as far as biochemical, histological and behavioral aspect is concerned (Padma-priya *et al*., 2012; Padma-Priya and Maruthi, 2013; Rajput *et al*., 2012; Desai *et al*.,2013; Parikh *et al*., 2014 and Patel *et al*., 2016).Genotoxic Potential of the IMI has also been studied in *Oreochromis niloticus* by Ansoar-Rodríguez, *et al*., 2015 and have reported primary DNA damage at the chromosomal level and proved the potential risk of IMI in a non-target organism. CZ is a unique cyanoacetamide, chemically unrelated to any other commercial disease control agent and the biochemical mode of action is also different. The chemical has got systemic action for cymoxanil and moderate persistancey for mancozeb (Roy *et al*., 2010). Because of its major metabolite ethylenethiourea, recently it has come under close scrutiny of health protection agencies due to its carcinogenic, teratogenic and goitrogenic effects in mammals (Ulland *et al.,* 1972; Keppel, 1971; Das *et al.*, 2009). These studies suggest that Mancozeb and Cymoxanil have been individually studied in various animal models and found to be mild to moderately toxic. Bearing studies conducted by Desai and Parikh (2013. 2014 1nd 2015), where they have reported the biochemical, Behavioural and Histological alterations on exposure of Curzate, apart from this no studies have been recorded on CZ with reference to fresh water teleost fish.

Unlike pesticides, which directly kill the organism/s, the plant nutrients may boost the growth of one organism and cause imbalance in the ecosystem leading to extinction of one or more species. Moreover, the studies conducted till date have been focused on the metal toxicity, the toxicity of the plant nutrient Librel^{TM} on edible fishes has been thoroughly studied by Sadekarpawar *et al.,* (2013.2014.and 2015). PE a herbicide is also widely used for selective post-emergence control of annual, perennial grasses and broad-leaved weeds in cereals, and is currently recommended for use on some relevant crops in over 30 countries (Singh *et al.,* 2012; Giovanni *et al.,* 2011). Due to its widespread use, it has become a potential water pollutant and presents environmental risk, especially for aquatic organisms, owing to its fairly high water solubility which result in its high mobility. It has been detected in surface and groundwater (Battaglin *et al*., 2000). Phytotoxicity of chlorsulfuron, sulfometuron-methyl and metsulfuron-methyl has been reported for higher

plants (Sabater and Carrasco, 1997). Toxicity of tri-sulfuron on aquatic organisms has been reported earlier (Baghfalaki *et al*., 2012). ***However, the toxic potential of all the agrochemicals as far as gene expression as well as alterations in the hormonal titers on fresh water teleost O.mossambicus is lacking and hence the present study was accounted to fill the gap of our understanding of the adverse effects of the agrochemicals on gene expression and alterations in the HPG, HPI and HPT axis.***

The hypothalamic–pituitary interrenal (HPI) axis is responsible for releasing corticosteroids and catecholamines in response to a stressor. Cortisol is the major corticosteroid in teleost fish and most mammals (Miller , 2006). When a fish perceives a stimulus as a stressor, the hypothalamus releases corticotropin releasing hormone (CRH) that stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH enters the bloodstream and stimulates the steroidogenic interrenal cells in the anterior head kidney to synthesize cortisol (Hontela 2003). Cortisol regulates its own production through a negative feedback loop by altering ACTH secretion at the pituitary and hypothalamus (Hontela 2006). Cortisol affects a variety of systems that regulate homeostasis (Dorval, 2003). High plasma cortisol levels indicate the animal is under acute or sub-chronic stress, while low levels indicate no stress, interrenal exhaustion or impairment of the HPI axis (Miller and Hontela, 2011; Martinez-porchas *et.al*.,2009). Several studies have corroborated the impairment in the cortisol synthesis and secretion due to the action of agrochemicals (Gravel and Vijayan, 2006;Hontela, 2006; Aluru *et al*., 2005). The impairment is either at the level of CRH, ACTH or at the level of StAR protein , and N6, 2'-o-dibutryl adenosine 3': 5'-cyclic monophosphate (dbc-AMP) (Leblond *et al*., 2001) which is the common target of many environmental pollutants ranging from pesticides to pharmaceuticals (Hontela, 2006; Martinez-porchas *et al.*, 2009). Therefore many pollutants halt cortisol secretion and even if the fish is under stress this will probably not be reflected in the cortisol response.

In the present study cortisol, level in *O. mossambicus* was found to be lower than that of control level on exposure to IMI and CZ, which were parallel with the decrease in the GR receptors (**Chapter II**) suggesting an impairment of the interrenal exhaustion or impairment of the HPI axis (Miller, 2003; Martinez-porchas etal., 2009). On the other hand, decreased levels of serum cortisol as a response of fish under toxic conditions were also reported in fish. *Fundulus heteroclittus* registered a drop in the serum cortisol level following exposure to 2 mg/l of

naphthalene concentration which was considered as a result of severe necrosis of interrenal tissues (DiMichelle and Taylor,1978). Hontela and her co-workers (1997), while investigating the impact of toxicants on fish interrenal tissues based on the *in vitro* studies, also reported inhibiting effects of pollutants on interrenal function and cortisol secretion. *Oreochromis mossambicus*, under sub-lethal thiodon exposure registered significant reductions in serum cortisol levels following 1, 6 and 12 hours of exposures (Parvatham *et al*., 2004). Similar reductions in serum cortisol level were also reported in *Oreochromis mossambicus* exposed to sub-lethal concentration of dimecron (Karthikeyan *et al*., 2004), in *Channa striatus* exposed to lethal and sub-lethal concentrations of sevin (Sumathiral, 2006), in *Labeo rohita* fingerlings exposed to endosulfan and dietary pyridoxine (Akhtar *et al*., 2010), in *Clarias gariepinus* exposed to varying concentrations of endosulfan (Ezemonye and Ikpesu, 2010) and in *Sarotherodon mossambicus* under endosulfan toxicity (Thangavel *et al*., 2010). Wedemeyer and Yasutake (1997) stated that too low levels of cortisol are indicative of interrenal exhaustion from severe stress, whereas, too high levels indicated the fish to be under chronic or acute stress. Similar reductions in cortisol level as a function of interregnal exhaustion were also reported in fish exposed to various pesticides (Parvatham *et al*., 2004; Karthikeyan *et al*., 2004; Sumathiral, 2006 and Thangavel *et al*.,2010; Swarnalatha, 2015) and during *in vitro* exposure of head kidney tissues of fish to pesticides (Leblond *et al*., 2001) and to heavy metals (Brodeur *et al*., 1997). The reduced cortisol level in the present study, together with similar reports of interrenal exhaustion and reduction in cortisol level in other fish under different types of toxic conditions could be considered as an adaptive response by the fish by way of maintaining low metabolic rate under pesticide. However, at this juncture as ACTH, StAR and 17-β hydroxylase are not being studied and hence the actual mode of action cannot be elucidated.

Corticosteroids hormones produced by adrenocortical cells in fish, synergize with TH at target tissues to promote morphogenesis (Denver, 2009). Further as reported by Terrien and Prunet (2013) TH and CS can regulate each other, in a positive or negative way depending on molecular, cellular and physiologic context. This cross-regulation is important to amplify hormone signals, regulation of hormone activity, and coordinate hormone action. Thyroid hormone disruption can also result in negative impacts on foetal brain development (Ghisari & J.Bonefeld, 2005). Plasma levels of T_3, and T_4, and T_3/T_4 ratio can be used as suitable indices for

estimating the metabolism state with respect to growth, rate of protein synthesis, and oxygen consumed by tissues (Fontainhas-Fernandes, 2000; Gad, 2008).Changes in T3/T4 ratio were just observed in the immature fish in each experiment, which was due to their greater sensitivity. Organic pollutants may decrease thyroid hormones production and change in their circulating concentrations (Hood *et al*., 2003; Teles *et al*., 2004). However, the exact mechanism of effect of these pollutants is not clear. Decreased thyroid hormones and T_3/T_4 ratio variations in regions polluted with crude oil are reported earlier. Increased deiodination, biliary excretion of thyroid hormones (Gad *et al*., 2008), negative feedback in the hypothalamo-pituitary-thyroid axis, and disruption in rate limiting function of brain and hepatic deiodination enzyme (5'-monodeiodinase) (Brown *et al.,* 2004; Yarahmadi 2016) would decrease T_3 and T_4 in plasma, and change T_3/T_4 ratios.

The present studies on *O.mossambicus* showed that exposure of agrochemicals at sub-lethal concentration induce alternations in plasma concentration of T_3, T_4 and TSH in comparison to the normal control group. In particular, possible impairment of thyroid function was demonstrated by the significant decrease in plasma T_3, T_4 and TSH levels in CZ and IMI exposed fishes. Reduction in T_3, T_4 and TSH hormone levels indicates reduced thyroid function and reduced metabolic activity. Similar results have been reported in *Salmo salar* exposed to endrin toxicity (Freeman and Sangalong, 2000). Similar reductions in thyroid hormone levels were also reported in *Cyprinus carpio* exposed to sub-lethal endosulfan (Jenkins *et al*., 2003), in *Oreochromis mossambicus* (Karthikeyan *et al*., 2004), in *Sarotherodon mossambicus* (Thangavel *et al*., 2005a) exposed to dimecron and in *Channa striatus* exposed to lethal and sublethal concentrations of sevin (Sumathiral, 2006), in fresh water fish *Clarias batrachus* exposed to IMI (Padma priya *et al*., 2014. Furthermore, occurrence of T_3 in lesser amount may indicate the rapid turnover from T_4 to T_3 usage (Ray *et al*., 2015). The regulatory pathways involved in thyroid homeostasis are numerous and complex. As a consequence agrochemicals can act at many levels in the thyroid system (Ishihara *et al*., 2003). There are at least three independent, but possibly interacting mechanisms that may explain the ability of agrochemicals to reduce circulating and tissue levels of thyroid hormones. First, agrochemicals have been shown to change thyroid gland structure, possibly directly interfering with thyroid gland function and disrupting directly the hormone synthesis in the thyroid gland (Boas *et al*., 2006; Brown *et al*., 2004; Ishihara *et al*., 2003; Schnitzler *et al*., 2011). Second, agrochemicals can target thyroid hormone metabolism.

They may affect extrathyroidal iodothyronine deiodinases, enzymes that control the conversion of thyroid hormones and are thus essential in the regulation of levels of biologically active T_3 locally and systemically (Zoeller and Tyl, 2007). It has been shown that exposure of agrochemicals increases bile flow rate as well as the biliary excretion of T_4 (Collins and Capen, 1980a). Agrochemical exposure also induces the expression and activity of specific enzymes that utilize thyroid hormones as conjugate group acceptors and increase T_4 conjugation (Klaassen and Hood, 2001). These actions facilitate T_4 clearance by hepatic metabolism, reducing the biological half-life of T_4. Finally, agrochemicals can also competitively bind to thyroid hormone binding proteins like transthyretin (TTR) in blood (Boas *et al*., 2006; Ishihara *et al*., 2003; Wade *et al*., 2002) and can potentially displace thyroid hormones from their carrier molecules. Moreover, these may interact to produce summative effects. Besides these direct effects, indirect effects via disruption of thyroid hormone receptors and accessory proteins that directly control the gene expression through thyroid hormone responsive elements can also interfere with the thyroid system (Blanton and Specker, 2007; Ishihara *et al*., 2003).

Results of the present work confirm the observations of the previous authors and the reduced thyroid titers on exposure of CZ and IMI is possibly mediated through either direct effects or through indirect effects through receptors. The increase in T_3, T_4 and TSH levels in the plasma of *O. mossambicus* following PE exposure probably suggests the onset of an increased metabolic activity triggered by the pesticide exposure. The main metabolic pathways for thyroid hormones are deiodination, glucuronidation and sulfation (Brown *et al.,* 2004). Iodothyronine deiodinase are enzymes involved likewise in the activation of thyroid hormone. In fish, apparently more than in other vertebrates, these important thyroid hormone transformations are controlled outside the thyroid and occur mainly in peripheral tissues (liver, brain, kidney, gill) as proposed by Schnitzler *et al*., (2011), an increased titer of the thyroid hormones on PE exposure is probably routed through the peripheral tissues, and hence, the tissue specific thyroid titer will confirm and shade more light.

Agrochemicals at low concentrations may act as mimics or blockers of sex hormones, causing abnormal sexual development, feminization of males, abnormal sex ratios, and unusual mating behavior. The unique plasticity of sex differentiation in fish suggests that these animals may be very susceptible to disruption of sexual characteristics by pollutants (Murthy *et al*., 2013). Many

studies show a direct relationship between concentrations of pesticides and related chemicals in fish gonads and alter the hormone concentrations. Agrochemicals are reported to cause degenerative changes in gonads and arrest gametogenic processes either by acting directly on the gonads or by interfering with the secretary activity of the hypothalamo hypophyseal-gonadal/thyroid axis that regulates various reproductive events. Secretion of hormones such as gonadotropin-releasing hormone (GnRH), gonadotropin, growth hormone, adrenocorticotropic hormone (ACTH), testosterone, estrogens, 17,20β-dihydroxyprogesterone and thyroid hormones are in general lowered, leading to cessation of gametogenesis, vitellogenesis, oocyte maturation, ovulation, spermiation, etc. Adverse effects of pesticides have also been demonstrated on fecundity, fertilization, hatching, and postembryonic development. The effects are highly variable and depend on the nature, dose, and mode of application of the pesticides (Lal, 2007).

Ecotoxicological manifestation of a commercial pyrethroid, devicyprin, (cypermethrin- 25%) on gonadal impairment in freshwater edible fish, *Channa punctatus* has been studied by Srivastava *et al*., 2008. These substances have multiple modes of action since they can potentially act on the synthesis, secretion, transport, action and elimination of endogenous hormones (Segner *et al*., 2003). The reproductive system in vertebrates is regulated by the hypothalamic-pituitary-gonadal (HPG) axis, where the HPG axis begins in the hypothalamus and serves as the integration center through the release of gonadotropin releasing hormone (GnRH) into the hypophyseal portal system which stimulates the synthesis and secretion of the gonadotropins (luteinizing hormone (LH) and follicle stimulating hormone (FSH)) from the anterior pituitary gland (Plant, 2015). In females, LH exerts its function on the ovaries to promote ovulation; while FSH promotes follicular maturation and the synthesis of ovarian estrogens (Wang *et al*., 2015). In males, LH acts on Leydig cells to promote the synthesis and secretion of testosterone, while FSH binds to Sertoli cells in order to promote spermatogenesis (Jin and yang, 2014).

Estradiol, progesterone, and testosterone produced by the ovaries and testes regulate the positive and negative feedback loops for proper reproductive function. Estrogens are steroid hormones found in representatives of all classes of vertebrates, including fish, amphibians, reptiles, birds and mammals (Lange *et al*., 2003), 17b-estradiol (E_2) is the form responsible for most biological activities, such as its involvement in the control of sexual differentiation, maturation and reproduction. However, these hormones also exert numerous other effects on the development,

differentiation and homeostasis of diverse target organs. Estrogens are also involved in the control of the cell cycle and proliferation. Estrogens act via two main mechanisms: genomic via nuclear receptors and non-genomic that takes place through membrane receptors (Acconcia *et al*., 2005; Kelly and Levin, 2001; Loomis and Thomas, 2000). Genomic estrogen actions require a relatively long time (from hours to days) to be accomplished. However, more rapid effects (from seconds to few minutes) also exist, referred to as ‘‘non-genomic signaling’’ (Falkenstein *et al*., 2000). Several enzymes are involved in the process of estrogen biosynthesis. At the rate-limiting step, androgens are converted into estrogens via aromatization. This reaction is catalyzed by an enzymatic complex formed by the cytochrome P450 aromatase, a heme binding protein encoded by the cyp19 gene (aromatase CYP19), functioning in combination with the flavoprotein, NADPH-cytochrome P-450 reductase.

A variety of agrochemicals, many of which are in use throughout the world, have been shown to affect (generally inhibit) in vitro aromatase activity in different vertebrate systems (Heneweer *et al*., 2004; Mason *et al*., 1987; Sanderson *et al*., 2002; Vinggaard *et al*., 2000; Zarn *et al*., 2003). Controlled experimentation has demonstrated that aromatase inhibitors can produce profound effects on the reproductive endocrine system and spawning success of fish (Ankley *et al*., 2002), and recent studies suggest that altered steroidogenesis is associated with adverse effects observed in fish from the field (Lavado *et al*., 2004; Noaksson *et al*., 2003). Studies conducted by Diamanti-Kandarakis *et al*., 2009 have opined the agrochemicals in the form of EDCs, as they interact with the steroid hormones as analogs or antagonists and have suggested that these compounds are EDCs as well as more generalized toxicants. In line of these, the present studies have obtained a significant increase in estradiol and testosterone on exposure of CZ which is agreement of the work of Anlkey *et al*., (2005) along with the other scientists who have reported that fungicides may act as both aromatase inhibitors and androgen agonists (Noriega *et al*., 2005; Vinggaard *et al*., 2000, 2002, 2005). In contrast, the exposure of IMI and MN resulted in increase in testosterone titer and a decrease in estradiol indicating that both the agrochemicals may be acting on aromatase (CYP19) and thus can be regarded as EDCs as it has resulted in reducing the estradiol levels (Villeneuve *et al*., 2006; Shalaby *et al*., 2007; Argemi *et al*., 2013; Reyes *et al*., 2014). Exposure of PE resulted in a decrease in testosterone titer with a concomitant increase in estradiol, probably by stimulating the CYP enzymes, in addition to aromatase, involved in steroidogenesis (Wilson *et al*., 2004; Jensen *et al*., 2004; Leino *et al*., 2005; Ankley

et al., 2002; 2005 and 2009). Overall, the set of responses suggests that the PE is androgen antagonists and estradiol agonists, whereas, IMI and MN are androgen agonists and estradiol antagonists and the mode of action of CZ suggest that it is agonists to estradiol as well as testosterone, however, it does have a profound effect of the thyroid titer. ***(Chapter I).***

Agrochemicals as EDCs can act at multiple locations in the HPG-axis (Xi *et al*. 2011; He *et al*. 2012). For example, bisphenol A (BPA), a ubiquitous synthetic EDC causes up-regulation in Kiss-1, GnRH, and FSH mRNA in the brain and pituitary of mammals. In the gonads, it causes inhibition in testicular steroidogenic enzymes and increased expression of aromatase (CYP19) in the ovaries. Gonadotropin subunit genes are up-regulated in the brain and pituitary of adult fish exposed to BPA (Rhee *et al*. 2010). Transcripts and gene regulation in the brains of fish and mammals change in response to many EDCs (van der Ven *et al*. 2006; Zhang *et al*. 2008; Villeneuve *et al*. 2009; Rhee *et al*. 2010; Tian *et al*. 2010; He *et al*. 2012). Miaserin, like several other antidepressants, has estrogenic activity (van der Ven *et al*. 2006). This compound interferes with the serotonergic and adrenergic networks within the brain of zebrafish. Japanese medaka (*Oryzias latipes*) responds to EE_2, a component of pharmaceutical birth control, by downregulating the GnRH receptor I in the brain of males (Zhang *et al*. 2008). This causes changes in male behavior such as reduced competition for females and changes in nest protection that can lower fecundity. Female medaka respond to the anabolic steroid 17β-trenbolone (TRB) with up-regulation of GnRH receptor II in the brain. The fungicide fadrozole affects the transcription profile of the zebrafish (*Danio rerio*) brain, causing neurodegenerative stress, which leads to impairment of the endocrine system (Villeneuve *et al*. 2009).

Monocrotophos, an organophosphorus pesticide, also increases the expression of FSH beta-subunit mRNA in goldfish (*Carassius auratus*), increasing secretion of FSH, while decreasing the expression of LH beta subunit mRNA expression and secretion of LH. This changes the events of follicle maturation and steroidogenesis in the ovary downstream processes (Tian *et al*. 2010). Exposure to oil sands process-affected water increases gene expression for gonadotropins in the brains of fathead minnow (He *et al*. 2012). Many of these and other EDCs also affect the gonads, often through interference with steroidogenic enzymes. Fadrozole is another EDC that impacts the HPG-axis at numerous sites (Ankley *et al*. 2002; Villeneuve *et al*. 2009). Fadrozole is a fungicide that is well established in toxicology as an aromatase inhibitor. It also changes

gene expression in the ovary and follicle maturation is reduced due to vitellogenesis impairment in the liver. MCP works as an EDC in female goldfish by increasing gonadal expression of aromatase mRNA (Tian *et al*. 2010). This causes increased production of E_2 and changes the ratio of E_2:T in the plasma. OSPW exposed male fathead minnow up regulate gonadotropin receptors in the gonads, as well as genes for enzymes.

Teleost particularly *O.mossambicus* are known to have three isoforms of *GnRH (GnRH-I ,GnRH-II ,GnRH-III)* that are distributed in various tissues, till date *GnRH-I* is identified to regulate HPG (Nocillado and Elizur 2008; Maruska and Fernald 2011; Sempere *et al*., 2012) and that GnRH I neurons are known to be regulated by kiss 2 neurons of discrete nuclei of hypothalamus in some teleost (Oakley *et al.,* 2009), thus intimately regulating each other (Parhar *et al.,* 2004; Clarkson *et al* .,2010). In the present study, of all the agrochemicals exposed, the expression of GnRH-I was found to be up regulated in MN, CZ and IMI exposed group with a significant down regulation except in PE exposed group compare to control. In fish GnRH-I is known to be regulated by negative feedback of circulating hormonal levels through its receptor located in hypothalamus and pituitary (Weltzien *et al*.2004, Zohar *et al*. 2010). PE being a herbicide belonging to the group of Sulphonyl urea has elevated the levels of hormones (Estradiol and Testosterone Chapter I) which directly confirms the mechanism, suggesting that the GnRH-I fibers present in the pituitary, probably through its primary hypophysiotropic role has a strong correlation between GnRH-I expression in brain and gonadal activity. Thus, our results are in agreement with the results of Khan *et al.,* (2001) where atlantic croaker was exposed to aroclor 1254, Piazza *et al.,* (2011) where fish larvae was exposed to endosulphan.

Kiss2 mRNA expression was also found to be significantly up regulated under the exposure of MN, IMI and CZ, implying that it is exerting its effect by up-regulating GnRH-I and Kiss2 neurons. Furthermore, MN exhibited the maximum alteration in the Kiss2 gene expression pattern, possibly due to the nature of MN, which is an amalgamation of trace metal ions (Zn^{2+},Fe^{2+},Cu^{2+},B^{+},Mn^{+}) proposing the synergistic or individual action of metal ions (Brian *et al*., 2005, Correia *et al*., 2007; Filby *et al*., 2007; Finne *et al*., 2007; Zhang *et al*., 2009; Mebane *et al.,* 2012; Sadekarpawar *et al.,* 2015). IMI belongs to neonicotinoid group, is known to exert its effect by blocking the actylcholinesterase activity in brain. In the present study, the increase in GnRH-I and Kiss2 proves the genotoxic potential of IMI in altering the activity of gonads and

thereby on reproduction apart from its usual mode of action (Andersen *et al.,* 2004; Kitahashi *et al*., 2009;Desai and Parikh 2013; Bharadwaj and Sharaf 2014; Gibbons *et al.,* 2015; Crosbya *et al.,* 2015; Ansoar-rodriguez *et al*., 2015; Dang *et al.,* 2015). However, these responses, although critical in understanding the mechanism of agrochemicals, need to be fully examined and integrated in a broader system to support more reliable prediction of it.

Phosphotidol Inositol (PI) and Protein kinase C (PKC) are important factors in downstream signaling pathway. Inhibition of PKC has been reported to block the gene activity, proving its role at transcriptional level (Ghosh and Ray 2012). GnRH activates multiple signal transduction pathways such as Ca^{2+} and cAMP signaling through binding to GnRH-R (Ruf *et al*., 2003; Millar *et al*., 2004), which stimulates phospholipase C to generate inositol trisphosphate and diacylglycerol. Increases of these signaling messengers lead to activation of protein kinase C (PKC) and also an increase in intracelullar Ca^{2+} concentration. These two secondary signal mediators are involved in GnRH-induced GTH release and synthesis (Klausen *et al*., 2002; Ando and Urano 2005). An up regulation of PKC gene (IMI, MN and CZ) and down regulation (PE), thus indicate the multiple signaling pathway through which the agrochemicals are altering the expression. Our results are parallel with earlier reported work of Yaron *et al.,* 2003; Ando and Urano 2005; levavi-Sivan *et al.,* 2010 where they have reported the molecular mechanism of divergent physiological strategies of reproductive success in various teleost.

Kisspeptins are a group of peptides that stimulate GnRH release and are required for puberty and maintenance of normal reproductive function. Studies in teleosts have revealed the presence of multiple kisspeptin forms (Kiss1, Kiss2) in the brain. It has been suggested that there is a double site of Kisspeptin action in the brain, either in the hypothalamic-hypophyseal region or in the median eminence, an area located outside the blood brain barrier (Nocillado *et al.,* 2008). The important role of Kiss 1 has also been established to regulate gonadotropin secretion, confirming the pivotal role in regulation of reproduction (Akazome *et al*., 2008). Neurons expressing kissspeptins are key players in controlling the cyclic activity of the reproductive axis, possibly by activating GnRH neurons (Roa and Tena-Sempere 2007; Tena-Sempere 2010; Escobar *et al*., 2013). Expression of kisspeptins and its receptors exhibits interspecies variation (Escobar *et al*., 2013). In line of this, the results of the present study where, mRNA expression and western blot analysis have confirmed the expression of kiss 1 in *O.mossambicus* for the first time. A

significant down-regulation of kiss 1 under PE exposure, suggests its non-essential role (unpublished data) for reproduction (Servili *et al*., 2011; Ogawa *et al*., 2012 Tang *et al*., 2015). However, the exact mechanism by which it happens is still illusive. Immuno-histochemical and cloning studies will be able to shed more light on the same. These results thereby suggest that of all the agrochemicals; PE, IMI and MN are capable of interfering with kiss 2 and GnRH system thereby altering the HPG axis.

The endocrine system functions due to the presence of hormone interaction with cognate receptors (Casals-Casa and Desvergne 2011). The receptors are classified as membrane-bound receptors (GtH Ir and GtHIIr) and nuclear receptors (ER I, ER II, ARI and AR II). The membrane-bound receptors typically bind peptide hormones, while the nuclear receptors bind small lipophilic hormones including the sex steroids. *GnRH-I* upon activation acts on cells of anterior pituitary, thus initiating the release of GtH-I (FSH like) and GtH-II (LH like) peptides. This in turn binds to its receptor (GtH-Ir, GtH-IIr) present either on gonads (Yaron *et al.,* 2003; Chen and Fernald 2008; Maruska and Fernald 2011). Receptor profile of GtH-Ir and GtH-IIr was found to be up regulated on IMI exposure; whereas CZ exposure resulted into up regulation of GTH Ir , while MN exposure produced an up regulation of GTH IIr. An up regulation in GtHs suggest either an operation of kiss 2 or PKC mediated pathway leading to an increase in GtHrs culminating into either vitellogenesis/spermatogenesis in the gonads (Yousefian and Mousavi 2011). Our results are in accordance with earlier reports in zebra fish (Hoo *et al*.,2003; Kitahashi *et al*., 2009).

Many EDCs are small lipophilic compounds and are capable of interacting with nuclear receptors (Casals-Casa and Desvergne 2011). This often causes changes in gene expression of the hormones involved in steroidogenesis. Agrochemicals in the form of EDCs often target receptors as either agonists, which mimic a naturally occurring hormone or antagonists and blocks the action of a naturally occurring hormone. These can affect receptors throughout the HPG-axis. Some of these chemicals disrupt a variety of hormone regulated physiological pathways, including reproductive responses mediated by ER and AR in vertebrates (Bowman *et al*., 2002; Larkin *et al*., 2003; Johnston 2013;Nelson *et al*., 2013; Baker and Hardyman 2014). Both estradiol and androgens have been shown to directly regulate pituitary expression of gonadotropin subunit in several fish species (Huggard-Nelson *et al*., 2002). *ER-I* and *ER-II*

analogous to mammalian estrogen receptors α and β (Nagler *et al.,* 2007; Guiguen *et al.,* 2010; Nelson and Habibi 2013) were studied in ovary, brain and testes. Among all the agrochemicals exposed, there was significant up regulation of *ER-I* in ovary, brain and testes of IMI and MN exposed groups, possibly governing the action by some downstream signaling mechanism (Selin *et al.,* 2009), confirming the endocrine disrupting action of these chemicals. *ER-II* did not show same pattern of regulation as CZ and PE exposure resulted in a significant up regulation of *ER-II* mRNA in brain and ovary, while IMI accredited the higher expression of *ER-II* only in brain tissue. CZ being the mixture of cymoxanil and mancozeb, has resulted into constitutive receptor activation leading to its up regulation (Villeneuve *et al*. 2009; Coumailleau *et al.,* 2015), which may be due to its mimicking action as that of estrogen. Apart from the conventional studies done on various groups of herbicide (Parikh *et al.,* 2014), very few studies are accounted for the negative effects of PE on any organism. PE which belongs to the group of sulfonylurea too expressed the parallel effect as that of CZ, suggesting its mimicking role to that of estrogen which was well supported by an increase in plasma level of 17- β estradiol (chapter I). Our result are parallel to the result of Kim *et al.,*(2014); Servili *et al.,* (2011). In teleost estrogen signaling is mediated through three ER subtypes and each subtypes is likely to show differential responses (cAMP, MAPK, directly activation of transcription factors) to ligands which ultimately results in a deleterious effect on those pathways to affect the physiological functions. In the present study, there was a down regulation of MAPK suggestive of activation of either of the two canonical pathway (Cabas *et al*.,2013; Tohyama *et al.,* 2015).However, full elucidation of mechanistic molecular pathways by which agrochemicals are modulating the estrogen signaling, requires a better understanding of distinct roles of each ER subtypes.

In the present study, there was an up regulation of AR on exposure of all the agrochemicals, however significant up regulation was noticed only in CZ and IMI exposed fish probably impairing the reproduction in testes and ovary, which leads to 17-β estradiol (E_2)/ 11-keto testosterone (11kt) imbalance. (Kubota *et al*., 2003; Loutchanwoot *et al*., 2008; Martinovic *et al*., 2008; Eustache *et al*., 2009; Hatef *et al*., 2012; Golshan *et al*., 2014,2016).

In all vertebrates, gluco-corticosteroids play a key regulatory role in stress responses, growth and general metabolism, reproduction and immunity (Stolte *et al.,* 2008). Studies have investigated the role of cortisol with GR and/or MR in fish osmoregulation, primarily through

pharmacological approaches; however, some of the results are conflicting. Genome duplication event occurred in teleost fish (Jaillon *et al.,* 2004), leading to two distinct GR genes (Bury *et al*., 2003; Greenwood *et al*. 2003; Bury & Sturm 2007; Stolte *et al.,* 2008). Transcripts encoding cortisol and its associated receptors are maternally deposited as an indication of major developmental necessity (Alsop *et al*.,2008). In the present work, focus was mainly on the GR, as it has high affinity to bind its peptide cortisol (Basu *et al*. 2003**;** Shelly *et al.,*2013). Indeed, several studies have shown that the xenobiotics disrupt cortisol and its receptor response to stress by targeting multiple sites along the HPI axis, including impaired steroidogenesis and brain glucocorticoid signaling (Aluru *et al.,* 2004; Hontela, 2005; Vijayan *et al.,* 2005; Aluru and Vijayan, 2006).In the present study, tissue specific receptor expression was studied under the exposure of agrochemicals, among which, significant damage was encountered by IMI, which down regulated the mRNA of GR in all the organs followed by CZ in ovary and testis only. The exposure of IMI and CZ resulted in sensitization of cortisol receptor and may be its peptide (cortisol), substantiating the receptor down regulation probably due to its self regulation (Sathiyaa and Vijayan 2003**)**. Thus, the mechanism that may be operative is the increased in the cortisol peptide which binds to its receptor leading to activation of other growth regulated transcriptional factors and thus maintaining the physiological stress caused by the agrochemicals (Gravel and Vijayan 2006).Moreover, earlier studies have been already established the primary, secondary and tertiary responses of these agrochemicals (Upadhyay *et al*., 2014; Sadekarpawar *et al*., 2015, Patel *et al*., 2016).

In common with the reproductive steroid hormones, the synthesis and release of the thyroid hormones is under the control of a central Hypothalamus-pituitary-thyroid (HPT) axis (Scholz and Mayer 2005; Eales, 2006, Blanton and Specker, 2007 and Zoeller *et al*., 2007) .Thyroid stimulating hormone is released by pituitary is the primary physiological regulator of thyroid gland function, stimulating thyroid hormone synthesis/ release, and exerting trophic effects on thyroid tissue. In teleost two isoforms of TSHr (TSH-αr and TSH-βr) have been established. In line of this, TSH-βr expression pattern was studied in thyroid tissue under the exposure of agrochemicals, where IMI exposure resulted in a significant increase, while PE, CZ and MN exposed groups showed non-significant down regulation. Our results are in agreement with the work of Ghisari *et al*., (2015) where they have checked the effect of 13 pesticides on thyroid profile *in-vitro* and Rossi *et al*., (2007) ; Picchietti *et al*., (2009) who have reported interference

of DDT metabolites with TSH receptors on thyroid follicular cells. Overall, this study indicates that gene expression is a promising tool for assessing the biological condition of fish exposed to agrochemicals.

The following schematic diagram summarizes the possible mode of action of agrochemicals:

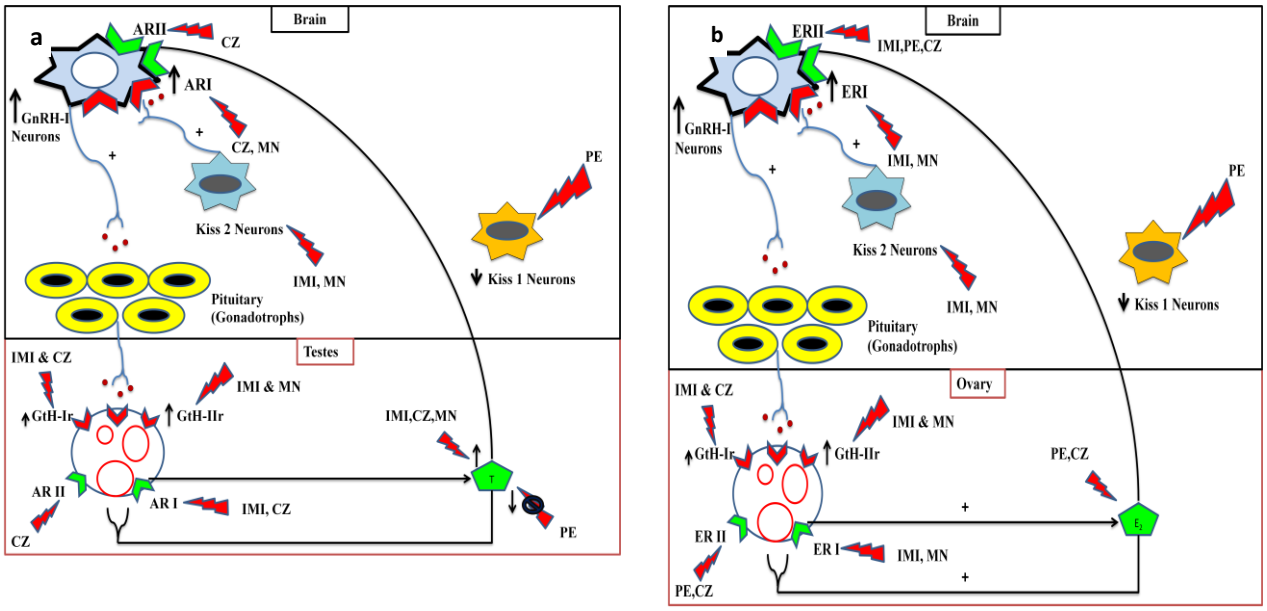

Figure XIII (a,b): Schematic representation of Hypothalamus-pituitary Gonadal Axis (HPG) under the exposure of Agrochemicals (PE-Pyzosulfuron ethyl, IMI-Imidacloprid, MN-Micronutrient Mixture & CZ-Curzate). GnRH-Gonadotropin releasing Hormone, Kiss-Kisspeptin, GtHIr& GtHIIr-Gonadotropins receptors, ERI and ERII-Estrogen receptors , ARI and ARII-Androgen Receptor, E_2-Estradiol,T-11-ketotestosterone.

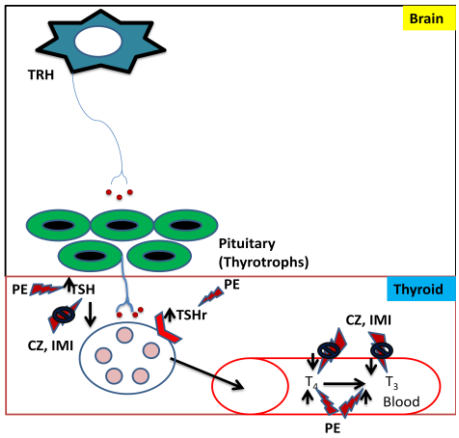

Figure XIV: Schematic representation of Hypothalamus-pituitary thyroid Axis (HPT) under the exposure of Agrochemicals (PE-Pyzosulfuron ethyl, IMI-Imidacloprid, MN-Micronutrient Mixture & CZ-Curzate). TRH-Thyrotropin releasing Hormone, TSH-Thyroid Stimulating Hormone, T_4-Thyroxine, T_3-Triiodothrionine.

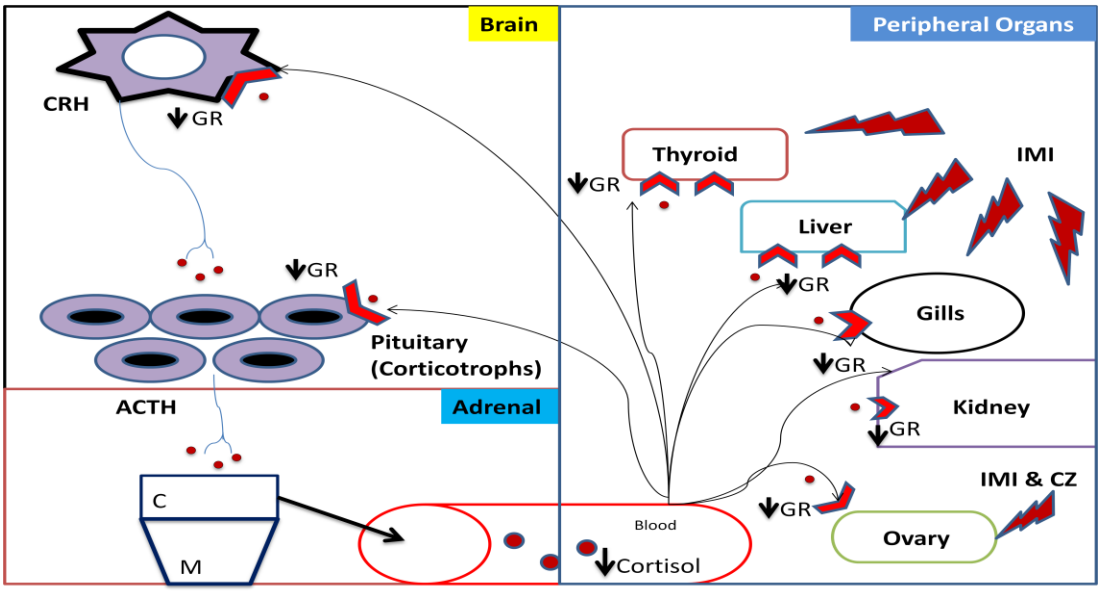

Figure XIV: Schematic representation of Hypothalamus-pituitary interenal Axis (HPI) under the exposure of Agrochemicals (PE-Pyzosulphuroethyl, IMI-Imidacloprid, MN-Micronutrient Mixture and CZ-Curzate). CRH-Cortocotropin Releasing Hormone, ACTH-Adrenocorticotropic Hormone, GR-Glucocorticoid Receptor, C-cortical region, M-medullary region

Future prospects /recommendations:

The information gathered from the present investigation is thus valuable and has probed the extent to which the agrochemicals have an impact at genetic level. These data can be especially important in aquatic systems for which the fate and effect of many chemicals are largely unknown.

The gene expression studies have postulated the possible targets of agrochemicals; however with the help of advance technologies the exact target based mechanism can be validated:

- Like microarrays can be done because they gauge changes in the expression of a large suite of genes, allowing simultaneous screening of multiple biological processes. Thus, gene microarrays will help to detect biological responses.
- Tissue localization of candidate markers can be done with the help of Immuno-histochemistry which will throw more light on the detection of biomarker in *O.mossambicus* on exposure to the agrochemicals.

- Sequencing analysis can be done to screen out the possible point mutations occurring in the promoter region of the genes. Further, *in-situ* hybridization can be employed for specific localization of DNA/RNA sequences to screen out possible mutations.