3. LITERATURE REVIEW

3.1. Pesticide in aquatic ecosystems

The aquatic environment is the ultimate sink for many of those chemicals as the end stage collectors due to direct discharges of domestic and industrial wastes, urban and agricultural runoff, discharges from ships or hydrological and atmospheric process. The aquatic environment covers 70% of the earth, can neutralize some chemical wastes, however, many of the chemicals introduced into the environment during the last 50 years are highly persistent compounds such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), pesticides, alkyltin compounds and heavy metals (i.e. Pb, Hg, Cd, etc.). Because of their chemical stability and persistency, they tend to accumulate in the different compartment of the aquatic environment. The result is the exposure of the aquatic organisms to highly toxic chemicals (Havelkova *et al*., 2007).

Since pesticides are indispensable for agriculture to curtail the damage of crops, a wide range of hypotheses about the effects of pesticide on aquatic ecosystems should be considered, including impacts on ecosystem structure and function and its interactions with the abiotic environment. The main points regarding the ecological consequences of pesticide contamination are: 1) temporary and reversible reductions in plant productivity, 2) damage to the community structure of macrophytes and reduction of its ability to sustain populations of other organisms, 3) adverse effects on survival, growth, and/or reproduction of herbivores and carnivores, including fish (Solomon *et al*., 1996). In cases where pesticide seems not to be directly toxic to aquatic fauna, indirect effects through alterations of the algal food resource may provide the best explanation for observed faunal population and community responses in natural systems (Denoyelles *et al*., 1982).

In a developing nation like India where the agricultural sector is vast and occupies a pivotal role in the economy, the nation runs at a high risk of problems related to pesticide contamination. Positive detections of pesticides have been made in water samples of river Kaveri (Rajendran and Subramanian, 1997), sediment samples of lake Kolleru, Andhra Pradesh (Rao and Pillala, 2001), drinking water in Delhi (Mathur *et al*.,2003) and river water and fish samples of river Ganga around Kolkata (Aktar *et al*., 2009). Although the trend of pesticide accumulation differs between species and tissues depending on the fat content, yet residues tend to accumulate and its toxicity can be biomagnified (Kannan *et al*., 1995).

In a study conducted by Malik *et al*. (2007), organochlorine pesticide residue, viz. DDT metabolites were detected in fish muscle samples of river Gomti in Uttar Pradesh showing contamination of the river with persistent organochlorines reflecting present or earlier usage of organochlorine pesticides. Kaur *et al*. (2008) have also reported organochlorine pesticide residue in Indian major carps and exotic carps in Punjab showing inland water contaminated by the use of pesticides in the agricultural fields.

North Bengal is one of the major tea growing areas of India and comprises of vast stretches of tea plantations particularly in the areas of Darjeeling, Terai and Dooars. Bishnu *et al*. (2009) in their study reported the presence of pesticide residues in tea soil and water bodies of the Dooars region, showing that these plantation areas serve as primary source of contamination of water bodies in this region. Pal *et al*. (2011) have also reported the presence of pesticide residues in the sediment and water of rivers flowing through the tea gardens from the Terai regions of Darjeeling district. Similarly, Singh *et al*. (2015) also reported the presence of chlorpyrifos, ethion and dicofol residues in the sediment and water of Deomani river flowing through the tea gardens of Terai region of West Bengal along with the presence of pesticide residues in the muscles of fish *Puntius sp*. collected from the river.

According to Hall *et al*. (2001), there are presently 900 pesticide products and 600 active ingredients in the market. Out of the millions of tons of pesticide that are applied annually, however, less than 5% are estimated to reach the target organism and the bulk gets deposited on the soil, water and non-target organisms (Pimental and Levitan, 1986).

3.2. Biological response of aquatic organism to environmental contaminants

Toxic organic contaminants such as PAHs, PCBs and dioxins found in the ecosystem, can be taken up by the organisms through the direct uptake from water by gills or skin (bioconcentration), through the uptake of suspended particles (ingestion) and through the consumption of contaminated food (biomagnification). Those chemicals are highly lipophilic in nature, and can easily enter the cell (Ejobi *et al*., 2007).

The first stage of the effects of a pollutant on the organisms is the interactions with endogenous molecules. These interactions can be divided into three main groups. In the first group, the contaminants can be sequestrated and then neutralized in the neutral lipid fraction. In the second group, contaminants can bind specifically to some cellular molecules such as receptors, for example, some xenoestrogenic chemicals like DDT, a kind of organochlorine

pesticides largely used for agricultural purposes, bind to estrogen receptor and show estrogenic effects (Adeyemi *et al*., 2008). In the third group, contaminants can interact with the biotransformation enzymes. These enzymes either metabolize contaminants into nonreactive hydrophilic compounds which are easily excreted from the body or convert them into reactive metabolites which are more toxic than the parent compounds. All these interactions can result in accumulation of foreign chemicals in the body of organisms and lead to direct or indirect (via its metabolites) toxic effects or excretion of the parent compound and/or its metabolites.

Pollutants which are bioaccumulated in the organism, first cause changes at the molecular and cellular level. This may lead to the adverse effect on the organism which in turn may cause changes in the population and the community level in the years to come (Arinc *et al*., 2000). As stated above, for example, xenoestrogenic compounds first bind to the estrogen receptor at the cellular level and cause feminization of male species at the individual level and finally, this may threaten the existence of susceptible species at the population even community level (Gimeno *et al*., 1996). Growing number of toxic organic chemicals in the aquatic environment may cause deleterious effects on populations. For this reason, it is very important to detect the relationship between contaminant level (exposure) and their biological effects before any detrimental effect has been established at higher organization level (Van der Oost *et al*., 2003).

3.3. Xenobiotic biotransformation

Xenobiotic biotransformation is the process by which lipophilic foreign compounds are metabolized to hydrophilic metabolites that are eliminated directly or after conjugation with endogenous cofactors by renal or biliary excretion. These metabolic enzymes are divided into two groups, Phase I and Phase II enzymes (Oesch *et al*., 2000).

The Phase I involves reactions giving rise to more polar compounds. This may either result in reducing the effectiveness of the metabolized xenobiotic, also called as bioelimination or produce an active metabolite, also called as bioactivation in terms of both pharmacology and toxicology (Siroka and Drastichova, 2004). Phase I reactions, considered as functionalization reactions, add or uncover functional groups on xenobiotics with the purpose of increasing the polarity or nucleophilicity that create a suitable substrate for Phase II metabolism. Phase I reactions generally takes place by oxidation, reduction or hydrolytic reactions. These

reactions are mediated primarily by the CYP 450 family of enzymes, but other enzymes (e.g. flavin monooxygenases, dehydrogenases, peroxidases, amine oxidases, xanthine oxidases) also catalyze the oxidation of certain functional groups. In addition to the oxidative reactions, there are different types of hydrolytic reactions catalyzed by enzymes like carboxylesterases and epoxide hydrolases (Hodgson and Goldstein, 2001; Parkinson, 2001).

Phase I products are not usually eliminated rapidly, but undergo a subsequent reaction in which an endogenous substrate such as acetic acid, glucuronic acid, sulfuric acid or an amino acid combines with the newly formed functional group to make a highly polar conjugate which can be easily excreted. Sulfation, glucuronidation and glutathione conjugation are the most prevalent classes of phase II metabolism, which may directly act on the parent compounds, or, as is usually the case, on functional groups added or exposed by phase I reactions (LeBlanc and Dauterman, 2001; Rose and Hodgson, 2004; Zamek-Gliszczynski *et al.*, 2006).

3.4. Biomarkers

A biomarker is a measurable indicator of some biological state or condition. Most chemical analyses for environmental contaminants only establish their presence. Although chemical analyses are able to quantitatively measure a wide range of pollutants, these analyses are unable to reveal potential activity within an organism. Exposure to contaminants may produce increased activity of specific cytochrome P450 enzymes, which makes it a potential biomarker of contaminant exposure. The use of biochemical biomarkers fulfills this purpose (Guengerich, 2001).

Biomarker responses are of two kinds: those that measure only exposure to a pollutant (bio-indicators), and those that measure both exposure and toxic effect of environmental chemicals (biochemical biomarkers) (Peakall, 1992).

Van der Oost *et al.* (2003), mentioned that special attention ought to be given to interpret the data obtained through the use of a biomarker to differentiate between laboratory and field results, the biological and toxicological relevance of the induction of enzymatic activity, and possible confounding factors not related to the exposure, thereby producing the same effect as the causal factors (e.g., stress by excessive manipulation). Therefore, it is recommended to use several biomarkers and combine them with the quantification of accumulated xenobiotics to determine cause-effect relationships. In fish, physiological (e.g., gonadosomatic index,

liver somatic index and morphometric parameters), reproductive (e.g., plasmatic vitellogenous level) and molecular (e.g., CYP1A1 activity and AChE inhibition) biomarkers have been frequently used (Orrego *et al.*, 2006).

3.4.1. Bioindicators

A bioindicator is any biological species or group of species whose functions, population or status can reveal the qualitative status of the environment. A bioindicator response provides information on both the presence of a contaminant in the environment and that it has reached the affected tissue or organ in sufficient amounts for a period of time, long enough to cause the observed response (Perry *et al.*, 1998). Freshwater biomonitoring uses organisms as indicators primarily of organic pollution in streams, lakes, ponds and rivers.

According to Manly (1995) and Beeby (2001), the model organisms, also called bioindicators, should be easily identifiable and abundant in the field representing a short life cycle, an accumulator of pollutants with capacity to reflect levels of pollutants and sources of emission, and with a well-known biology, ecology and distribution.

Fish are among the most important members of the aquatic ecosystems and represent about half of the currently known vertebrate species (Bucheli and Fent, 1995). The sudden death of fish may indicate heavy pollution and the effects can be measured in terms of physiological, biochemical or histological responses of the fish organism after exposure to sub-lethal levels of pollutants (Mondon *et al.*, 2001). Furthermore, they provide a vital protein source and, hence, are of crucial importance for the nutrition of mankind. Therefore, the health and protection of these organisms are of pivotal importance. The fish plays an increasingly important role in the monitoring of water pollution as it responds with great sensitivity to changes in the aquatic environment (Saha and Kaviraj, 2003). Among fish, the main species for monitoring programs and toxicological studies are zebrafish, rainbow trout, channel catfish, Japanese medaka, mummichog, Atlantic salmon, scup and Nile tilapia (Arellano-Aguilar *et al.*, 2009).

3.4.2. Biochemical markers

The biochemical marker is any biochemical compound like enzymes, antibody, antigen or hormone that is sufficiently altered and serves as an aid in predicting susceptibility.

Biochemical markers are a response induced in the tissues by the presence of a specific group of contaminants that have the same mechanism of toxicity. These kinds of markers are measurable responses to the exposure of an organism to xenobiotics. Once a xenobiotic enters into an organism, it binds to specific receptors that may induce cellular processes that have toxic or other adverse effects at the cellular level (Siroka and Drastichova, 2004). Biochemical markers have been used in toxicology, ecotoxicology and pharmacology research, and involve both *in vitro* and *in vivo* assays.

One of the most intensively studied biomarkers, in both laboratory and field conditions, is cytochrome P450. Cytochrome P450's represent an inducible enzyme system, and increased amounts of specific CYP 450 isozymes are observed after treatment of organisms with a variety of environmental contaminants (Scott, 1999). Its levels have been studied as a response of the organism to the presence of pollutants in an aquatic environment. The first knowledge of CYP 450 activity in aquatic organisms was reported in 1972 (Khan *et al*., 1972). Since then, cytochrome P450 enzymatic activities have been reported in aquatic arthropods, annelids, cnidarians, molluscs, poriferans, platyhelminths, echinoderms and insects (Livingstone, 1985; Chipman *et al*., 1991; Feyereisen, 1993; James and Boyle, 1998). Payne and Penrose (1975) were among the first to make use of this enzyme system as a biochemical biomarker, reporting elevated monooxygenase activity in fish from petroleum-contaminated sites in the aquatic environment. Payne (1976) suggested that level of monooxygenase activity (benzo-a-pyrene hydroxylase) is the indicator of environmental exposure of aquatic animals to pollutant mixtures. Cytochrome P450 has been used in fish cell lines as a biochemical biomarker of polychlorinated hydrocarbon contamination in sediments and landfill leachates (Fent, 2004) and also to detect contamination of toxic carcinogen pollutants, such as polychlorinated biphenyls (Arinc *et al*., 2000).

3.5. Cytochrome P450 (CYP 450)

3.5.1. Cytochrome P450 characterization

The name cytochrome P450 originated from its spectral properties before its catalytic function was known. Klingenberg (1958) and Garfinkel (1958) first reported that hepatic microsomes contain a pigment that binds carbon monoxide with unusual visible absorbance maxima of 450nm in its CO-reduced difference spectrum. Omura and Sato (1964) discovered that this pigment is a b-type cytochrome and called it Cytochrome P450. CYP 450 is believed

to be more primitive than those considered and may even have emerged even before the advent of atmospheric oxygen probably very early in chemical evolution (Wickramasinghe and Villee, 1975).

CYP 450 belongs to one of the most intensively studied enzyme systems and represents a class of hemoproteins present in virtually all lineages of life (Wickramasinghe and Villee, 1976). The presence of a heme-thiolate bond in the enzyme active site is responsible for the various unique spectroscopic and catalytic properties of this class of enzymes (Estabrook and Werringloer, 1996). CYP 450 catalyzes a variety of reactions in structurally diverse compounds that serve as substrates for the enzyme. Endogenous substrates for the enzyme include cholesterol, steroid hormones and fatty acids and exogenous compounds (xenobiotics) such as drugs, food additives, polyaromatic hydrocarbons, pesticides and chemicals used for industry. CYP 450 is also involved in inactivation or activation of therapeutic agents; conversion of chemicals to highly reactive molecules that produces unwanted cellular damage, cell death or mutations; production of steroid hormones; metabolism of fatty acids, and enzyme inhibition or induction that results in drug-drug interactions and adverse effects (Okita and Masters, 1997).

Since the main function of CYP 450 involves the activation of molecular oxygen, the significance of the heme-thiolate linkage was suggested to be intimately involved in oxygen atom transfer (Wickramasinghe and Villee, 1976). CYP 450 is a one-electron acceptor, and the oxygen activation occurs in two steps (Peterson *et al.*, 1977):

$$\mathbf{1.\ O_2 + P450\text{-}Fe^{2+} \rightarrow P450\text{-}\ Fe^{2+}O_2 \leftrightarrow P450\text{-}\ Fe^{3+}O_2^-}$$

$$\mathbf{2.\ P450\text{-}\ Fe^{3+}O_2^- + e^- \rightarrow P450\text{-}\ Fe^{2+}O_2^- \leftrightarrow P450\text{-}\ Fe^{3+}O_2^=}$$

The catalytic cycle of CYP 450 mediated reactions involves the transfer of electrons from NADPH via. NADPH cytochrome P450-reductase, a flavoprotein that is present in much lower concentration than CYP 450. The CYP 450 cycle starts with the binding of the substrate. A second step is accomplished when the heme iron is reduced from the ferric form (Fe^{3+}) to the ferrous (Fe^{2+}) by the addition of an electron from cytochrome P450-reductase. Oxygen then binds to the CYP 450 in its ferrous state. The complex is converted to a $Fe^{2+}OOH$ state by the addition of a proton (H^+) and a second electron derived from the cytochrome P450-reductase or from cytochrome b_5. A second H^+ cleaves the $Fe^{2+}OOH$ and produces H_20 and the $(FeO)^{3+}$ complex which transfers its oxygen atom to the substrate.

When the oxidized substrate is released, the CYP 450 molecule is ready for a next cycle (Parkinson, 2001).

CYP 450 enzymes have the ability to react with a multitude of different chemical substrates. This mechanism of reaction includes oxygen activation, splitting and formation of very reactive oxygen. The catalytic power of cytochrome P450 is demonstrated in the following thermodynamic equation (Rein and Jung, 1993):

$$\mathbf{RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+}$$

Where (R) is the substrate and (ROH) is the hydroxylated product.

The overall reaction is called monooxygenation due to the incorporation of only one of the two oxygen atoms into the substrate.

CYP 450 system contains a number of versatile isozymes in that they catalyze many types of reactions, including aliphatic and aromatic hydroxylations and epoxidations, N-oxidations, sulfoxidations, dealkylations, deaminations, dehalogenations, and others (Matsumura, 1985). These isozymes are responsible for the oxidation of different substrates or for different types of oxidative reactions of the same substrate.

CYP 450's are typical monooxygenases and catalyze oxygenation of various organic substrates using NADPH and molecular oxygen as co-substrates. At the same time, these enzymes are known to be capable of utilizing organic hydroperoxides and hydrogen peroxide as co-substrates in hydroxylation reactions. CYP 450's can also reduce certain compounds, acting as an electron carrier. CYP 450 dependent monooxygenations are accompanied by the release of superoxide radicals and hydrogen peroxide (Londono, 2005).

3.5.2. Cytochrome P450 distribution and function

CYP 450's are membrane-bound monooxygenase proteins involved in the biotransformation of many endogenous and exogenous compounds. They have been characterized in both prokaryotic (Urlacher and Schmid, 2002; Lewis and Wiseman, 2005) and eukaryotic species such as plants (Bolwell *et al.*, 1994); aquatic invertebrates (Snyder, 2000); annelids (Rewitz *et al.*, 2004); crustacea (James and Boyle, 1998); fish (Andersson and Forlin, 1992); birds (Walker, 1998); and mammals (Seliskar and Rozman, 2007), with relatively few exceptions,

like the enteric bacteria *Escherichia coli* and *Salmonella typhimurium*, are devoid of CYP 450 genes (Guengerich and Isin, 2008).

These enzymes are found predominantly in the endoplasmic reticulum and mitochondria of liver and adrenal glands, however, they are also distributed throughout the body in diverse areas such as the brain, heart, intestine, kidney, lung and skin (Sarasquete and Segner, 2000). In teleost fish, CYP 450's are present in liver, intestine, gonads, kidneys, brains, gills, blood, spleen, skin (fins) and eyes (Arellano-Anguillar *et al*., 2009). Owing to the absence of mitochondrial P450's in nematodes (*C. elegans*) and lower eukaryotes, it has been suggested that mitochondrial CYP 450's did not originate independently but was picked up from the microsome, presumably by mistargeting of the latter gene (Nelson, 1998).

Functionally, CYP 450 enzymes may be broadly divided into those involved primarily in drug and other foreign xenobiotic metabolism, those functioning in steroidogenesis and those participating in other important endogenous functions. Despite a large number of CYP 450's, the bulk of drug metabolism is catalysed by a relatively small number of CYP 450 enzymes found in families 1, 2, 3 and to a lesser extent by family 4. The substrates for cytochrome P450 include a host of xenobiotics, including substances that occur biologically although are foreign to animals, such as antibiotics and unusual compounds in plants, as well as synthetic organic chemicals, and a variety of steroids and other physiologically occurring lipids (Wickramasinghe, 1990; Ryan and Levin, 1990; Guengerich, 1991). CYP 450 enzymes comprise 70% - 80% of all Phase I xenobiotic metabolizing enzymes (Evans and Relling, 1999). The number of man-made environmental chemicals has been estimated as greater than 200000, most of which are thought to be potential substrates for CYP 450 and many may also serve as inducers or inhibitors of various isoforms (Murray *et al*., 2012). Since the synthetic modification of new and existing drugs and xenobiotics are possible, there is no upper limit on the number of compounds acted on by this enzyme family.

3.5.3. Nomenclature

Cytochrome P450 enzymes are known under a variety of names, including monooxygenases, mixed function oxidases (MFOs), poly-substrate monooxygenases (PSMOs), microsomal oxidases and heme-thiolate proteins (Feyereisen, 1999). The nomenclature of these enzyme designates all gene members of a cytochrome P450 superfamily with a CYP prefix, followed by Arabic numeral for the family, a letter for the subfamily and a numeral for the individual

gene (Nebert and Gonzalez, 1987), for e.g. 1A1, 2B1, 3A4, etc. In the term CYP 450, the first two letters represent the cytochrome and the first letter in P450 is used as a preface to designate a gene or protein with a characteristic soret peak formed by absorbance of light at wavelengths near 450 nm when the heme iron is reduced (often with sodium dithionite) and complexed to carbon monoxide. Thus cytochrome P450 1A1 and P450 2B1 are designated CYP1A1 and CYP2B1 respectively in this nomenclature system. All members of a family share >40% identity at the amino acid sequence level, members of a subfamily share >55% identity and members that share more than 97% identity are considered to represent alleles unless there is evidence to the contrary (Nelson *et al*., 1996).

CYP 450's are greatly conserved across vertebrate lineages. CYP 450 families and subfamilies, with the exception of subfamilies within CYP2's, are nearly identical in vertebrate taxa and the total number of CYP 450 genes in vertebrate species is similar. For example, fish and mammalian lineages share all families except CYP39, which has arisen within mammals (Nelson, 2003). All other 17 CYP families have clear orthologs in both fish and mammals and the intron-exon boundaries are well conserved within CYP 450 families. CYP2's are the least conserved family with extensive subfamily diversification (Nelson, 2003).

Among the many families of cytochrome P450, family 1–4 are the main contributors in the metabolism of xenobiotic compounds and are most widely studied throughout the world (Nelson *et al*., 1996). CYP 450's are under the complex and distinct control of endogenous signals, and various xenobiotic compounds have been shown to influence their activity. As a result, individual CYP 450's can show diverse expression patterns related to life stages, sex, tissues, strains or diet (Scott and Wen, 2001). Some forms are expressed at all life stages, such as CYP4D1 and CYP6A1, while others are adult specific, such as CYP6D1 or larval specific such as CYP6B2 (Scott *et al*., 1998). Similarly, CYP 450's show great variation in response to inducers and some CYP 450's have several known inducers, while others have none (Amichot *et al*., 1998).

3.5.3.1. CYP1 family

This family represents one of the most studied families in fish and have been reported in 64% of the total teleost fish studied (Arellano-Anguillar *et al*., 2009). It stands as the most searched for with respect to other isoforms due to its extensive use as a biomarker of

xenobiotics exposure (Whyte *et al*., 2000; Siroka and Drastichova, 2004). Research on CYP 450's in fish is more limited than that reported for mammals. The CYP1A family members, CYP1A1 and CYP1A2 have been well characterized as inducible genes and historically are linked to the aryl hydrocarbon receptor (AhR) (Dogra *et al*., 1998). In fish, the presence of only single CYP1A gene is reported and is considered ancestral to both mammalian CYP1A1 and CYP1A2 (Schlenk *et al*., 2008). Fragmented CYP1B1 research exists in fish with only a handful of reports of studies published to date. The presence of CYP1B like form in fish has been established through comparative studies with the mammalian isoform and by northern blot. CYP1B1 is constitutively expressed in most tissues but also inducible through the AhR pathway. CYP1B1 is involved in the metabolism of endogenous estrogens, as well as active in the biotransformation of heterocyclic amines found in charcoal broiled meats (Crofts *et al*., 1997). The CYP1C subfamily of cytochrome P450, which is present in fish but not in mammals, has only recently been discovered and so limited research on the subfamily is available. The vertebrate CYP1C subfamily was first described by Godard *et al*. (2005) when they identified a CYP1C1 and CYP1C2, the two CYP1C paralogs in fish, in scup and carp liver and head kidney.

3.5.3.2. CYP2 family

CYP2 is the largest CYP 450 family comprising 16 genes, 5 families and 13 subfamilies (Nebert and Russell, 2002). CYP2C and CYP2E1 account for more than 15% of liver CYP 450 enzymes, whereas the corresponding value for CYP2A6 is approximately 10% and for CYP2D6, CYP2C8, CYP2C19 less than 5% (Pelkonen *et al*., 2008). CYP2B6, CYP2C9, CYP2C19 and CYP2D6 presents the most important CYP 450 polymorphism clinically with ethnic differences in their distributions. Approximately 30% of all drugs are CYP2D6 substrates such as codeine and tramadol (Desmeules *et al*., 1991; Dayer *et al*., 1997). CYP2D6 can be inhibited by quinidine, fluoxetine or paroxetine (Otton *et al*., 1993; Heiskanen *et al*., 1998; Lemberg *et al*., 2010), but CYP2D6 seems not to be inducible by other drugs. In mammals, induction of CYP2B proceeds through activation of the constitutive androstane receptor (CAR) (Honkakoski and Negishi, 1997) but in fish, unlike their mammalian counterpart, the presence and expression of CYP2B are contradictory, in spite of the fact that this subfamily is important for the metabolism of foreign compounds such as pesticides and drugs (Arellano-aguillar *et al*., 2009). On the basis of metabolism of prototypical mammalian CYP2B substrates such as aldrin, benzphetamine, ethylmorphine,

aminopyrine and alkoxyresorufins, the existence of piscine CYP2B-like enzymes have been supported (Stegemann, 1981; Eisele *et al.*, 1984; Goksoyr *et al.*, 1987; Buhler and Williams, 1989; Elskus and Stegemann, 1989; Kleinow *et al.*, 1990; Haasch *et al.*, 1994). Furthermore, protein purification followed by immune-cross-reactivity and sequence analysis with other fish species and rat CYP2B1 have extensively proven the occurrence of CYP2B form in fish such as scup (Stegemann and Hahn, 1994), rainbow trout (Miranda *et al.*, 1990), tomtate, pinfish, Bermuda chub and sergeant major (Stegemann *et al.*, 1997). Even though, CYP2B forms do exist in fish there seems to be an apparent lack of induction in response to the mammalian prototypical CYP2B inducer phenobarbital (Stegemann, 1981; Eisele *et al.*, 1984; Goksoyr *et al.*, 1987; Buhler and Williams, 1989; Haasch *et al.*, 1994; Iwata *et al.*, 2002).

3.5.3.3. CYP3 family

The CYP3A subfamily is the most important CYP 450 subfamily consisting of four isoenzymes CYP3A4, CYP3A5, CYP3A7 and CYP3A43 (Finta and Zaphiropoulos, 2000). CYP3A4 exhibits a dominant role among the CYP3A proteins. The CYP3 family accounts for the metabolism of 50% of the currently used pharmaceutical agents (Parkinson, 1996). In fish, they are regulated by the same receptors as in mammals, namely, through the pregnane X receptor (PXR) (Bresolin *et al.*, 2005). Although several variant alleles of the CYP3A4 gene have been defined, most of them seem to be too rare to contribute significantly to the CYP3A4 variability (Eiselt *et al.*, 2001). CYP3A4 is undetectable in fetal liver, but rapidly rises after the birth, and becomes the predominant CYP 450 in the adult liver (Schuetz *et al.*, 1994). CYP3A4 accounts for approximately 30% of total CYP 450 proteins (Shimada *et al.*, 1994). The CYP3A4 expression represents high inter-individual heterogeneity and its function has been estimated to vary over 11-fold in both liver microsomes and duodenal biopsies. The expression of CYP3A4 diminishes along the intestinal tract, thus the greatest CYP3A4 content is determined in the duodenum and lowest in the ileum (Paine *et al.*, 1997). As in the liver, CYP3A4 is the most abundant cytochrome also in the intestine, representing approximately 50–70% of its total intestinal CYP 450 content (Paine *et al.*, 2006).

3.5.3.4. CYP4 family

The CYP4 family consists of 11 subfamilies (CYP4A- CYP4M) (Simpson, 1997), with the CYP4A subfamily perhaps being the most highly studied. The CYP4A subfamily is of interest in toxicology, due to its inducibility by multiple chemicals, including agents used as plasticizers, herbicides and solvents, e.g., di-(2-ethylhexyl) phthalate, 2,4-dichlorophenoxyacetic acid and trichloroethylene (Simpson, 1997). In the case of fish, the function of CYP4 family is unknown to date (Arellano-aguillar *et al*., 2009).

3.6. Mechanisms of induction of cytochrome P450 in fish

The induction of CYP 450's is highly conserved and is found not only in humans but also in many other species. The inducible CYP 450's (CYP1 - CYP3) make up a large percentage of CYP 450's in the liver. In most cases, induction of CYP 450's occurs by a process involving *de novo* RNA and protein synthesis through ligand activation of key receptor transcription factors including aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR) and others ultimately leading to increased transcription (Daujat *et al*., 1991). An alternative mechanism of CYP 450 induction involves compounds that stabilize translation or inhibit the protein degradation pathway (Woodcraft and Novak, 1998).

For many years, the mechanisms governing cytochrome P450 gene induction following xenobiotic exposure remained elusive. AhR–CYP1A interactions were well described, but the processes by which induction of CYP2, CYP3 and CYP4 families occurred had yet to be determined (Hahn *et al*., 2005). With the discovery of the nuclear receptors CAR and PXR, the mechanistic understanding of CYP 450 regulation was greatly enhanced. Both CAR and PXR are low-affinity (high substrate concentration) xenosensors in mammals, capable of regulating genes associated with the metabolism, transport and elimination of exogenous substrates.

3.7. Role of cytochrome P450 in fish

Initially, it was thought that fish lacked CYP 450 linked monooxygenases, but studies carried out in the late 1960s by Buhler and Rasmusson (1968) and Dewaide and Hendersson (1968) showed that these enzymes were present in the livers of rainbow trout and other fish. Subsequently, as observed in mammals, multiple CYP 450 forms were also discovered in

fish, predominately in the liver, but also found in lower concentrations in other tissues, like kidney, gut, gall bladder, gonads, nervous tissue, endocrine cells, gills, etc. (Goksoyr and Forlin 1992; Sarasquete and Segner 2000). Since then a large number of CYP 450's have been characterized in different species that have been studied (Nelson, 2009). Lester *et al.* (1993) and Lewis (2001) reported high levels of CYP 450 in fish liver, accounting for 1 to 2% mass of hepatocytes. Cytochrome P450 is not only of crucial importance for the transformation of xenobiotic or exogenous substances, but it also plays a major role in metabolizing various endogenous substances (Siroka and Drastichova, 2004). Even though there are differences between mammalian and fish monooxygenase systems (e.g., generally lower enzyme activity, different isoenzyme composition and lack of phenobarbital-type induction for the fish enzyme system), the overall enzyme system and characteristics seem to be very similar (Arellano-Aguilar *et al.*, 2009).

It has been known for some time that various species of fish possess the enzymes involved in the biotransformation of xenobiotic chemicals, this area of research did not enjoy the intensity of effort in the past as did its mammalian counterpart. Recent interest in the aquatic environment and in aquatic toxicology has led to investigations with *in vitro* tissue preparations from several species of fish and it is now well documented that fish possess mixed-function oxidase enzymes and conjugating enzymes and that the former are inducible by many of the agents which induce mixed-function oxidases in mammals (Lech and Bend, 1980)**.**

Schlenk *et al*. (1993) reported the presence of at least two constitutive forms of CYP 450 in channel catfish by performing western blots, utilizing polyclonal antibodies raised against five purified rainbow trout liver CYP 450 enzymes and revealed at least two protein bands that were approximately 50 kDa and 53 kDa. Schlenk (1994) reported the expression of CYP1A and CYP2K from liver and kidney in channel catfish (*Ictalurus punctatus*) exposed to 2-Methylisoborneol, which is a natural product produced by a mixture of microorganisms that inhabit ponds used for raising various fish species, using anti-trout CYP 450 polyclonal antibodies.

Although the full catalytic function of many fish CYP 450's is still unknown, organ and cell-type distribution of major fish CYP 450's have been investigated. Immunohistochemistry, PCR, Northern and Western blots are among the common tools used to determine mRNA and protein expression levels of CYP1A and 3A in fish tissues. CYP1A is found in the liver, heart, gill, kidney, and intestinal tract of fish (Husoy *et al.*, 1994) and CYP3A is highly

expressed in the liver and intestinal tract (Lee *et al*., 2001; Tseng *et al*., 2005). By genomic sequence analysis, the presence of CYP1B1 was reported in scup (*Stenotomus chrysops*), plaice (*Pleuronectes platessa*) and striped dolphin (*Stenella coeruleoalba*) (Godard *et al*., 2000). Hassanin *et al*. (2009) reported that Nile Tilapia CYP1A genomic sequence shows similarities of 50.8, 70.7, 71.8, 77.2, 72.4, 74.3, 77.6, 77.8, 79.1 and 80.5% with CYP1A of common carp, red sea bream, European eel, toad fish, Japanese eel, rainbow trout, European sea bass, butterfly fish, killifish, scup and European flounder respectively.

In fish, as in other animal species, there are great differences in cytochrome P450 catalytic activity among individuals of one population as well as among populations (Navas and Segner, 2001; Machala *et al*., 1997; Schlenk and Di Giulio, 2002). In addition, there are large inter-species differences and, in different species, the same substrate can be metabolized by different CYP 450 isoforms. Van der Oost *et al*. (2003) and Orrego *et al*. (2006) have considered CYP 450 as one of the most valuable and stable fish biomarkers for evaluating environmental risk assessment and environmental monitoring.

Studies in numerous fish species have revealed multiple cytochrome P450 proteins that have different physicochemical as well as catalytic properties. Procedures including protein purification, immunological reactivity and amino acid sequencing have been used to identify and classify different CYP 450 genes in fish. Stegemann *et al*. (1997) studied multiple CYP 450 expression in 10 tropical fishes from Bermuda, viz. Graysby bass, Grey snapper, Yellowtail snapper, Parrotfish, Slippery dick, Blue-striped grunt, Tomtate, Pinfish, Sergeant major and Bermuda chub. Bainy *et al*. (1999) have also reported multiple cytochrome P450 forms in Tilapia (*Oreochromis niloticus*) from Billings reservoir, Sao Paulo, Brazil. The induction of this enzyme system in fish is highly specific for certain classes of xenobiotics (e.g., PAHs and planar PCBs) and clearly attributes a biological reaction with exposure to those chemicals.

The more studied fish families are Cyprinidae, Ictaluridae, Salmonidae, Fundulidae, Cichlidae, Sparidae and Adrianichthyidae. These families include species with trade importance, easily maintained in captivity or ubiquitously distributed (Burr and Mayden, 1992). Zebrafish, channel catfish, rainbow trout and Japanese medaka are examples. As of 2009, at least 34 isoforms of CYP 450 have been described in fish, which comprise 19 subfamilies and 8 families, viz, CYP1, CYP2, CYP3, CYP4, CYP11, CYP17, CYP19 and CYP26 (Arellano-Aguilar *et al*., 2009). At least one CYP 450 enzyme has been described in more than 57 teleost fish species. The CYP1A isoform has been reported in over 64% of

these, while CYP19A has been reported in 17.5%, CYP17C in 15.7% and CYP1B1, CYP1C, CYP17C and CYP2E1 between 14 - 15%. CYP2K and CYP2M isoforms have been reported in 10.5% and enzymes from CYP2N, CYP2P, CYP2X, CYP3A, CYP4T1, CYP11A1, and CYP26 subfamilies in smaller percentages. The high percentage of CYP1A compared to other isoforms is due to its broad application as a biomarker of exposure and thus making it the most studied one (Whyte *et al.*, 2000; Siroka and Drastichova, 2004; Arellano-Aguilar *et al.*, 2009).

Hepatic monooxygenase activities in rainbow trout (*Oncorhynchus mykiss*), flounder (*Platichthys felsus*) and dab (*Limanda limanda*) have been used as a monitoring tool for water bodies in northern Germany by industrial effluents (Pluta, 1993). CYP 450's, principally the CYP1A1 isoform have been used to detect the presence of pollutants in environmental monitoring programs due to its high affinity to xenobiotics (Livingstone *et al.*, 2000).

It has also been reported that enzymatic activity is higher in the field than in laboratory tests which are related to physiological and physical factors (Pluta, 1993; Schmitt *et al.*, 2005; Hinck *et al.*, 2006; Kammann *et al.*, 2008). Hence, it has been suggested that monitoring programs should set up *in situ* basal levels of enzymatic activity in organisms from reference and polluted habitats. In other cases, CYP 450 enzymatic activity can also be compared between fish populations from reference clean sites to evaluate a possible risk of polluted sites. Another common practice in biomonitoring studies is the organism transplantation from unpolluted to polluted sites.

According to Whatley *et al.* (2009), channel catfish (*Ictalurus punctatus*) from field studies, Troy wastewater treatment plant (Alabama) induced transcription of mRNA for CYP1A enzyme production and increased EROD activity in channel catfish compared to catfish exposed to water from upstream of the wastewater treatment plant. Again, Whatley *et al.* (2010) suggested that *in situ* exposure to wastewater pollutants using caged experimental organisms provide a much more convenient local monitor of pollutant exposure and biological impact than *ex situ* toxicological studies. They also reported the induction and gene expression of CYP1 in channel catfish to be stronger in the field exposures over laboratory exposures on all sampling days and also reported the presence and induction of CYP1B when quantified by real-time reverse transcription polymerase chain reaction.

The CYP1 gene family represents one of the most studied families of CYP 450 in fish, primarily with regard to the role that CYP1A subfamily members play in the

biotransformation of environmentally persistent aromatic hydrocarbons (e.g., PCBs, PAHs) and their relationship with disease processes resulting from exposure to compounds of this nature (Stegmann and Hahn, 1994). CYP1A induction is mediated by Ah receptor (AhR), a xenobiotic-binding protein present in the cytosol (Lewis, 2001). Pacheco and Santos (2002) reported that naphthalene, a class of PAH, have been found to be specific for the induction of CYP1A in European eel. Induction of CYP1A in fish has been widely used as a sensitive and convenient early warning signal of exposure to aryl hydrocarbon agonists such as polychlorinated biphenyls, polyaromatic hydrocarbons, dioxins, furans and organochlorine pesticides (Jonsson, 2003). In fish, CYP1A activities are often used as a marker to determine the quantities of persistent organic pollutants (Havelkova *et al*., 2007).

Willett *et al.* (2006) reported the induction of CYP 1B in channel catfish in both the laboratory treated with benzo-a-pyrene (BaP) and from the three field site, Lake Roebuck in Itta Bena, Bee Lake in Thornton and Sunflower River in Indianola. The CYP1B transcript was higher in gills compared to other tissues in both laboratory and wild catfish when quantified with real-time reverse transcriptase PCR. 3-methylcholantrene (3-MC), a specific inducer of CYP 1A in mammals is also reported to induce CYP 1A in catfish, *Ancistrus multispinis* (Klemz *et al*., 2010).

As in mammals, induction of the cytochrome P450 2B family in fish by phenobarbital is unclear, however, over the last decades, fish liver microsomes have been shown to metabolize prototypical mammalian CYP2B substrates, like aldrin, benzphetamine, ethylmorphine, aminopyrine and alkoxyresorufins (Stegemann, 1981; Eisele *et al*., 1984; Goksoyr *et al*., 1987; Elskus and Stegemann, 1989; Buhler and Williams, 1989; Kleinow *et al*., 1990; Haasch *et al*., 1994; Sadars *et al*., 1996; Iwata *et al*., 2002). A CAR immunoreactive protein was also detected in scup liver cytosol and nucleus using antibodies against human CAR. Studies on scup (Klotz *et al*., 1986), rainbow trout (Miranda *et al*., 1990) and some tropical fishes from the Bermuda Archipelago (Stegemann *et al*., 1997) have also been shown to metabolize prototypical mammalian CYP2B substrates. CYP2B1 and CYP2B2 are the primary members expressed in rats. In rodents, phenobarbital and other barbiturates usually induce enzymes from this subfamily and are inhibited by metyrapone (Mimura *et al*., 1993). A piscine CYP2B gene ortholog has so far not been reported, however, the existence of piscine CYP2B-like enzymes was further supported by protein purification and immuno-cross-reactivity studies. *N*-terminal analysis of scup CYP 450B showed 50% sequence identity with rat CYP2B1 and CYP2B2, furthermore, proteins from

different taxa, including several fish species, show cross-reactivity with antibodies against both scup CYP 450B and rat CYP2B1 (Stegemann and Hahn, 1994; Stegemann *et al*., 1997).

The possible existence of CYP2E1 form in fish (*Poeciliopsis monacha-lucida*) was proposed based on hybridization with rat CYP2E1 49-base oligonucleotide, antibodies to rat CYP2E1, as well as responsiveness to ethanol treatment. This CYP 450 form was suggested to be involved in the CYP 450 mediated dealkylation of the fish carcinogen diethylnitrosamine (Kaplan *et al*., 1991). Furthermore, metabolism of the mammalian CYP2E substrate chlorzoxazone by hepatic microsomes in winter flounder (*Pleuronectes americanus*) and in viviparous *Poeciliopsis monacha* and *Poeciliopsis viriosa* supported the presence of CYP2E-like enzymes in fish (Wall and Crivello, 1998; Kaplan *et al*., 2001). The CYP2E1 isoform is particularly induced by ethanol, acetone, isoniazid and by starvation in animal models (Lieber, 1997). CYP2E1 is responsible for the metabolism of a large number many endogenous or exogenous small molecules and also potential bioactivation of many low-molecular-weight pharmaceuticals and other xenobiotic compounds, like acetaminophen, glycerol, halogenated anaesthetics, carbon tetrachloride, nitrosamines and benzene (Lee *et al*., 1996; Gonzalez, 2005). Chlorzoxazone and p-nitrophenol are routinely used as substrate probes to measure CYP2E1 activity, although chlorzoxazone can also be metabolized by CYP1A2.

CYP3A-like proteins were initially purified from several teleost species including scup, rainbow trout, and Atlantic cod (Klotz *et al*., 1986; Celander *et al*., 1989; Miranda *et al*., 1989). CYP3A are the major constitutive CYP 450 forms expressed in the liver and intestine of fish and is considered to play a vital role as a biochemical defence to prevent bioaccumulation of xenobiotics (Celander *et al*., 1989). CYP3A enzymes are among the most versatile forms of CYP 450's as they have unusually broad substrate specificities for both endogenous and exogenous substrates, including steroids, bile acids, eicosanoids, retinoids, xenobiotics such as pharmaceuticals and procarcinogens (Aoyama *et al*., 1990; Gillam *et al*., 1993; Li *et al*., 1995; Waxman *et al*., 1998). James *et al*. (2005) also reported the presence and expression of CYP3A family isozymes in the intestine of channel catfish (*Ictalurus punctatus*).

3.8. Cytochrome P450 in air breathing teleost fish

Studies on cytochrome P450 in teleost fish have been rapidly progressing after it was first reported about 4 decades ago. In fish, cytochrome P450 induction has been widely used as a

biomarker in analyzing exposures and responses of aquatic organisms to contaminants. Of all the teleost fish species, however, studies on cytochrome P450 in air breathing fish have been reported only in a few species.

The best studied example of CYP 450 is found in the CYP1A subfamily, which has been shown to be highly inducible in numerous aquatic species (Bucheli and Fent, 1995). Al-Arabi and Goksoyr (2002) examined the phase I cytochrome P450 monooxygenase activity and response in two tropical fish species, riverine catfish (*Rita rita*) and marine mudfish (*Apocryptes bato*) for the first time. Ethoxyresorufin O-deethylase **(**EROD) activity mediated by CYP1A was shown to be significantly induced in two tropical fish species after they were given a single intraperitoneal injection of two selected inducing compounds, β-naphthoflavone (β-NF) and a polychlorinated biphenyl mixture (Clophen A50) for 3 and 10 days. But the fishes when treated with the heavy metal compound cadmium chloride, showed significant inhibition of CYP1A activity in both the treated fish.

Yu (2000) reported dose-effect induction of gut and hepatic cytochrome P450 content and CYP1A dependent EROD activity in *Channa striatus* when exposed to deltamethrin, malathion and endosulfan for 48 hours. Mdegela *et al*. (2006) also reported the induction of CYP 1A in liver and gills of African sharptooth catfish (*Clarias gariepinus*) exposed to waterborne benzo-a-pyrene for 24 hours at different concentrations of 1.60, 3.44, and 18.21 gm/L. Liver genes that regulate detoxification are important in the metabolism of xenobiotics in various aquatic organisms. Similarly, Hassanain *et al*. (2007) also reported the induction of EROD activity in the liver of African sharptooth catfish, *Clarias gariepinus* exposed to benzo-a-pyrene while inhibition of the same was seen with cadmium. Qun *et al*. (2008) obtained the CYP1A cDNA of Taiwan snakehead (*Channa maculata*) by RT-PCR method. They reported that the cloned cDNA fragments were 908, 902 and 684 bp in length encoding 302, 300 and 228 amino acids.

In India, very little work has been done on cytochrome P450. Sadat *et al*. (2009) reported the increased level of hepatic and renal microsomal protein content in *Heteropneustes fossilis* which was correlated with increased N, N-dimethylaniline demethylase and aniline hydroxylase activity when treated with sub-lethal concentration of cypermethrin. The cytochrome P450 band intensity in SDS-PAGE was also higher in treated fish in contrast to the control fish. Radhakrishnan (2010) reported the induction of CYP 4501A in gills of *Heteropneustes fossilis* after exposure to benzo-a-pyrene for 45 days. Similarly, Pal *et al*. (2011) reported the induction of multiple forms of CYP 450 (viz. CYP1A, CYP2B, CYP2E1,

CYP3A4) in *Channa punctatus* exposed to sub-lethal concentrations of dicofol and chlorpyrifos for a period of 15 days. According to Ghosh and Ray (2013), in *Heteropneustes fossilis*, carbofuran augments total phospholipid in the liver and accelerates CYP1A activity by changing its structural conformation and thus controls the detoxification of xenobiotics.

3.9. Cytochrome P450 and pesticide interaction

There are many pathways by which pesticides leave their sites of application and distribute throughout the environment and enter the aquatic ecosystem. The major route of pesticides to water ecosystems is through rainfall runoff and atmospheric deposition and another source of water contamination by pesticides are from domestic and industrial discharge. Most pesticides eventually find their route into rivers, lakes and ponds and have been found to be highly toxic to non-target organisms that inhabit natural aquatic environments apart from their target organisms (Vryzas *et al.*, 2009; Werimo *et al.*, 2009; Arjmandi *et al.*, 2010; Maqbool and Ahmed, 2013). The contamination of water bodies by pesticides is known to have deleterious effects on the reproduction, survival and growth of aquatic animals. In the past few years, the problems caused by pesticides runoff coupled with intense agricultural practices has drawn scholars attention due to the increase in death among the fish in various ponds, lakes and streams of around the world because of their high sensitivity to the environmental contamination of water. Different concentrations of pesticides are present in many types of wastewater and numerous studies have found them to be toxic to aquatic organisms, especially fish species (Uner *et al.*, 2006; Banaee *et al.*, 2008). Certain physiological and biochemical processes may be significantly damaged when pollutants such as pesticides enter into the organs of fishes (John, 2007; Banaee *et al.*, 2011).

After exposure to different concentrations of pesticides in water, the fish absorbs them in its gills, skin or gastrointestinal tract. In other words, due to their lipophilicity, most pesticides easily permeate the biological membranes and it increases the sensitivity of fish to aqueous pesticides. Then, these compounds are rapidly metabolized and may be bioconcentrated in various tissues of fish. Bioaccumulation occurs if the pesticides are slowly metabolized or excreted from the body. As the amount of pesticide increases, it becomes more harmful to the consumer or animal. Accumulated pesticides can cause death or long term damage. The low biodegradation and the high lipid solubility of some pesticides such as organochlorine pesticides have led to problems with the bioconcentrations of these compounds in different

tissues of fish. In addition, since some fish are lower on the food chain, bioaccumulation of pesticides may increase in tissues of their predators and consumers, such as humans and thus affect their health and survival. So, the bioaccumulation of these contaminants in fish and the potential biomagnification in humans are perceived as threats (Favari *et al*., 2002). Ballesteros *et al*. (2011) showed that pesticides may be transformed in various tissues of fish during the initial 24 hours of exposure. However, some difference in the distribution of the original compounds and their metabolites may exist between tissues relating to differences in metabolism rates.

Bioaccumulation rate of pesticides in fish depends on the species, life stages, the amount of fat reserve in different tissues and diet of fish, chemical and physical properties of pesticides and of water. For the elimination and detoxification of toxic compounds, fishes have developed detoxification mechanisms involving the liberation of biotransforming enzymes collectively termed xenobiotic metabolizing enzymes. Enzymatic biotransformation of pesticides can potentially alter their activity and toxicity. Enzymes participating in the biotransformation of pesticides are classified into phase I and phase II enzymes. The phase I enzymes, particularly cytochrome P450 involving CYP1A and CYP3A, are commonly involved in the biotransformation of both exogenous and endogenous compounds, thereby creating a more polar hydrophilic compound. The common pathways of biotransformation of different kinds of pesticides include three cytochrome P450 mediated reactions: *O*-dealkylation, hydroxylation and epoxidation of pesticides (Kitamura *et al*., 2000; Straus *et al*., 2000; Behrens and Segner, 2001; Nebbia, 2001).

Cytochrome P450 isoenzymes or isoforms are of vital significance in the metabolism of many xenobiotics and endogenous compounds (Wickramasinghe, 2017), and levels of total cytochrome P450 have been employed to detect the presence of pollutants in aquatic environment owing to its high sensitivity to xenobiotics, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), and have been so far proved to be the most responsive indicator (Schlenk and Di Giulio, 2002). EROD activity have been seen to be induced in liver microsomes of rainbow trout exposed to dieldrin and endrin (Janz *et al*., 1992), *Ancistrus multisipinis* exposed to deltamethrin (Assis *et al*., 2009), roundnose grenadier in response to DDT exposure (Lemaire *et al*., 2010) and Indian major carp, *L. rohita* exposed to sub-lethal concentrations of chlorpyrifos (Rai *et al*., 2010). Pal *et al*. (2011) also reported the induction of CYP1A, CYP2B, CYP2E1 and CYP3A4 in *Channa punctatus* exposed to chlorpyrifos and dicofol.

In general, the metabolic transformation of pesticides in living organisms goes through various metabolic reactions. Phase I reactions primarily carried out by microsomal mixed function oxidase is responsible for converting xenobiotics into more soluble products. Although these products are generally less toxic than the parent compound, more toxic metabolites may also occur, as in the cases of parathion (Chambers *et al*., 1991) and malathion (Buratti *et al*., 2005) and endosulfan (Lee *et al*., 2006). Such *in vitro* studies with phase I enzyme provide explicit details of the chemical nature of metabolites and intermediates, the pattern of their formation and the metabolic pathways of xenobiotics (Kim *et al*., 2005).

Perkins *et al*. (1999) reported an induction of several cytochrome P450 isoforms with low specificity in *Ictalurus punctatus* upon exposure to aldicarb, a carbamate pesticide and the fungicide propiconazole has also been reported to induce the expression of hepatic cytochrome P4501A in brown trout (*Salmo trutta*) (Egaas *et al*., 1999). Strauss *et al*. (2000) showed the induction of CYP1A activity in fingerling channel catfish when exposed to chlorpyrifos and parathion and also reported that aroclor 1254 did not induce the CYP 450's responsible for the metabolism of the phosphorothionate insecticides. According to Stuchal *et al*. (2006), CYP1 and CYP3 family isozymes in channel catfish demethylated methoxychlor in liver and intestine. Their results suggested that the formation of estrogenic metabolites from methoxychlor would be more rapid in catfish co-exposed to CYP1A inducer, 3-methylcholanthrene. Some of the pesticides are extremely poisonous to fish species and may affect the fish population largely due to the inhibition of cytochrome P450 enzyme which reduces the capability of fish to face chemical stress. One such pesticide carbofuran has recently been reported to inhibit EROD activity in sea bass (*Dicentrarchus labrax*) (Moreno *et al*. 2011). Manar *et al*. (2011) have reported induction and inhibition of cytochrome P450, mainly of the family 1-3 by 18 pesticides belonging to organophosphorous, pyrethroids and benzoyl urea pesticides.

CYP2E1 activity shows induction as well as inhibition in humans and other experimental animals, based on the type of xenobiotic exposure. Studies have reported an induction of CYP2E1 activity upon exposure to pesticides such as carbaryl (Tang *et al*., 2002), imidacloprid (Schultz-Jander and Casida, 2002) and parathion (Mutch *et al*., 2003) while inhibition of the same upon exposure to pesticide chlorpyrifos have also been reported in humans (Tang *et al*., 2002). A similar result has been published in Atlantic cod (*Gadus*

morhua) exposed to ketokonazole, ethynylestradiol and nonylphenol (Hasslberg *et al*., 2005). Rai *et al*. (2010) also reported inhibition of CYP2E1 by chlorpyrifos in fish, *Labeo rohita*.

Liver being the main organ of detoxification, effects on fish liver are of increasing importance to environmental toxicologists and have been the subjects of many recent reports. Scientists from throughout the world have reported the effects of pesticides on hepatic cytochrome P450 experimenting with different fish species. The diversity of these reports is illustrated by the following examples: Barnhill *et al*. (2003) reported that dieldrin stimulated the expression of CYP 450 in Rainbow Trout, *Oncorhyncus mykiss* when fed with 0.3 mg dieldrin/kg/day for 9 weeks and then an intraperitoneal (ip) dose of benzo-a-pyrene (10 µmol/kg). Female Nile Tilapia, *Oreochromis niloticus*, showed higher induction of EROD activity than their male counterpart when exposed to herbicide, paraquat (Figueiredo-Fernandes *et al*., 2006). Mortensen and Arukwe, (2006) reported CYP3A and PXR mRNA induction in the liver of Atlantic salmon, *Salmo salar* exposed to p, p' DDE (Dichlorodiphenyldichloroethylene). Barberm *et al*. (2007) reported the induction of CYP2 and CYP 3 families in largemouth bass *Micropterus salmoides* after exposure to p, p'-DDE. Similarly, Blum *et al*. (2008) also reported an induction of CYP3A in largemouth bass *Micropterus salmoides* exposed to methoxychlor. *Gasterosteus aculeatus*, a three-spined stickleback showed moderate induction of EROD activity in a transient manner after treatment with different doses of prochloraz fungicide for different periods of time suggesting an extensive metabolization (Sanchez *et al*., 2008).