PART-I

Xenobiotics and Enzymatic Detoxification
Xenobiotics: their detoxification in green mussel *Perna viridis* (Linnaeus, 1758) and the nano level impact in *Oreochromis mossambicus* (Peters, 1852) reproduction.

PREAMBLE

It is well known that xenobiotics impair the aquatic organisms at different levels. The potent and virulent xenobiotics at the higher concentrations cause organism toxicity i.e. killing the animals, the moderate concentrations cause organ toxicity i.e. impair the physiology of related organs, where as the trace (< nano) level concentrations (usually undetectable by even sophisticated instrument, thus we feel safe therefore allowed with in the limits of National and international standards, as safe) impair cellular/molecular system. In most cases the exposed animal able defend against the xenobiotics through different mechanism (avoid by escaping from the area, mucous production, reducing its physiology etc) for their existence. In the cellular level metabolic enzymes come for rescue. The secrete enough specific enzyme to neutralize the effect of xenobiotics to its capacity, otherwise the failed animal subsequently die.

Further the long time exposure to trace concentration (or one time exposure to higher also moderate concentrations) can express it's impact after a long time at molecular level i.e. they majoritily get disrupted their endocrine functioning either as agonist or antagonist. Thus body regulatory mechanism become tilted and most particularly affect the reproductive system, leads to irregular sex disorder causing imposex, ambiguous sex and even sex reversal in the susceptible organism. Continuation of such sex reversal in a particular population results with the development of unisex population. Generally sexual differentiation/dimorphism is essential in many animals to proceed nuptial activity, thus the morphologically unisex population unable to breed and propagate, ultimately leads to the extinction of the species. Therefore trace level xenobiotics are more dangerous in the eradication of a species.

Therefore the present study was designed to understand the entire nature of sublethal levels of pollutants effect in aquatic organism. In this regard two different environment and two different animals were chosen and each is dealt differently under two part of the thesis.

The Part-I of the thesis deals about the predominant coastal pollution (oil) and their defense by employing different enzyme, and the responsive enzymes are recognized as biomarker enzymes and the best responsive one is designated as 'robust biomarker'. In this study the marine green mussel *Perna viridis* was considered as an animal model.

In Part-II, the universally dominant pollution sewage was dealt with and even the treated sewage water impact in the riverine system was studied using *Oreochromis mossambicus* as an animal model.

The amalgamation of representative major aquatic systems (ie coastal and freshwater), pollutants (hydrocarbon and sewage) in the animal models (invertebrate and vertebrate), the impacts [in cellular (enzyme defense) and whole animal (morphological variation)] are expected to furnish a full insight onto the xenobiotics exposure through this study.
1. GENERAL INTRODUCTION

Pollution becomes omnipresent in the living world and only the concentration and pollutant type vary with places. Once Antarctica was considered as a datum point (reference point ie Standard for purity) to compare the pollution level. Now many reports reveal the pollution in Antarctica. In 1950’s it was said that ‘pollution was an illusion’, and during mid 60’s the scenario changed polluted and non polluted areas were discriminated and suggested ‘solution to pollution is dilution’ but during the on set of 90’s the saying was totally changed as regions of polluted and least polluted (no non polluted areas). Thus pollution become omnipresent, however many organism are also living in the biosphere with the circumstance that even the mother milk contains some pesticide residues. Although many eco-development and pollution abatement programme are enforced all over the world to compare pollution and delimited National and International standard for maximum allowable limits of each pollutants in waters for different purposes.

Therefore the present study was propounded to understand and try to get some answers the following interrogation.
1. How the organism able to thrive in polluted water bodies?
2. Is there any defense mechanism available to safe guard them?
3. If so are they totally relieved of the ill effect of pollutants or they are also get affected but survive?!
Or they may have any longterm ill effect.

1.1 Bioindicators and Biomarkers for Pollution

It is paradox that some organism flourish well in certain polluted condition. They formed as pollution indicators for example the prevalence of Brachionus sp and Cyanobacteria indicated the organic (sewage)pollution. Similarly many organism's exerts some physiological or biochemical response to pollutant
exposure such responsive biomolecules, specific to particular pollutant become the molecular biomarker. When compared with chemical residue analysis, biomarkers have an advantage of being a measure of the stress incurred in the organism, and so, are more biologically relevant. However, chemical analysis for defined pollutants gives well defined concentration data, biomarkers are often more difficult to interpret. When compared with population parameters, the opposite is the case. Biomarkers are often more easily quantifiable than population parameters, such as growth and reproduction, but whereas measurements of population parameters gives an accurate picture of the ecological effects of the pollutant. Biomarkers are not necessarily indicative of a deleterious effect. That is, although all population stresses are necessarily preceded by a biochemical response, all biochemical responses need not be associated with a population stress.

1.2 Mechanisms of Toxicity

Biochemical and physiological mechanisms underlying the toxicity of environmental pollutants. The interaction of toxicants with subcellular components and macromolecules with emphasis on mechanism of action, in particular the type of toxicity (neuro, haemotoxicity etc.), carcinogenesis, endocrine disruption, mutagenesis, and teratogenicity due to pesticides, chemicals, heavy metals, etc.

1.3 Xenobiotics in Aquatic environment

The life is the gift of nature to the planet Earth and is gradually destructed by many man made activities. The human necessity culminates new discovery in every field giving new product and also waste materials after their use. That waste must be added up as pollution factory at every time in all over the world. These pollutants subsequently reach the water bodies like lakes, rivers, ponds, estuaries and ultimately to marine. Each has various sub-ecosystems with marginal overlap
of inhabitants. Most of the hazardous disposals into the aquatic medium that causes serious ill effect on the aquatic life especially the sedentary animals like oysters, mussels, barnacles, anemones etc., of the region (Robert and Van Hook, 1978; White, 1980; Schwarzenbach et al., 2006) The river has lotic water system and the current flow change the availability of its dispersed pollutants. Further, could not be constant that might be bleached by the continuous flowing of water. Moreover the survival organisms have much possibility to escape from this transit pollution while the marine, lake and other lentic water system can change whenever entered into the pollutants or xenobiotics. Xenobiotic is a chemical (or, more generally, a chemical mix) which is not a normal component of the organism which is exposed to it. Xenobiotics, therefore, include most drugs (other than those compounds which naturally occur in the organism), as well as other foreign substances. The term xenobiotic was coined to cover all organic compounds that were foreign to the organism under study. In some situations this is loosely defined to include naturally present compounds administered by alternate routes or at unnatural concentrations.

Xenobiotics are molecules that are not produced in vivo but which are introduced into the body from the environment and subsequently metabolized by the body. Routes of introduction to the body include inhalation (e.g. aromatic hydrocarbons in cigarette smoke), intravenous (e.g. various anesthetics), transdermal and, for most pharmaceutical agents, ingestion (Black, et al., 1995). The metabolism of xenobiotics in our environment is an important field of investigation. However, to minimize risk and optimize therapeutic benefits, it is critical that the metabolism of candidate pharmaceutical agents be elucidated, including identification and properties of key metabolites. Most pharmacologically active agents are lipophilic and their elimination from the body is enhanced by enzymatic modifications that render them more hydrophilic. This accelerates renal or biliary excretion and reduces back adsorption from tubular urine. All major
routes of exposure (liver, lung, nasal mucosa, gastrointestinal tract, and skin) have substantial xenobiotics metabolism capabilities. Since ingestion is a primary route of exposure to xenobiotics, and xenobiotics adsorbed by other routes typically pass quickly through the hepatic portal circulation, the liver is a key site for detoxification and most studies of xenobiotic metabolism have focused on the liver. The increasing levels of industrialization day to day since the end of Second World War. The hydrocarbons are two major groups; they are aliphatic and aromatic compounds. The low molecular weight of the both compounds is toxic then higher molecular weight compounds. The low molecular weighted compounds are gas, liquid and semi-solid in nature but the higher molecular weight compounds are solid and that not easily dissolved in alcohol and water. The aliphatic compounds final products in the form of wax while the aromatic compounds final product in the form of tar or coal. The environment impact is the most important ill effect in all living things, these came from pollution that causative factor are named as ‘pollutant’. These pollutants are coming under the abiotic factors. They are most rapidly using chemical such as Polycyclic Aromatic Hydrocarbons (PAHs), Polybrominated biphenyl (PBB), Halogenated compounds, pesticides etc.

Polycyclic aromatic hydrocarbons (PAHs) and heavy metals are two prevalent classes of persistent contaminants in aquatic and terrestrial environments (Bruce et al 2002). In most cases, the xenobiotic are bioactivated by insertion of molecular oxygen catalyzed by mixed-function oxidases of the cytochrome P-450 system.

The predominant xenobiotics are Polycyclic Aromatic Hydrocarbons (PAH) Planar Halogenated hydrocarbon (PHH), Pesticides, pharmaceutics chemicals. A group of compounds consisting of carbon and hydrogen and having 2 or more condensed benzene ring structures formed various PAHs like Low molecular weight PAHs, such as naphthalene, methyl-naphthalene and
acenaphthene, that have two or three rings, are acutely toxic but noncarcinogenic (Andrew Daugulis and Colleen McCracken., 2003; Ramesh Sharma, et al., 2002) to a broad spectrum of organisms. Sediments in freshwater and marine harbours are frequently contaminated with industrial organic compounds, including polynuclear aromatic hydrocarbons (PAH). PAH are derived from petroleum, spilled fuel, street run-off, and coal tar from coal gasification, creosote treatment of wood, or coke ovens at steel plants (Ringuette et al., 1993).

1.4 Xenobiotic metabolism

Polycyclic aromatic hydrocarbons (PAHs) are potent inducers of several genes; including some encoding “Phase I” and “Phase II” xenobiotic-metabolizing enzymes. These enzymes include cytochrome P450 (P450), glutathione-S-transferases, NADPH quinone reductases or cytochrome c reductase, and UDP-glucuronosyl transferases. The risk of the aquatic life is very complicated to assess due to the complex nature of the hydrocarbon mixtures and the co-occurrence of other contaminants and heavy metals. The presence, abundance and community compositions of benthic invertebrate species are common indicator; the effects and bioassay of the toxicity using invertebrates have been developed and applied some success to discriminate zone of degradation (Bailey et al., 1995).

1.4.1 Phase I Metabolism

Phase I metabolism is the first stage in the elimination of xenobiotics, it is simply stated oxidation, reduction or hydrolysis of a foreign compound. Usually, these are the non-polar and highly lipophilic compounds; it does this directly and indirectly with the ultimate goal of facilitating excretion by the kidneys. Many xenobiotics are lipophilic and almost chemically inert (e.g. PAHs). Phase I is the predominant biotransformation pathway. It generally involves the addition or exposure of functional groups on the xenobiotic, for example by oxidation or hydrolysis. By far the most extensively examined system, from the point of view
of biomarkers, is the mixed function oxidase system (MFO) which involves oxidation by a variety of isozymes of cytochrome P-450 (Sipes, and Gandolfi, 1991). Some compounds can be eliminated solely by Phase I enzymes. Phase I metabolism is of concerned with functionalization that is the introduction or exposure of functional groups (reactive oxygen) on the chemical structure of a compound, making it more polar and thus more water-soluble (Langrand and Toutain, 2000; Lim, et al 2005). Unfortunately some lipophilic compounds are so lipophilic (by size of the molecule or composition of the atoms) that inserting one oxygen in a large molecule is not enough to make the compound water-soluble enough to excrete by water-mediated excreting organs. An option is to “Phase I” it again, inserting another oxygen atom into the molecule, which further increases the polarity of the molecule, which makes it more water-soluble, and do this over and over again until the molecule can be water-mediated and excreted.

1.4.1.1 How the Phase I metabolism is dominated by cytochrome P450.

There are two reasons that explains priority and dominance:
1. The vast majority of compounds metabolized in phase I is processed by cytochrome P450. The reason is the broad substrate specificity of cytochrome P450, compared to other enzymes. Other phase I enzymes can have a high turnover of a specific substrate, but are unable to metabolize the next compound.

2. Cytochrome P450 can generate reactive metabolites (like epoxides) that are more toxic than the mother compound. Whenever toxic reactions by metabolism occur, cytochrome P450 is often responsible. Cytochrome P450 derives it name from its absorption maximum at 450 nm, showing that it is a heme-containing protein.
1.4.2 CYP 450 systems

The P450 isozyme system is the major phase 1 bio-transforming system involved in most of the living system, it was accounting for more than 90% of drug bio-transformations, this system has huge catalytic versatility and a broad substrate specificity. It is also called the mixed-function oxidase system, the P450 monooxygenases and the heme-thiolate protein system. All P450 enzymes are a group of heme-containing isozymes which are located on the membrane of the smooth endoplasmic reticulum. They can be found in all tissues of the human body but are most concentrated in the liver.

Cytochrome P450 (P450) is the collective term for a large superfamily of heme-containing proteins that play an important role in the oxidative metabolism of numerous endogenous and foreign compounds (Nelson et al., 1996). Cytochrome P450 monooxygenases (P450s) are a supergene family of enzymes involved in the biotransformation of a wide range of both endogenous and exogenous compounds. P450s play important roles in the metabolism of many drugs and in the activation of a variety of chemical toxicants and carcinogens in both humans and animals. The name "cytochrome P-450" is derived from the spectral absorbance maximum at 450nm when carbon monoxide binds to CYP in its reduced (ferrous, Fe2+) state. The basic reaction catalyzed by P450 is monooxygenation, that is the transfer of one oxygen atom from molecular oxygen to a substrate. The other oxygen atom is reduced to water during the reaction with the equivalents coming from the cofactor NADPH. The end results of this reaction can be (N-) hydroxylation, epoxidation, heteroatom (N-, S-) oxygenation, heteroatom (N-, S-, O-) dealkylation, ester cleavage, isomerization, dehydrogenation, replacement by oxygen or even reduction under anaerobic conditions.
Function of P450s are involved physiological changes of endogenous substrates like steroid hormone such as cortisol, testosterone, estradiol synthesis in their respective glands of adrenals, testes and ovary from body cholesterol. Another important function is xenobiotic metabolism of exogenous compounds. They react with key enzyme system in the activation of foreign compounds for conjugation, elimination and excretion. It also involved inducible and genetic differences and it may be in part in cancer susceptibility and drug responses

1.4.2.1 Reactions of Cytochrome P 450

\[ \text{RH (substrate)} + \text{O}_2 + \text{NADPH} + \text{H}^+ = \text{ROH (product)} + \text{H}_2\text{O} + \text{NADP}^+ \]

The P450 catalytic cycle involved the following steps when any substrate binds to the enzyme.

(1) The normal state of a P450 with the iron in its ferric [Fe3+] state.
(2) The substrate binds to the enzyme.
(3) The enzyme is reduced to the ferrous [Fe2+] state by the addition of an electron from NADPH cytochrome P450 reductase. The bound substrate facilitates this process.
(4) Molecular oxygen binds and forms an Fe2+OOH complex with the addition of a proton and a second donation of an electron from either NADPH cytochrome P450 reductase or cytochrome b5. A second proton cleaves the Fe2+OOH complex to form water.
(5) An unstable [FeO]3+ complex donates its oxygen to the substrate.
(6) The oxidised substrate is released and the enzyme returns to its initial state. (Dawson 1988)

1.4.3 Cytochrome P450 C Reductase

Mammalian NADPH-cytochrome P450 reductase (CPR) was first identified by Horecker in 1950 as an NADPH-specific cytochrome c reductase. Later studies (Williams, et al.1962) showed that this flavoprotein was situated on
the endoplasmic reticulum (microsomes); although cytochrome c, a mitochondrial protein. Studies in the 1960's linked CPR to the newly discovered microsomal electron transport chains, cytochromes P450 and b5, involved in drug and steroid hydroxylations. This was definitively demonstrated by Lu and Coon when reconstitution of a microsomal fatty acid hydroxylase was shown to require cytochrome P450, CPR, and phospholipid for activity (Lu et al, 1968).

1.4.4 Cytochrome c oxidase

The enzyme cytochrome c oxidase or Complex IV is a large transmembrane protein complex found in bacteria and the mitochondrion. Cytochrome c oxidase, the terminal enzyme in the respiratory chain, is located in the inner membrane of mitochondria and bacteria. It catalyses the reduction of dioxygen to water and pumps an additional proton across the membrane for each proton consumed in the reaction. The resulting electro-chemical gradient is used elsewhere, for instance in the synthesis of ATP (Pecina et al, 2004). It receives an electron from each of four cytochrome c molecules, and transfers them to one oxygen molecule, converting molecular oxygen to two molecules of water. In the process, it binds four protons form the inner aqueous phase to make water, and in addition translocates four protons across the membrane, helping to establish a transmembrane difference of proton electrochemical potential that the ATP synthase then uses to synthesize ATP(Tsukihara et al, 1995). It plays a vital role in enabling the cytochrome a3-CuB binuclear center to accept four electrons in reducing molecular oxygen to water. The mechanism of reduction was formerly thought to involve a peroxide intermediate, which was believed to lead to superoxide production. However, the currently accepted mechanism involves a rapid four electron reduction involving immediate oxygen-oxygen bond cleavage, avoiding any intermediate likely to form superoxide(Voet and Voet, 2004).

\[
4 \text{Fe}^{2+}-\text{cytochrome c} + 8 \text{H}^+ + \text{O}_2 \rightarrow 4 \text{Fe}^{3+}-\text{cytochrome c} + 2 \text{H}_2\text{O} + 4 \text{H}^+_\text{out}
\]
1.4.5 Antioxidant enzymes
The principal enzymatic antioxidants are Catalase, superoxide dismutase, glutathione reductase, glutathione oxidase etc and the non enzymatic factors includes vitamins, elements etc.. These antioxidants serve as an excellent scavenger against potential oxidative stress products (Verlecar et al., 2007). Thus, fight against the cellular damaging free radicals by the way of dismuting into hydrogen peroxide and further converted to water molecules and also fulfilled the loss of electron of free radicals (neutralized). Antioxidant defenses constitute both enzymatic and non-enzymatic parameters, however the predominant enzymes alone used as biomarkers in environmental monitoring studies.

1.4.6 Phase II metabolism

Phase II metabolism conjugates highly water-soluble moieties like glucuronic acid, sulfate, glutathione and others to lipophilic compounds. Thus a new molecule with a lipophilic part and a water-soluble part is generated. As a whole the molecule becomes enough water-soluble that the kidney can easily excrete the compound. Also because of the increased hydrophilicity, reabsorption is reduced or eliminated. For instance oxidating benzene to phenol changes its partition coefficient about 4-fold to a more water-soluble compound. However, in the case of morphine, conjugation (phase II metabolism) to morphine-glucuronide changes its hydrophilicity 187-fold.

Phase III excretes water-soluble compounds out of the cells. Once outside the cell the compounds reach the blood, which transports them to the kidney from where they are excreted into urine. Phase III is not a metabolizing process: it does not chemically change the molecule as in phase I and phase II. Phase III enzymes are especially found on the cells that excrete into bile and urine.
1.5 Polycyclic aromatic hydrocarbons (PAH’s)

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants (Harvey, 1991). PAHs usually occur in complex mixtures containing parent compounds and substituted homologues (Luthy et al., 1994). The PAH composition of a mixture depends on the type of source; for example, PAHs from petroleum are primarily Petroleum hydrocarbons (PHCs) are complex mixtures in both composition and molecular structure, mostly originated from crude oil. PHCs contain a wide range of chemical products such as gasoline, kerosene, fuel oil, jet oil, heavy oil and lubrication oil. PHCs may enter the environment through accidents, spills or leaks, from industrial releases, or as byproducts from commercial or domestic uses. PAHs may arise from PHC pollution (Shouming., et.al, 2004) and from incomplete combustion of organic materials, such as wood, coal and oils. Polycyclic aromatic hydrocarbons (PAH) are organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. Polycyclic Aromatic Hydrocarbons (PAHs) occur naturally in fossil fuels and are a by-product of combustion practices involved in incineration and power generation. PAHs are classified as carcinogens and are closely monitored in the environment. Particularly in drinking and waste water, furnace emissions, soil, and hazardous waste. They belong to a group of omnipresent environmental contaminants formed and released during incomplete combustion or by industrial processes. They are characterized by high mutagenic (Adonis and Gil, 2000) and carcinogenic (Menzie.,et.al., 1992) potential. PAH can arise both naturally and as a result of anthropogenic activity. The latter is a much more important contributor of environmentally hazardous compounds. PAHs have high molecular weight and low volatility, their molecular structure comprises several inlaid benzene rings, present as methylated versions (Youngblood and Blumer, 1975). The health effect of particular concern from exposure to PAHs is cancer (IARC, 1987; U.S. EPA,
It has been found that several methylated PAHs have a greater carcinogenic potential than the parent counterparts (Weis et al., 1998).

Exposure to polycyclic aromatic hydrocarbons (PAH) and planar halogenated aromatic hydrocarbons (PHAH) variously results in carcinogenesis, as well as immune system, reproductive, endocrine and developmental toxicity. PAHs are a group of chemicals that are formed during the incomplete burning of coal, oil and gas, garbage, or other organic substances. PAHs can be formed through natural processes or those related to human activities. More than 100 different PAHs reported. Most PAHs do not occur alone in the environment. Rather they found as mixtures of two or more PAHs. PAHs can occur in the air attached to organic particles, in the soil, or in the sediments as solids. They can also be found in substances such as crude oil, coal, creosote, and road / roofing tar. Evaporation into air does occur very easily. Examples of Polycyclic Aromatic Hydrocarbons are on overhead. Examples: Acenaphthene, Anthracene, Benz[a]anthracene, Benzo[a]pyrene, Benzo[b]fluoranthene, Benzo[ghi]pyrene, Benzo[k]fluoranthene, Chrysene, Dibenz[a,h]anthracene, Fluoranthene, Fluorene, Indeno[1,2,3-cd]pyrene, Phenanthrene, and Pyrene. The carcinogenic potency of PAHs, and other carcinogens and the extent of binding of their ultimate metabolites to DNA and proteins are correlated with the induction of cytochrome P450 isozymes (Sheweita , 2000).Although unmetabolized PAHs can have toxic effects, the major concern in animals is the ability of reactive metabolites to bind to proteins and DNA. Four, five and six ring PAHs have greater carcinogenic potential than two, three or seven ring PAHs. The addition of alkyl groups to PAHs enhances the carcinogenic potential of these compounds.

1.5.1 PAHs exposure through inhalation:

In the environment exposure to PAH vapor or PAHs attached dust and other particles in the air is common. These can come from vehicle exhausts, coal burning, wildfires, agricultural burning, and hazardous waste sites. Other
inhalation exposures come from PAHs present in tobacco smoke, smoke from wood burning fireplaces, and creosote-treated wood products. The naphthalene like petroleum byproducts are induce carcinogenicity through inhalation (NTP., 1992).

1.5.2 PAHs exposure through ingestion:

Cooking meat or other foods at high temperatures those results in charring of the food increases the amount of PAHs in the food (Brittebo and Brandt, 1990;). Mostly the aquatic organisms consume light molecular weight forms because they are on the surface and subsurface of water medium and the higher molecular weight forms are in the sediment. The aquatic birds and mamals got mainly through the exposure to oil spills. These highly lipophilic compounds are quickly absorbed by all routes of exposure. Its storage is mostly in kidney, liver and fat tissue. PAHs do not have long half-lives, usually measured in days. Excretion is primarily by urine and feces.

1.5.3 Biodegradation of PAHs in the environment

Biodegradation, the primary mechanism of PAH removal from the environment(NRC, 2003), is a complex process that involves action of microbial consortia on multiple substrates.

\[
\begin{align*}
R\text{CH}_2\text{-}O\text{-}R' & \xrightarrow{\text{oxidation}} R\text{CH}_2\text{-}O\text{-}R' \rightarrow R\text{CHO} + \text{HOR}' \\
& \xrightarrow{\text{H}^+} \xrightarrow{\text{H}_2\text{O}} R\text{CHO} + \text{HOR}' \\
& \xrightarrow{\text{H}^+} \xrightarrow{\text{H}_2\text{O}} R\text{CHO} + \text{HOR}' \\
\text{OCH}_3 & \rightarrow \text{OH} \\
& \xrightarrow{\text{H}^+} \xrightarrow{\text{H}_2\text{O}} \text{OH} (\text{anisoles only}) \\
R\text{-}S\text{-}R' & \xrightarrow{\text{oxidation}} R\text{-}S\text{-}R' \rightarrow R\text{-}S\text{-}R' \\
& \xrightarrow{\text{reduction}} \xrightarrow{\text{H}^+} \xrightarrow{\text{H}_2\text{O}} \text{R-S-R'} \\
& \xrightarrow{\text{H}^+} \xrightarrow{\text{H}_2\text{O}} \text{R-S-R'} \\
\text{thioether} & \xrightarrow{\text{oxidation}} \xrightarrow{\text{H}^+} \xrightarrow{\text{H}_2\text{O}} \text{sulfoxide} \rightarrow \text{sulfone}
\end{align*}
\]
N-DEALKYLATION

RNHCH₃ → RNH₂ + H₂C=O (esp. R = H)

Imipramine
(anti-depressant)

N-OXIDATION

PhNH₂ → PhNHOH (hydroxylamine)

PhNHR → PhN(OH)R (hydroxylamine)

PhNR₁R₂ → PhNR₁R₂ (amine N-oxide or N-oxide)

DEHYDROGENATION

RCH₂NH₂ → RCH=NH (imine)

imine → RCH=O and NH₃
1.6 Structure and chemical formula of naphthalene

- Formula: C_{10}H_{8}
- MW: 128.17
- SMILES String: c1ccc2ccccc2c1
- Synonyms: Camphor tar; Mothballs; Naphthene; white tar
1.6.1 Properties of Naphthalene

Naphthalene is produced from either coal tar (which contains approximately 10% naphthalene) or petroleum. It is produced by condensation and separation of coal tar from coke-oven gases, or from petroleum by dealkylation of methylnaphthalenes. It occurs as white monoclinic plates, scales, powder, balls, or cakes, with the distinctive odor usually associated with mothballs. Naphthalene melts at 80.2°C and boils at 217.9°C. It has low solubility in water (31.7 mg/L at 25°C), but is more soluble in organic solvents (e.g., alcohol, benzene, ether, and acetone). Naphthalene has a log octanol-water partition coefficient of 3.3. It may degrade some forms of plastics, rubber, and coatings. Naphthalene is sensitive to heat and volatilizes at room temperature, with a vapor pressure of about 0.09 mm Hg. The vapor is heavier than air, with a density of 4.42. Naphthalene sublimes at temperatures above its melting point (ATSDR 2003, HSDB 2003). The general public is potentially exposed to naphthalene through inhalation of ambient and indoor air. The average daily intake of naphthalene from ambient air has been estimated to be 19 µg, based on an average naphthalene concentration of 0.95 µg/m³ in urban and suburban air and an inhalation rate of 20 m³/day (ATSDR 2003). Accidental ingestion of household products containing naphthalene, mainly by children, has been reported. Dermal exposure to naphthalene may occur through handling or wearing of clothing stored with moth repellents containing naphthalene (ATSDR 2003; EPA 1980).

1.6.2 Sources of Naphthalene and its possible way to interact with the environment

Naphthalene is a fused ring bicyclic aromatic hydrocarbon and thus serves as a model for understanding the properties of a large class of environmentally prevalent polycyclic aromatic hydrocarbons (PAHs). Naphthalene and its substituted derivatives are commonly found in crude oil and oil products. Certain PAHs are strong human carcinogens leading to widespread interest in the
microbial metabolism of these compounds. Naphthalene and methyl-naphthalenes occur naturally in fossil fuels such as petroleum and coal, and are produced when organic materials (e.g., fossil fuels, wood, tobacco) are burned. Naphthalene is also produced commercially from either coal tar or petroleum. Naphthalene is frequently present in industrial and automobile emissions and effluents besides in various media in the general environment due to its natural occurrence in coal and petroleum products and emissions, its use as an intermediate in the production of plasticizers, resins, and insecticides, and its use in a variety of consumer products such as moth repellants. In 2002, environmental releases of naphthalene reported under the EPA Toxics Release Inventory (TRI) program reported about 2.07 million pounds in air emissions, 0.03 million pounds in surface water discharges, 0.23 million pounds in underground injection discharges, and 0.37 million pounds in releases to land. Most naphthalene are entering into the environment and discharged to the air, with the largest releases associated with the combustion of plant material and fossil fuels and volatilization from naphthalene containing consumer products.

Since the present work was pivoted around xenobiotics (hydrocarbon pollution) and enzyme molecular defense strategy, an elaborate details on hydrocarbons PAHs (naphthalene) was furnished. Moreover, the marine green mussel *Perna viridis* was used as an animal model for the evaluation of the said defense (detoxifying) enzymes, towards the development of molecular biomarker.