Chapter 1: Introduction and Review of the Literature....
Metallic elements are ubiquitous environmental constituents and some of these are found in all the living organisms where they play a variety of roles. These metals play a dual role in the biological system. In trace amounts they are essential for the entire living organism due to their involvement in numerous physiological processes. They may be structural constituents of biomolecules, enzyme activators, stabilizers as well as component of control mechanism and redox system. On the other hand, these metals when present in excess amount may become toxic. Pollution of the environment and human exposure to metallic elements may occur naturally by erosion of surface deposits of the metallic minerals, volcanic activity, forest fires, from bedrock and surface run off as well as from human activities.

1.1. ESSENTIAL ELEMENTS

Based on the present evidence, 26 of the naturally occurring elements are essential to life. Major elements are carbon, hydrogen, oxygen, nitrogen, sulphar, calcium, potassium, phosphorus, sodium, chlorine and magnesium, while trace elements include iron, iodine, copper, zinc, manganese, cobalt, nickel, molybdenum, boron, selenium, chromium, fluorine, tin, silicon and vanadium. They are essential because they form an integral part of one or more enzymes involved in metabolic or biochemical processes in the body.

1.2. HEAVY METALS

The common definition of the heavy metals is that they are metals with a density greater than 4 g/cm³ (Connell and Miller, 1984). Specific gravity some well-known toxic metallic elements with are Arsenic, 5.7; Cadmium, 8.65; Lead, 11.34; and Mercury, 13.546 (Lide, 1992). There are 35 metals that concern with
Introduction and Review of the Literature

occupational or environmental exposure; 23 of these are the heavy metals. They are antimony, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, zinc and metalloid arsenic (Glanze, 1996). Living organisms require trace amounts of some heavy metals, including cobalt, copper, iron, manganese, molybdenum, vanadium, strontium, and zinc (Kennish, 1992). Non-essential heavy metals are generally toxic to living organisms in fairly small concentration while excess of trace/essential metals may also result in toxic manifestations.

Heavy metal pollution is a problem, which involves the total ecosystem. Trace amount of most heavy metals occur naturally in the environment. Industrial and technical uses of nonessential metals by humans have resulted in an unnaturally high concentration of heavy metals in our environment. Heavy metals become toxic because they are not metabolized by the body, disturb the normal metabolism and accumulate in the soft tissues. They may enter the body through food, water, air or absorption through the skin when they come in contact with humans. When speaking of toxicity due to heavy metals we generally mean, lead, mercury, iron, copper, manganese, cadmium, arsenic, nickel, aluminum, silver, and beryllium because they are so pervasive, or produce toxicity at low concentration. Amongst heavy metals, mercury is one of the most hazardous pollutants of the environment (Du et al., 2005; Augusti et al., 2008).
Mercury is a unique poison in that it incapacitates numerous enzymes in cells, including those used to neutralize free radicals.

200 micrograms of mercury would fit on the head of a pin. According to the Environmental Protection Agency (EPA), dropping that pinhead of mercury into 23 gallons of water would make it unsafe for human consumption.
## Introduction and Review of the Literature

### General

<table>
<thead>
<tr>
<th>Name, Symbol, Number</th>
<th>mercury, Hg, 80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical series</td>
<td>transition metals</td>
</tr>
<tr>
<td>Group, Period, Block</td>
<td>12, 6, d</td>
</tr>
<tr>
<td>Appearance</td>
<td>silvery</td>
</tr>
<tr>
<td>Standard atomic weight</td>
<td>200.59 g·mol⁻¹</td>
</tr>
<tr>
<td>Electron configuration</td>
<td>1s² 2s² 2p⁶ 3s² 3p⁶ 4s² 3d¹⁰ 4p⁶ 5s² 4d¹⁰ 5p⁶ 4f¹⁴ 5d¹⁰ 6s²</td>
</tr>
<tr>
<td>Electrons per shell</td>
<td>2, 8, 18, 32, 18, 2</td>
</tr>
</tbody>
</table>

### Physical Properties

<table>
<thead>
<tr>
<th>Phase</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (near room temperature)</td>
<td>(liquid) 13.534 g·cm⁻³</td>
</tr>
<tr>
<td>Melting point</td>
<td>234.32K (-38.83°C, -37.89°F)</td>
</tr>
<tr>
<td>Boiling point</td>
<td>629.88K (356.73°C, 674.11°F)</td>
</tr>
<tr>
<td>Critical point</td>
<td>1750 K, 172.00 MPa</td>
</tr>
<tr>
<td>Heat of fusion</td>
<td>2.29 kJ·mol⁻¹</td>
</tr>
<tr>
<td>Heat of vaporization</td>
<td>59.11 kJ·mol⁻¹</td>
</tr>
<tr>
<td>Heat capacity</td>
<td>(25°C) 27.983 J·mol⁻¹·K⁻¹</td>
</tr>
</tbody>
</table>
The chemical symbol for mercury, Hg, was derived from its Latin name HYDRARGYRUM, meaning LIQUID SILVER. This name reflects its appearance and its ability to impact a silvery color to the other metals. Occasionally mercury is also referred to as QUICKSILVER, a derivative from the Latin translation of ARGENTUM VIVUM, meaning live or quick silver (D’Itti, 1972).

Mercury is a naturally occurring heavy metal found throughout the environment. It has no known biological function and is highly toxic to humans (Williams et al., 1999). Mercury cycles in the environment as a result of natural and human (anthropogenic) activities. The amount of mercury mobilized and released into the biosphere has increased since the beginning of the industrial age (ATSDR, 1999). Due to industrialization and changes in the environment during the twentieth century, humans and animals are exposed to mercury compounds (Fitzgerald and Clarkson, 1991). Mercury can exist in 3 oxidation states viz. elemental mercury (Hg⁰), mercurous mercury (Hg⁺) and mercuric mercury (Hg²⁺). Mercurous and mercuric mercury can combine with other elements to form either organic or inorganic mercury compounds (Health and Safety Executive, 1996a; Hu, 1998). Mercury occurs in the atmosphere in the form of elemental mercury vapor, which circulates in the atmosphere for up to a year, and hence can be widely dispersed and transported thousands of miles from the sources of emission. In the sediments of rivers, lakes, and the ocean, metallic mercury is transformed within microorganisms into methyl mercury. Most of the mercury in water, soil, sediments, or plants and animals is inorganic mercury salts and organic forms of mercury (e.g., methyl mercury). This methyl mercury biomagnifies in the marine food chain to reach very high concentration in predatory fish such as swordfish, tuna, king mackerel, and shark (Dietz et al.,
2000; Gilmour and Riedel, 2000). Consumption of contaminated fish is the major route of human exposure to methyl mercury. All forms of mercury cause toxicity in a number of tissues and organs, which depends on the chemical form of mercury, the level of exposure, the duration of exposure, and the route of exposure (Zalups, 2000). The distinction between elemental, inorganic and organic mercury is much more important than oxidation states in determining toxicity, as organic mercury compounds are the most toxic (Health and Safety Executive, 1996b; Hu, 1998; Williams et al., 1999).

1.3. PROPERTIES

Mercury is the only metal which is liquid at room temperature over a wide range of temperature, coupled with its almost constant thermal coefficient of expansion over a temperature range from 0°C to 300°C (D’ltri, 1972). This silvery liquid metal is very dense, yet has a high surface tension that causes it to form tiny little perfect spheres in the pores of the rocks it is found in. Mercury has low vapor pressure (0.002 mm Hg at 25°C) due to which, at 25°C, an atmosphere that is fully saturated with mercury vapors (approximately 18mg/m³), which are released from liquid metallic mercury. The levels attainable in indoor air at room temperature can therefore greatly exceed safe levels and result in poisoning (ATSDR, 1999). Mercury vapors are colorless and odorless. Mercury metal is poor conductor of heat, but it is a moderately a good conductor of electricity. Mercury easily forms alloy (known as amalgam) with almost all common metals, including gold, aluminium and silver, but not iron. When frozen, mercury forms crystals in the rhombohedral system at low pressure, and in the tetragonal system at high temperature. Elemental mercury is soluble in lipid and nitric acid while insoluble in hydrochloric acid (Zalups, 2000).
Inorganic mercury compounds are formed when mercury combines with other elements such as sulphur, chlorine and oxygen. These are also called mercury salt. Most of inorganic mercury compounds are in the form of white powder or crystals, except for mercuric sulfide (also known as cinnabar), which is red, and turns black after exposure to light. Divalent salts (mercuric) are more soluble in water than monovalent salts (mercurous) (ATSDR, 1999).

Mercury forms covalent bonds with carbon and the relating compounds show a variety of physical, chemical and toxicological properties. Some microorganisms (bacteria and fungi) and natural processes can change the mercury in the environment from one form to other. Organic mercury compounds are volatile at room temperature (as metallic form). Alkyl mercury compounds (methyl or ethyl mercury) are more toxic than aryl mercury compounds (phenyl mercury) (ATSDR, 1999; Williams et al., 1999).

1.4. USES

Elemental mercury is used in thermometers, barometers, batteries, electric lamps and pressure-sensing devices. It is also used in chlor-alkali industry, refining processes, preparation of lubrication oils, and in dental amalgams. Another important use of elemental mercury is to extract gold from its ore or impurities by amalgamation (ATSDR, 1999).

Inorganic mercury was used in the past in laxatives, skin lightening creams and soaps, and in paint industries, color industries, in pharmaceutical industries for the preparation of drugs and disinfectants. It is also used as fungicide. Mercuric chloride is a topical antiseptic or disinfectant agent while mercuric sulfide is used in vermilion and in antifouling paints (Health and Safety Executive, 1996a;
Organomercurial compounds were widely used as bactericide-fungicide agents to protect water based paints from bacterial fermentation and also used to control fungus disease through their application as seed dressing. They were also used in the manufacture of mercury fulminate; this was used as a constituent of detonators for the production of explosives. But these are banned due to their adverse health effects (Health and Safety Executive, 1996a).

1.5. SOURCES OF EXPOSURE

Mercury found in the world's environment comes from two major sources. First, it occurs in the nature as metallic mercury (in the form of minute droplets in rock layers) and inorganic mercury compounds in the form of mercury sulfide (concentrated mercury ore). Mercury is an element in the earth's crust and transported to the oceans as a result of natural land erosion. Due to high vapor pressure of metallic mercury, it is likely to be evaporated from the soils into the atmosphere and widely distributed into the entire environment (D’Itri, 1972).

The second source is the direct or indirect human activities, which are related to various uses of mercury. A major source of exposure to humans for elemental mercury is through inhalation in occupational settings (US EPA, 1993a; ATSDR, 1999) and for general population is elemental mercury released in the mouth from dental amalgam fillings (US EPA, 1993b; US Public Health Service, 1993). Occupational exposure to mercury is generally to mercury vapor and can occur in dentistry, mining, and the manufacture of electrical equipment and medical instruments (Evnas, 1998). Thimerosal, a mercury-containing preservative, is a
Introduction and Review of the Literature

component of some vaccines but has been phased out from most routine childhood vaccinations. Mercury can also be found in ayurvedic medicinal products (Goldman and Shannon, 2001; Saper et al., 2004). The general population is usually not exposed to inorganic mercury compounds to any significant extent today, as most products containing these compounds have now been banned. Limited exposure could occur through the use of old cans of latex paint, which until 1990 could contain mercury compounds to prevent bacterial and fungal growth. The most important organic mercury compound, in terms of human exposure, is methyl mercury. Methyl mercury exposure occurs primarily through the diet, with fish and fish products as the dominant source. Sources of past exposure to methyl mercury include fungicide-treated grains and meat from animals fed such grain. However, fungicides containing mercury are banned today, and this source of exposure is now negligible (ATSDR, 1999).

1.6. BIOGEOCHEMICAL CYCLE

Mercury occurs naturally in the elemental form and/or its sulfide ore known as cinnabar (HgS). A small concentration of mercury is found throughout the lithosphere, the atmosphere, the hydrosphere and the biosphere. Although volcanoes and other natural sources release some elemental mercury to the environment, anthropogenic emissions from coal-fired electric power generation facilities, chlor-alkali production, waste incineration, and other industrial activities now account for approximately 70% of the 5,500 metric tons of mercury that are released into the earth's atmosphere each year (UNEP, 2002). Elemental mercury is readily aerosolized because of its low boiling point, and once airborne it can travel long distances to eventually deposit into soil and water. The inorganic form of mercury, when either bound to airborne particles or in a
gaseous form, is readily removed from the atmosphere by precipitation and is also dry deposited. Wet deposition is the primary mechanism for transporting mercury from the atmosphere to surface waters and land. Even after it deposits, mercury commonly is emitted back to the atmosphere either as a gas or associated with particles, to be redeposited elsewhere. Natural transformations and environmental pathways of mercury are very complex and are greatly affected by local conditions.

There are two main types of reactions in the mercury cycle that convert mercury through its various forms: oxidation-reduction and methylation-demethylation. In oxidation-reduction reactions, mercury is either oxidized to a higher valence state (e.g. from relatively inert Hg\(^0\) to the more reactive Hg\(^{2+}\)) through the loss of electrons, or mercury is reduced, the reverse of being oxidized, to a lower valence state.

### 1.6.1. Mercury Oxidation

The oxidation of Hg\(^0\) in the atmosphere is an important mechanism involved in the deposition of mercury on land and water. Elemental mercury (Hg\(^0\)) can volatilize relatively easily and be emitted into the atmosphere, where it may be transported on wind currents for a year or more and be re-deposited in the environment for further cycling. Atmospheric oxidation or reduction of elemental mercury vapor (in the air), may occur in the presence of dissolved ozone, hydrogen peroxide, hypochlorite, or organoperoxy compounds. In contrast, Hg\(^{2+}\) has an atmospheric residence time of less than two weeks due to its solubility in water, low volatility and reactive properties. In rain water, mercury undergoes oxidation by ozone to Hg\(^{2+}\) and other forms. Hence, when Hg\(^0\) is converted to Hg\(^{2+}\), it can be rapidly taken up in rain water, snow, or
adsorbed onto small particles, and be subsequently deposited in the environment through "wet" or "dry" deposition.

In the Arctic, the conversion of Hg⁰ to Hg²⁺ in the atmosphere occurs very rapidly in a phenomenon known as "mercury depletion" at the end of dark polar winters. Some inorganic mercury compounds, such as mercuric hydroxide [Hg(OH)₂], undergo rapid reduction to monovalent mercury by sunlight (Munthe and McElroy, 1992). When the sun rises in the spring, atmospheric Hg⁰ is converted photochemically to Hg²⁺ in the presence of reactive chemicals released from sea salt (for example, bromine and chlorine ions) and mercury levels in the atmosphere are "depleted" as the Hg²⁺ is then deposited on snow and ice surfaces.

1.6.2. Mercury Methylation

In the environment, mercury is transformed into methyl mercury when the oxidized or mercuric species (Hg²⁺), gains a methyl group (CH₃). The methylation of Hg²⁺ is primarily a natural, biological process resulting in the production of highly toxic and bioaccumulative methyl mercury compounds (MeHg⁺) that build up in living tissue and increase in concentration up the food chain, from microorganisms like plankton, to small fish, then to fish eating species like otters and loons, and humans. The organomercurials also bioaccumulate and biomagnify so that top predators tend to carry much higher levels of organomercurials than organisms lower down the food chain. Many fish contain organomercurial compounds at high levels to be a health hazard to humans consuming them.
The process by which methyl mercury is accumulated in aquatic environments by fish is shown in Figure 1.1, and involves the conversion of inorganic mercury into organic mercury which is accumulated up through the food chain. The process of environmental mercury methylation involves prokaryotic microbes, with the first evidence that methanogenic Archaea were involved being shown by Wood and coworkers (Wood et al., 1968). Subsequently, sulfate-reducing bacteria in anaerobic sediments have also been shown to methylate mercury (Champier et al., 2004), with vitamin B12 (cobalamin) acting as the methyl carrier in the methylation reaction in *Desulfovibrio desulfuricans* (Choi and Bartha, 1993). The formation of methyl mercury is enhanced at low pH and higher mercury concentrations in the sediment (Gilmour and Henry, 1991). Some yeast species (e.g., *Candida albicans* and *Saccharomyces cerevisiae*) are also capable of methylating mercury at lower pH and can reduce ionic mercury species to elemental mercury.
as well. Lakes that have been acidified by acid rain or industrial runoff favor the methylation of mercury, although such conditions also decrease the abundance of fish species, which biomagnify mercury in the food chain. Formation and breakdown of organic mercury compounds appear to be dependent upon the same microbial and abiotic processes as in water (Andersson, 1979), and the methylation of mercury is decreased by increasing chloride ion concentration (Olson et al., 1991), although the presence of chloride ions has been suggested to increase the rate of mercury release from sediments (Wang et al., 1991). In soil, the complexing of elemental mercury with chloride ion and hydroxide ion to form various mercury compounds is dependent upon pH, salt content, and soil composition.

Alkalinity, or pH, plays a strong role in regulating the process because it is affected by, and in turn affects, the adsorption of various forms of mercury on soil, clay and organic matter particles, thus influencing mercuric ion availability. Acid rain may increase biomethylation as more MeHg is formed under acidic conditions. Mercury can be bound by sulfide ions and made unavailable for methylation; however, sulfate may stimulate growth of certain methylating microbes. Organic matter can stimulate microbial populations, reduce oxygen levels, and therefore increase biomethylation. Biomethylation increases in warmer temperatures when biological productivity is high, and decreases during the winter. Land use changes affecting some of these variables can result in increased rates of mercury methylation. For example, the construction of hydroelectric dams can mobilize mercury stored in the submerged forest floor and vegetation. The presence of organic matter (in the form of newly submerged vegetation) in combination with anaerobic conditions can stimulate microbial growth and lead to elevated methyl mercury levels.
In general, the form of mercury in the environment varies with the season, with changes in organic matter, nutrient and oxygen levels and hydrological interactions within an ecosystem. In addition, the quantity and forms of mercury are, to a large extent, a function of emission sources and transportation processes. All of these variables in turn affect the global mercury budget.

1.7. SYMPTOMS/CLINICAL FEATURES

1.7.1. Elemental Mercury

Acute inhalation of elemental mercury vapor produces symptoms similar to metal fume fever like cough, dyspnoea, tightness or burning pain in chest and pyrexia. Severe cases have other symptoms including respiratory distress, pulmonary oedema, pneumonia, fibrosis and death may sometimes occur. Neurotoxicity may also occur with symptoms such as tremors, emotional lability, headaches and polyneuropathy (damage to many nerves) (Goldwater and Clarkson, 1972; Hu, 1998; Baldwin and Marshall, 1999). Chronic exposure to elemental mercury has the characteristic symptom of tremor. The other symptoms are known as "mercurial erythremia", and this definition contains a wide constellation of symptoms, including excitability, memory loss, insomnia, timidity and sometimes delirium (Goldwater and Clarkson, 1972; Hu, 1998; Baldwin and Marshall, 1999; Williams et al., 1999). This disease was found in occupational exposure in the felt-hat industry, and lead to the expression "mad as a hatter" (Hu, 1998). The extensive use of mercury in the fur, felt and hat industry was the cause of "mad hatter disease", a condition characterized by delirium and hallucinations and inspiration for the Mad Hatter in Lewis Carroll's Alice in Wonderland (Baldwin and Marshall, 1999).
1.7.2. Inorganic Mercury

Inorganic mercury mainly causes poisoning by ingestion, and symptoms of an acute intake are due to the severe gastro-intestinal (GI) corrosion, which are haemorrhagic gastroenteritis, stomatitis (inflammation of the mouth), nausea, vomiting (may contain blood), diarrhea (may contain blood), abdominal pain and finally acute renal failure, cardiovascular collapse and shock. A lower level chronic intake may cause milder GI inflammation, gingivitis (inflammation of the gums), loosening of the gums, hypertension, tachycardia, nephrotic syndrome and also the symptoms similar to erethism. Skin exposure to inorganic mercury salts may cause exfoliative dermatitis (Health and Safety Executive, 1996b). Proteinuria, a condition in which urine contains an abnormal amount of protein, is one of the primary symptoms associated with mercuric mercury exposure (Hu, 1998).

1.7.3. Organic Mercury

Acute ingestion of organic mercury compounds can cause diarrhea, tenesmus, and blisters in the upper GI tract and there may also be symptoms of neurotoxicity (nerve damage) like paraesthesia, impaired peripheral vision, muscle weakness, irritability, memory loss and depression. There is also an increased risk of fetal toxicity, with effects including mental retardation and retention of primitive reflexes. The fatal dose of organic mercury is estimated to be between 10 and 60 mg/kg of body weight, but symptoms start when greater than 1.7 mg/kg has been ingested (Health and Safety Executive, 1996b). The sign of toxicity is a neurological syndrome caused by severe mercury poisoning. Symptoms include ataxia, numbness in the hands and feet, general muscle weakness, narrowing of the field of vision and damage to hearing and speech. In
extreme cases, insanity, paralysis, coma and death follow within weeks of the onset of symptoms. A congenital form of the disease can also affect fetuses in the womb (Clarkson and Magos, 2006). Chronic exposure to organic mercury may cause symptoms similar to chronic inorganic mercury poisoning, but by the time tremor develops, paraesthesia and muscle weakness are often also present. In advanced cases, ataxia and dysarthria are accompanied by a loss of peripheral vision, hearing impairment and sensory deficits in the limbs (Matthew and Lawson, 1979; Hu, 1998).

1.8. TOXICITY

Although all forms of mercury are to a greater or lesser extent toxic. The exception to this has been thought to be metallic mercury (Hg\textsuperscript{0}), but ingestion of this over long time periods has adverse health effects (Yoong, 2006), the alkyl organic mercury compounds, methyl mercury and dimethyl mercury are generally considered to be the most toxic. This is because they are readily absorbed by the gut, can cross the blood-brain barrier, bioaccumulate, and are not easily eliminated from the body (Gochfeld, 2003). Organomercurials cause serious neurological disorders and their effect on neonates is more severe than on adults.

Mercury exerts its toxicity by the metal or its ions binding to sulphydryl groups in the body. These groups may be part of some enzymes, and hence mercury and its compounds are potent inhibitors of some enzymes (Goldwater and Clarkson, 1972; Timbrell, 1995; Williams et al., 1999). Mercury also blocks the transport of potassium into cells and also blocks the transport of sugars. These effects are due to the binding of mercury to the -SH groups in or on the cell membrane. Once inside the cell, the metal may be sequestered in an inactive combination, or it
may react with enzymes or other compounds to elicit other toxic effects. Once inside the body, mercury undergoes a process called biotransformation. The carbon-mercury bond in organic compounds is broken, which in alkyl mercury compounds is known as demethylation, but methylation also occurs. Intakes of elemental and divalent mercury cause the interconversion of the forms to each other (Baldwin and Marshall, 1999). An intake of elemental mercury vapor is absorbed through the lungs then is oxidized in red blood cells to Hg$^{2+}$. It is also taken up by the brain and the fetus and is also metabolized to Hg$^{2+}$ in these tissues, and the mercury then becomes trapped in these sites as it is ionized. Organic mercury is able to distribute to the central nervous system (CNS), where it is oxidized (demethylation of alkyl mercury compounds) to Hg$^{2+}$ and leads to neurological damage. Exposure of the body to mercury in any of its forms stimulates the kidney to produce metallothionein, a metal-binding protein that affords partial protection against mercury toxicity (Hu, 1998).

1.8.1. Elemental Mercury

Elemental mercury is not well absorbed by the GI tract, but its vapor is well absorbed by the lungs. Its half life in the blood is around 60 days, but as mentioned above, it is fat soluble so it is able to cross the blood-brain barrier and the placenta. In these tissues and in the kidney it may be oxidized by catalase and hydrogen peroxide into mercuric mercury, and may be retained by the kidney and brain for years (Health and Safety Executive, 1996b; Hu, 1998; Baldwin and Marshall, 1999).

1.8.2. Inorganic Mercury

Inorganic mercury compounds can be absorbed through the GI tract but also through the skin. Because large overdose damages the GI mucosa, the loss of this
barrier further enhances the absorption through this route. Once it has been absorbed, it breaks down to metallic and mercuric mercury, but relatively little crosses the blood brain barrier. Its half life in the blood is around 40 days but it may be retained in the kidney as mercuric mercury (Health and Safety Executive, 1996b; Hu, 1998; Brodkin et al., 2007).

1.8.3. Organic Mercury

Organic mercury compounds, particularly methyl mercury, can evaporate and undergo pulmonary absorption, and are also well absorbed by ingestion. However, very little of these compounds is absorbed through the skin. As it is lipid soluble, once it enters the body it crosses the blood-brain barrier and the placenta and appears in breast milk. It also concentrates in the kidney and the CNS. The half life of organic mercury is around 70 days in the body (Hu, 1998; Brodkin et al., 2007).

1.9. DIAGNOSIS

Diagnosis of mercury poisoning is usually made by obtaining a complete history and performing a physical examination. In addition, laboratory tests may demonstrate increased mercury levels. Background blood mercury levels, however, do not exclude mercury poisoning, because it has a relatively short half-life in blood.

1.9.1. Elemental Mercury

Increased mercury vapor concentrations can be measured in exhaled air from people with dental amalgams, but the biological significance is uncertain. Also unclear is the significance of the slight increase in urinary mercury excretion detected after dental amalgams are placed.
1.9.2. Inorganic Mercury

Inorganic mercury exposure can be measured by determining urinary mercury concentration, preferably using a 24-hour urine collection. Results greater than 10 to 20 μg/L are evidence of undue exposure, and neurologic signs may be present at values greater than 100 μg/L. However, urinary mercury concentration also does not necessarily correlate with chronicity or severity of toxic effects, especially if the mercury exposure has been intermittent or variable in intensity. Whole blood mercury concentration can be measured, but values tend to return to normal (20 μg/L) within 1 to 2 days after the exposure to metallic mercury vapor ends.

1.9.3. Organic Mercury

Although methyl mercury can be measured in blood or hair specimens, collection of specimens requires special mercury-free collection materials and rigorous control of contamination. Such testing is usually conducted in a research setting. In the general population, the mercury level in hair is usually 1 ppm or less.

1.10. TREATMENT

The most important and most effective treatment involves identifying the mercury source and ending the exposure. Children who have had mercury poisoning should undergo periodic follow-up neurologic examinations by a pediatrician.

1.10.1. Elemental and Inorganic Mercury

Mercury accumulates in the blood, CNS, and renal tissues and is very slowly eliminated. Severe or symptomatic mercury poisoning can be treated by
chelation therapy, but whether it decreases toxic effects or speeds recovery in people who have been poisoned is unclear. Indications for chelation therapy after mercury intoxication are not firmly established (Baum, 1999). However, chelation therapy is typically reserved for those with evidence of a large mercury burden demonstrated by biological monitoring (measurement in hair, urine, or blood) or clinical manifestations of severe poisoning. Elimination of elemental and inorganic mercury is greatly enhanced by chelating agents, including succimer, D-penicillamine, and dimercaptopropanesulfonate. Chelating agents increase urinary mercury excretion, but their efficacy is uncertain. Severe mercury poisoning should be treated by or in consultation with a physician who has experience in this area. The only effective complexing and chelating agents that remove mercury from the body contain thiol groups (Kostyniak and Clarkson, 1981).

1.10.2. Organic Mercury

There is no chelating agent approved by the US Food and Drug Administration (FDA) that is effective for methyl mercury or ethyl mercury poisoning. Chelation has been used in cases of severe intoxication. Compared with other forms of mercury, organic mercury is significantly more resistant to removal from the body. Moreover, chelation therapy for organic mercury intoxication can be harmful; the agent dimercaprol appears to increase brain mercury concentrations and is contraindicated in the treatment of organic mercury poisoning. The chelator proven to be most effective in the treatment of severe organic mercury poisoning is succimer (Bates, 1998). Data have also identified a role for the drug N-acetylcysteine in the chelation therapy for methyl mercury poisoning (Ballatori et al., 1998).
1.11. PREVENTION

Organic mercury fungicides, including phenyl mercury (once used in latex paints), are no longer licensed for commercial use. Electronic equipment has replaced many mercury-containing oral thermometers and sphygmomanometers in medical settings (Table 1.1). The American Hospital Association agreed to phase out mercury use by its members (Goldman and Shannon, 2001). The purpose is to prevent pollution from mercury emissions from medical waste incinerators, because most of the mercury that is used in hospitals is likely to end up in the waste stream. In New Delhi, St. Stephen’s hospital has attempted to replace all mercury thermometers with digital alternatives. Sir Ganga Ram Hospital, New Delhi, Max Hospital, New Delhi and Holy Family hospital, New Delhi are also eliminating mercury from their organization in gradual manner (Toxics Link Factsheet, 2003).

The risks of exposure to methyl mercury from fish have to be balanced with the health benefits of eating fish. Fish is a source of high-quality protein as well as unsaturated fatty acids and other beneficial nutrients. For some populations, locally caught fish may be the only good alternative for a nutritious diet. If fish with lower mercury levels are available, then it is prudent to substitute these rather than eat fish that have methyl mercury advisories or commercial fish, such as swordfish and tuna, which are known to have higher mercury levels. Inorganic salts have limited use as antiseptics, although thimerosal is still available. As a precautionary measure, ethyl mercury in vaccines is being reduced or eliminated from vaccine preparations as quickly as manufacturers can alter their production processes and obtain FDA approval for the reformulated materials.
### Table: 1.1 Mercury Applications and Alternatives

<table>
<thead>
<tr>
<th>Mercury Applications</th>
<th>Alternatives Available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlor-alkali production (chlorine and caustic soda)</td>
<td>Membrane cell technology is the best available technology for production of chlor-alkali.</td>
</tr>
<tr>
<td>Dental amalgam</td>
<td>A number of new alternatives, such as gold, silver, gallium, ceramic and porcelain are available.</td>
</tr>
<tr>
<td>Medical thermometers</td>
<td>Electrical and electronic thermometers, glass thermometers containing a Ga/Sn alloy etc.</td>
</tr>
<tr>
<td>Pressure measuring and control equipment</td>
<td>Three main technologies that could be used to replace mercury in pressure gauges; piezoelectric crystals and other sensors; and fibre-optic pressure sensors, based on light transmission.</td>
</tr>
<tr>
<td>Electric and electronic switches</td>
<td>Manual/mechanical micro switches, solid-state switches, optical switches etc.</td>
</tr>
<tr>
<td>Pesticides (seed dressing)</td>
<td>Use of processes not requiring chemical pesticides/biocides and easily degradable, narrow-targeted substances with minimal environmental impact.</td>
</tr>
</tbody>
</table>
Currently, all vaccines in the recommended childhood immunization schedule do not contain thimerosal as a preservative (Goldman and Shannon, 2001). Newer enclosed methods for preparing mercury amalgams have decreased the likelihood of mercury spillage and exposure during dental amalgam preparation. There are a variety of materials, such as composite resins, stainless steel, and gold that do not contain mercury and are approved for use in dental restorations in children.

1.12. REGULATIONS AND ADVISORIES

An MRL of 0.0002 mg/m³ has been derived for chronic-duration inhalation exposure (365 days or more) to metallic mercury vapor in a group of 26 mercury-exposed workers from three industries exposed to low levels of mercury for an average of 15.3 years (range, 1-41 years) (Fawer et al., 1983). Although there are no regulatory standards for home air, the Agency for Toxic Substances and Disease Registry (ATSDR) suggests that acceptable residential air mercury levels should not exceed 0.05 µg/m³ (ATSDR, 1999). An MRL of 0.007 mg mercury/kg/day has been derived for acute-duration oral exposure (14 days or less) to inorganic mercury based on a NOAEL of 0.93 mg mercury/kg for renal effects (increased absolute and relative kidney weights) in rats exposed to gavage doses of mercuric chloride for 14 days (NTP, 1993). An MRL of 0.002 mg mercury/kg/day has been derived for intermediate-duration (15-364 days) oral exposure to inorganic mercury based on a NOAEL of 0.23 mg mercury/kg for renal effects (increased absolute and relative kidney weights) in rats (Dieter et al., 1992; NTP, 1993). An MRL of 0.0003 mg mercury/kg/day has been derived for chronic-duration (365 days or more) oral exposure to methyl mercury, based on
neuro developmental outcomes in a study by Davidson et al. (1998) of children exposed in utero to methyl mercury from maternal fish ingestion.

EPA has derived an oral RfD of 0.08 µg/kg/day for phenyl mercuric acetate as mercury (IRIS, 1997). The RfD is based on a LOAEL of 0.5 ppm mercury or 0.042 mg/kg/day phenyl mercuric acetate for detectable kidney damage in female rats after 2 years. EPA has derived an oral RfD of 0.3 µg/kg/day for mercuric chloride. The RfD is based on LOAELs of 0.226, 0.317, and 0.633 mg/kg/day of mercuric chloride. EPA has derived an oral RfD of 0.1 µg/kg/day for methyl mercury based on developmental neurological abnormalities in human infants (IRIS, 1997). The EPA inhalation reference concentration (RfC) for elemental mercury is 0.3 µg/m³. The RfC is based on a LOAEL of 0.025 ppm for human occupational exposure studies. Mercuric chloride and methyl mercury have been assigned EPA's weight-of-evidence classification of C, which indicates that they are possible human carcinogens (IRIS, 1997).

Mercuric cyanide, mercuric nitrate, mercuric sulfate, mercuric thiocyanate, mercurous nitrate, mercury, and mercury fulminate have been designated as hazardous substances pursuant to the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980 (US EPA, 1995a). Mercuric acetate, mercuric chloride, and mercuric oxide are mercury compounds that have been individually designated as extremely hazardous substances under Section 313 of Title III of the Superfund Amendments and Reauthorization Act (SARA) of 1986 (US EPA, 1995b). The EPA regulates mercury under the Clean Air Act (CAA) and has designated it as a hazardous air pollutant (HAP). Emission standards for release of mercury to the atmosphere have been promulgated for
mercury cell chloralkali plants, mercury ore processing facilities, major stationary sources, and municipal waste combustors (US EPA, 1996a).

In accordance with the authority of the Safe Drinking Water Act (SDWA), EPA has established a safe drinking water standard for mercury at 2 µg/L (FSTRAC, 1995). Under the Clean Water Act (CWA) EPA provides criterion concentrations for mercury as a priority toxic pollutant (US EPA, 1992). Mercury is regulated as a "priority pollutant" in accordance with the CWA. The CWA establishes the basic structure for regulating the discharge of pollutants to waterways and is designed to ensure that all waters are sufficiently clean to protect public health and/or the environment. However, if waters and their sediments become contaminated from sources such as atmospheric deposition and discharges from industrial, municipal, or agricultural operations, toxic substances could concentrate in the tissue of fish and wildlife. The fish or wildlife advisory is typically more restrictive to protect pregnant women, nursing mothers, and young children. To reduce children's exposure to methyl mercury, state advisory recommendations for fish consumption limits (meals per week or meals per month) should be strictly observed (ATSDR, 1999).

The FDA currently has advice for consumers recommending that pregnant women, and women of childbearing age who may become pregnant, limit their consumption of shark and swordfish to no more than one meal per month (FDA, 1998). Methyl mercury levels are much higher in these fish species than in the more commonly consumed species. The FDA advisory covers women of childbearing age who might become pregnant because dietary practices immediately before the pregnancy may have a direct bearing on fetal exposure during pregnancy. The FDA states that nursing mothers, who follow this advice,
will not expose their infants to increased health risks from methyl mercury (FDA, 1998). The FDA has also established an action level of 1 ppm for methyl mercury in fish (FDA, 1994). Several agencies have been working toward reducing methyl mercury exposure via food consumption. Guidelines for maximum exposure to mercury have been established by the EPA at 0.1 μg/kg/d (US EPA, 1997), by the FDA at 0.4 μg/kg/d (Tollefson and Cordle, 1986) and by the ATSDR at 0.3 μg/kg/d (ATSDR, 1999). These three guidelines are based on extrapolations from blood or hair concentrations of mercury in pregnant women and information about the pharmacokinetics of methyl mercury to calculate maximum daily oral intakes of methyl mercury during pregnancy that were not associated with measurable adverse outcomes in children (Goldman and Shannon, 2001).

1.13. EPISODES OF MERCURY TOXICITY

Despite the known acute toxicity of the alkyl mercurials, they, and in particular methyl mercury, were widely used during the middle of the twentieth century in agriculture, paper making, leather production, and paint formulations because they inhibit the growth of bacteria, fungi, and algae. Alkyl mercurials were found to be highly effective seed-dressing treatments for inhibiting fungal growth on grain, both in storage and after planting. Unfortunately, the widespread use of organomercurial seed dressings led to both ecological problems and some of the worst cases of mass poisoning of humans by mercury compounds.

Predatory birds were found to be suffering from organomercurial poisoning in Sweden in the 1950s, probably as a result of their ingestion of small mammals.
that had eaten treated grain (Clarkson et al., 2003). The list of alkyl mercury mass poisoning incidents and the numbers of affected people make grim reading, and have a common factor running through them. In each case the mass poisoning occurred because organomercurial treated wheat or barley seed, which was only intended for planting as a crop, was used directly for human consumption. During the 1950s and 1960s cases of accidental consumption of alkyl mercury treated cereal seed occurred in Iraq, Pakistan, and Guatemala. The worst reported case occurred in rural Iraq (1971–1972), where over 6000 people were affected and 500 died because they ate food containing flour produced from wheat or barley grains treated with methyl- and ethyl mercury compounds, with subsequent reports suggesting that up to 40,000 people may have been affected (Clarkson et al., 2003). The highly toxic nature of organic mercury compounds is now well recognized, and their use has been severely restricted in recent years.

Perhaps the most notorious case of mass alkyl mercury poisoning known as Minamata disease is that centered around Minamata in Japan in the late 1950s/early 1960s, and the lesser-known case that occurred around the Agano River in Niigata, Japan, in 1965. In both cases exposure to methyl mercury was again associated with food consumption by humans, but in this case the methyl mercury was ingested through contaminated fish that had bioaccumulated methyl mercury. The source of the mercury pollution in both cases was industrial effluent from acetaldehyde/vinyl chloride manufacture, in which Hg was used as a catalyst in the production of acetaldehyde from acetylene and was discharged into water bodies. Minamata disease was first discovered in Minamata city in Kumamoto prefecture, Japan in 1956. It was caused by the release of methyl mercury in the industrial wastewater from the Chisso Corporation's chemical factory, which continued from 1932 to 1968. This highly
toxic chemical bioaccumulated in shellfish and fish in Minamata Bay and the Shiranui Sea, which when eaten by the local populace resulted in mercury poisoning. Organomercurials led to severe methyl mercury poisoning with over 980 deaths recorded as a consequence in the Minamata incident (Clarkson and Magos, 2006).

1.14. MERCURY IN INDIA

Presently, India is one of the major users of mercury in the world (207-531 tones annually). While the developed world has an effective retrieval system and strict norms, regulations are still to be implemented in all parts of India. Chlor-alkali industries are the major source of mercury release in the atmosphere and surface water in India. Other contributors are coal-fired plants viz. thermal power plants, steel industries and cement plants. Plastic industry (where mercury is used as a catalyst), pulp and paper industry, medical instruments and electrical appliances, certain pharmaceutical and agricultural products account for additional consumption of mercury (Table 1.2). The government may have banned the setting up of new mercury-based caustic soda plants, but phasing out the existing ones is going to take some more time. The second-largest consumption of mercury in India is for the production of measuring instruments such as thermometers, barometers, etc. It is also used in manufacturing electrical apparatus, mercury vapor lamps, electrical switches, fluorescent lamps, etc (Toxics Link Factsheet, 2003). The presence of mercury in the environment of India can be traced back to the 1970's when various studies conducted showed the presence of mercury in our environment.
### Table 1.2: Major Consumers of Mercury in India

<table>
<thead>
<tr>
<th>Form of mercury</th>
<th>Consumers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elemental mercury</td>
<td>Chlor-alkali industry</td>
</tr>
<tr>
<td></td>
<td>(Mercury cell process)</td>
</tr>
<tr>
<td>Elemental mercury</td>
<td>Instrument manufacturing</td>
</tr>
<tr>
<td>Mercury oxide</td>
<td>1. Clinical thermometers</td>
</tr>
<tr>
<td>Mercury salts</td>
<td>2. Laboratory thermometers</td>
</tr>
<tr>
<td></td>
<td>3. Blood pressure monitors</td>
</tr>
<tr>
<td></td>
<td>4. Barometers</td>
</tr>
<tr>
<td></td>
<td>5. Other instruments</td>
</tr>
<tr>
<td>Mercury compounds</td>
<td>Electrical apparatus</td>
</tr>
<tr>
<td></td>
<td>1. Electric switches</td>
</tr>
<tr>
<td></td>
<td>2. Electric lamps</td>
</tr>
<tr>
<td></td>
<td>a) Fluorescent lamps</td>
</tr>
<tr>
<td></td>
<td>b) Mercury vapour lamps</td>
</tr>
<tr>
<td></td>
<td>3. Batteries</td>
</tr>
<tr>
<td>Mercury oxide</td>
<td>Fungicides</td>
</tr>
<tr>
<td>Mercury compounds</td>
<td>1. Phenyl mercury acetate</td>
</tr>
<tr>
<td></td>
<td>2. Methoxy ethyl mercury chloride</td>
</tr>
<tr>
<td>Mercury oxide</td>
<td>3. Others</td>
</tr>
<tr>
<td>Mercury compounds</td>
<td>1. Paints</td>
</tr>
<tr>
<td></td>
<td>2. Cosmetics</td>
</tr>
<tr>
<td>Elemental mercury</td>
<td>Health set-ups</td>
</tr>
<tr>
<td>Mercury oxide</td>
<td>1. Drugs, pharmaceuticals</td>
</tr>
<tr>
<td>Mercury oxide</td>
<td>2. Dental amalgams</td>
</tr>
<tr>
<td>Mercury oxide</td>
<td>3. Others</td>
</tr>
</tbody>
</table>
Groundwater fulfils about 80% of drinking water needs in India but now days both surface water and groundwater mercury concentration has increased in India. The Bureau of Indian Standards (BIS) has laid down safety limits for drinking water at 0.001 mg of mercury per liter (BIS, 1991).

<table>
<thead>
<tr>
<th>Table 1.3: Levels of Mercury (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permissible limit</td>
</tr>
<tr>
<td>Industrial Area, Panipat (Haryana)</td>
</tr>
<tr>
<td>Barsai Road, Panipat (Haryana)</td>
</tr>
<tr>
<td>Machua Village, Vatva (Gujarat)</td>
</tr>
<tr>
<td>Lali Village, Vatva (Gujarat)</td>
</tr>
<tr>
<td>Chiri Village, Vapi (Gujarat)</td>
</tr>
<tr>
<td>Sarangpur Village, Ankleshwar (Gujarat)</td>
</tr>
<tr>
<td>Bapunagar, Ankleshwar (Gujarat)</td>
</tr>
<tr>
<td>Pocharam Village, Patancheru (Andhra Pradesh)</td>
</tr>
</tbody>
</table>

A number of samples of groundwater in some industrial belts have shown concentrations of mercury higher than safe standards. A study shows levels of mercury to be very high as compared to the permissible limit (Toxics Link Factsheet, 2003). In 1999, a study was conducted to analyze ground water samples from eight places in three states (Haryana, Gujarat, Andhra Pradesh) where mercury concentration was reported to be above the permissible limits (Table 1.3). Other than these three states there are several other places where high level of mercury was found which include-Delhi, Mumbai, Bilai, Bhopal, Dhanbad, Singrauli etc. Higher levels of mercury were found near the chlor-
Introduction and Review of the Literature

alkali, thermal power plants, cement, dye, polymer and other chemical industries (Toxics Link Factsheet, 2003).

A study was conducted by the Community Health Cell, Bangalore, in Kodaikanal (Tamil Nadu) amongst former workers of the Hindustan Lever Co., mercury thermometer plant. In Kodaikanal, one of the largest thermometer manufacturing plants in the world producing 100,000 to 150,000 pieces a month, released 439 kg of mercury into the nearby area in 1997. During the monsoon season, the mercury would be washed away in run-off, contaminating the water bodies in the area.

Studies have shown that the total mercury pollution potential from coal in India is estimated to be 77.91 tones per annum, if average concentration of mercury in coal is assumed to be 0.272 ppm. About 59.29 tones of mercury per annum is mobilized from coal fired thermal power plants alone (CPCB, 2001). The seven super thermal power plants in the Singrauli area, which supply 10% of India's power, are responsible for 16.85% or 10 tones per annum of total mercury pollution through power generation.

Acute mercury poisoning in India is rare. Unlike some western countries, India does not have fish consumption advisories. A fish advisory informs the population about the effects of mercury on the human body. It tells people to avoid varieties of fish through which the probability of mercury poisoning is high. Due to this lack, people fishing for subsistence, low-income or poor fishermen and children remain at risk of mercury poisoning. There is consumer legislation in place - the Prevention of Food Adulteration Act 1954, - which aims to protect consumers from adulterated food and ensure food safety. The Rules declare mercury as a toxic metal and limit its concentration in fish to 0.5 ppm and
in other food items to 1.0 ppm. Methyl mercury concentration in all foods is limited to 0.25 ppm (PFA Act India, 1954 [amended 2005]). The WHO and the Food and Agriculture Organization (FAO), in their codex alimentarius guidelines, have also limited the concentration of methyl mercury to 0.5 mg/kg for all fishes except predatory fishes, and 1 mg/kg for predatory fishes such as shark, swordfish, tuna, pike, etc (UNEP, 2002). On the basis of the risk assessments, a number of countries have stipulated levels of daily or weekly mercury intakes. However, in a country like India where a large percentage of the population eat fish as a staple food, no provisions for daily or weekly mercury intake levels have been set down. Several studies on fish and prawns in Mumbai, Kolkata, Orissa, etc, have reported alarming rates of mercury concentrations (Tejam and Haldar, 1975).

Testing of seawater by the National Institute of Oceanography, Goa, found increased mercury concentrations in the Arabian Sea (Kaladharan et al., 1999). The Paryavaran Suraksha Samiti (PSS) collected samples from over 20 villages affected by industrial pollution in the Golden Corridor of Gujarat to investigate the water situation there. The samples were analyzed for mercury and chemical oxygen demand (COD). In Haria village and Atul Complex, mercury was shockingly high at 12 ppm - 1200 per cent more than the permissible limit of 1 ppm (Toxics Link Factsheet, 2003). Sample of fish in Ankleshwar (Gujrat) and also from Bhopal (Madhya Pradesh) showed mercury at a high level of 2 ppm which is 200 per cent above the standard. Samples in Vadodara-Nandesari ECP area also showed high mercury levels of 6 ppm and 1.3 ppm, which are 600 per cent and 30 per cent more than the prescribed standards, respectively. The water from the area is dangerous for human consumption as the groundwater contamination is increasing (Toxics Link Factsheet, 2003).
1.15. INORGANIC MERCURY

1.15.1. An Overview

The inorganic forms of mercury include liquid metallic mercury and its vapor, compounds of mercurous and mercuric mercury and dental amalgam. The ingestion of liquid metallic mercury does not appear to be toxic in itself. Health hazards from liquid metallic mercury are due to its potential to release mercury vapor. Dental amalgam releases mercury vapor that can be inhaled and presumably behaves toxicologically like mercury vapor inhaled from external sources such as in occupational exposures. However, other than rare cases of contact allergy, no convincing evidence is yet forthcoming that dental amalgam can cause adverse health effects. The other forms of mercury can give rise to kidney damage, including in some cases the nephrotic syndrome. Inhaled mercury vapor can also cause damage to the central nervous system due to its ability to cross the blood-brain barrier. Mercurous mercury, in the form of mercurous chloride or calomel, has a long history of medicinal uses, especially as a laxative and in infant teething powders. Both mercurous and mercuric compounds are believed to be the causal agents in the childhood disease of acrodynia or “pink disease.” The mercuric cation, $\text{Hg}^{2+}$, is believed to be the proximate toxic agent for all these inorganic forms of mercury. All species of inorganic mercury have the capacity to elicit idiosyncratic reactions. Such reactions require exposure to mercury, but the prevalence and severity do not appear to be dose related. The nephrotic syndrome and acrodynia are examples (Magos and Clarkson, 2006). The high mobility of inhaled mercury vapor in the body is assumed to be due to its physical properties as an uncharged, monatomic gas that can readily diffuse through the lipid monolayers of the cell membrane. The mechanisms of transport of mercurous and mercuric cations are not well
understood. Mercuric mercury is known to exit liver cells into bile as a complex with reduced glutathione. Mercuric mercury has a limited capacity to cross the blood-brain and placental barriers but is avidly accumulated by the kidneys.

1.15.2. Pathway of Human Exposure

A WHO expert committee (WHO, 1990) estimated the dietary intake of inorganic mercury in the European and North American general population to be approximately 4 μg Hg as compared to an estimated daily intake of all forms of mercury as 6.6 μg Hg. They estimated that 0.6 μg Hg came from methyl mercury in fish tissue and that the remainder was from non-fish sources. However, methyl mercury is partially converted to inorganic mercury in mammals, so that meat and poultry products may contain some inorganic mercury ultimately derived from the metabolism of methyl mercury from fish products used as animal food. An unusual dietary source is whale meat, where levels of inorganic mercury can be high (Grandjean et al., 1992). Medicinal uses of both mercurous and mercuric compounds have virtually disappeared, but inorganic mercury is the active principle in skin-whitening creams still used widely in many countries (Weldon et al., 2000). Mercury vapor is present in the ambient atmosphere at levels so low that human exposure is negligible (Fitzgerald and Clarkson, 1991). The two major pathways of human exposure to inhaled mercury vapor are occupational and from dental amalgam. Elemental mercury still has many industrial applications resulting in the escape of mercury vapor into the working atmosphere. Occupational exposures have historically provided a considerable amount of information regarding the health effects of chronic human exposure. Another less common pathway takes place in the homes of workers in mercury industries if care is not taken to decontaminate the workers’ clothing. Certain
Introduction and Review of the Literature

Religious and ethnic practices lead to the use of liquid mercury in the homes resulting in the release of mercury vapor. Until the late 20th century, home exposure to mercury vapor could occur from the use of latex paints, where mercury compounds were used as a preservative. It is possible that this use of mercury may still occur in certain parts of the world.

1.15.3. Toxicokinetics

1.15.3.1. Absorption

For inorganic mercuric compounds, absorption via the lungs is low, probably due to deposition of particles in the upper respiratory system and subsequent clearance by the mucociliary escalator. The extent of transport of inorganic mercury across the intestinal tract may depend on its solubility and/or how easily the compound dissociates in the lumen to become available for absorption (Endo et al., 1990). Absorption of mercurous compounds is less likely than absorption of mercuric forms, probably because of solubility. Using whole-body retention data, estimated mercuric chloride absorptions of 3–4%, 8.5%, and 6.5% were calculated for single oral doses of 0.2–12.5 mg/kg body weight, 17.5 mg/kg body weight, and 20 mg/kg body weight, respectively, in rats (Piotrowski et al., 1992). However, also using whole-body retention data to indicate absorption, an estimated absorption of 20–25% was calculated from single oral doses of 0.2–20.0 mg mercury/kg body weight as mercuric chloride in mice by comparing retention data after oral and intraperitoneal dosing and taking excretion and intestinal reabsorption into account (Nielsen and Andersen, 1990).

The rate of oral absorption of mercuric mercury compounds in laboratory rodents has been shown to be dependent on intestinal pH (Endo et al., 1990), age, and diet (Kostial et al., 1978). One-week-old suckling mice absorbed 38% of the
orally administered mercuric chloride, whereas adult mice absorbed only 1% of the dose on standard diets. Nutritional status might also contribute to the intestinal absorption of Hg^{2+}, through competition with nutritionally essential divalent cations (e.g., Cu^{2+}, Zn^{2+}) that might have insufficient body stores. Indirect evidence of dermal absorption in humans is provided by clinical case-studies in which mercury intoxication was reported in individuals following dermal application of ointments that contained inorganic mercury salts (Kang-Yum and Oransky, 1992).

Urine samples from young women using skin-lightening creams containing 5–10% mercuric ammonium chloride had a mean mercury concentration of 109 μg/L, compared with 6 μg/L for urine samples from women who had discontinued use and 2 μg/L for women who had never used the creams (Barr et al., 1973). Mercurous chloride laxative (calomel) ingested over a long period may produce toxic effects on the kidneys, gastrointestinal tract, and central nervous system (Wands et al., 1974). While insoluble mercurous chloride is not normally that readily absorbed, small amounts may be converted to mercuric ion, which is more likely to be absorbed, in the lumen of the intestine. In addition, the mercurous ion that is absorbed is subsequently oxidized to mercuric ion, which may induce cellular toxicity by binding to intracellular sulphydryl groups.

### 1.15.3.2. Distribution

The amount of inorganic divalent mercury that crosses the blood–brain and placental barriers is much lower, because of poor lipid solubility (Clarkson, 1989; Inouye and Kajiwara, 1990). In contrast, the liver and kidneys accumulate inorganic mercury readily (Yeoh et al., 1986; Nielsen and Andersen, 1990). Sin et al. (1983) found the kidney to have the highest mercury levels following repeated
oral exposure of mice to mercuric chloride (4–5 mg mercury/kg body weight) for 2–8 weeks.

1.15.3.3. Metabolism

Once absorbed, inorganic mercury enters an oxidation-reduction cycle. Absorbed divalent cation from exposure to mercuric mercury compounds can, in turn, be reduced to the metallic or monovalent form and released as exhaled elemental mercury vapor (ATSDR, 1999). There is evidence to suggest that the divalent inorganic mercury cation is reduced by mammalian tissue to metallic mercury after its oxidation. Rats and mice pretreated parenterally with mercuric chloride exhale metallic mercury vapor (Clarkson and Rothstein 1964; Dunn et al., 1981a). Liver and kidney homogenates in animals also release mercury vapor after exposure to mercuric chloride. The amount of mercury released increases upon treatment with ethanol (Dunn et al., 1981b). This increase suggests that glutathione reductase is responsible for mercuric ion reduction (Williams et al., 1982).

1.15.3.4. Elimination and Excretion

Elimination of mercury occurs primarily through the urine and faeces, with the exhaled air, sweat, and saliva contributing to a much lesser extent. The urine and faeces are the main excretory pathways of inorganic mercury compounds in humans, with an absorbed dose half-life of approximately 1–2 months (Clarkson, 1989). After a short-term high-level mercury exposure in humans, urinary excretion accounts for 13% of the total body burden. After long-term exposure, urinary excretion increases to 58%. Inorganic mercury is also excreted in breast milk (Yoshida et al., 1992). The overall rate of elimination of inorganic mercury
from the body is the same as the rate of elimination from the kidney, where most of the body burden is localized. In a sample of 1107 individuals from 15 countries around the world, Goldwater (1972) reported the following urinary mercury levels for subjects who had no known occupational, medicinal, or other exposure to mercury: <0.5 μg/L—78%; <5 μg/L—86%; <10 μg/L—89%; <15 μg/L—94%; <20 μg/L—95%. Elimination from the blood and the brain is thought to be a biphasic process, with an initial rapid phase in which the decline in the body burden is associated with high levels of mercury being cleared from tissues, followed by a slower phase with mercury clearance from the same tissues. An even longer terminal elimination phase is also possible because of accumulation or persistence of mercury, primarily in the brain. For high-level exposure to inorganic divalent mercury, the urine is probably the major elimination route (Inouye and Kajiwara, 1990).

Suzuki et al. (1992) estimated the elimination half-life from urine to be 25.9 days following a short-term exposure to a high level of mercuric chloride (13.8 mg/kg body weight). Age is a factor in the elimination of mercury in rats following inorganic mercury exposure, with younger rats demonstrating significantly higher retention than older rats. This age-dependent difference in the rate of mercury excretion may reflect differences in the sites of mercury deposition (i.e. hair, red blood cells and skin) (Yoshida et al., 1992).

1.15.4. Biomarkers of Exposure

Urine samples are considered to be the best determinant of body burden of mercury from long-term exposure to elemental and inorganic mercury. Urinary mercury measurement is reliable, simple and provides rapid identification of individuals with elevated mercury levels (Naleway et al., 1991). It is a more
appropriate marker of exposure to inorganic mercury, since organic mercury represents only a small fraction of urinary mercury. Blood samples are useful primarily in cases of short-term, higher-level exposures to these forms, but are not as reliable as an indicator of total body burden in longer-term exposures. Most analytical methods do not differentiate between inorganic and organic mercury; thus, total mercury concentrations in blood reflect body burden of total mercury. Inorganic forms of mercury are not excreted to any significant amount in scalp hair, making hair an inappropriate biomarker of inorganic mercury exposure. Occupational studies show that recent mercury exposure is reflected in blood and urine (IPCS, 1991; Naleway et al., 1991). However, at low exposure levels (<0.05 mg mercury/m³), correlation with blood or urine mercury levels is low (Lindstedt et al., 1979). Blood levels of mercury rise sharply during and soon after short-term exposures, indicating that measurements of blood mercury levels should be made soon after exposure (Cherian et al., 1978). The half-life of mercury in the blood is only 3 days, attesting to the importance of taking blood samples as soon after exposure as possible. In the case of low-level long-term exposure, urine samples provide the best indicator of body burden.

Urinary mercury levels have been found to correlate better with exposure than blood inorganic mercury concentrations following long-term, low-level occupational exposure to elemental mercury vapor (Yoshida, 1985). There may be marked diurnal variation in the urinary concentration of mercury (Schaller, 1996). Based on a systematic review of high-quality studies, the International Commission on Occupational Health and the International Union of Pure and Applied Chemistry Commission on Toxicology estimated that a mean value of 2 µg/L was the background blood level of mercury in persons who do not eat fish (Nordberg et al., 1992). These levels are "background" in the sense that they
represent the average levels in blood in the general population and are not associated with a particular source of mercury exposure. However, the intra- and inter individual differences in these biomarkers are substantial, possibly due to dental amalgam (urine) and ingestion of contaminated fish (blood) (Verschoor et al., 1988; IPCS, 1991). Several studies have reported a correlation between airborne mercury and mercury in blood and urine; however, results vary, and it is not known whether the ratio between concentrations in urine and blood is constant at different exposure levels (Roels et al., 1987). Limiting the analysis to studies in which the exposure had been assessed using personal breathing zone mercury measurements, it was estimated that in continuous 8 h/day occupational exposure, an airborne mercury concentration of 1 mg/m³ leads to an average urinary mercury concentration of 1.4 mg/L (variation between individual studies, 0.7-2.3 mg/L; seven studies) and to an average blood mercury concentration of 0.48 mg/L; six studies (Cross et al., 1995).

Relationships of urinary and blood mercury concentrations with signs and symptoms of exposure are less clear. Exposure to elemental or other inorganic forms of mercury can be verified by examining the urinary mercury concentration. Urinary mercury concentrations normally expected in an asymptomatic population would be <10 μg/L (Goldwater, 1972; ATSDR, 1999). Background levels of urinary mercury, adjusted for creatinine, in an unexposed population are generally expected to be 5 μg Hg/g creatinine (IPCS 1991; Schaller, 1996). Measurement of mercury in whole blood is the preferred test for exposure to organic mercury, since this form of mercury is excreted primarily in the feces rather than in the urine (Brodkin et al., 2007).
1.16. **MERCURY EXPOSURE: ANALYTICAL ASPECTS**

The proliferation of mercury in the environment has fuelled the search for analytical methods capable of measuring trace amount of this element in a variety of sample matrices. The highly toxic nature of mercury and its tendency to accumulate in tissues has made its quantitation in biological samples of particular interest. Numerous references detailing mercury determination in mammalian brain, liver, kidney and reproductive tissues can be found in the literature. Sample digestion schemes and modes of instrumental analysis for these methods vary greatly. The analysis of metals in biological and environmental samples is complicated by the different organic and inorganic forms of the metal that may be present. Analytical methods are available for the detection, and quantification of mercury in environmental and biological samples with high sensitivity and accuracy. Mercury has an additional problem of being relatively volatile and, therefore, easily lost during sample preparation and analysis. Since mercury is relatively volatile, care must be taken to avoid its loss during sample preparation and analysis. Lab ware should be thoroughly cleaned and acid-leached prior to use for trace-level analysis of mercury and its compounds, and due care should be taken to preclude the possibility of contamination by naturally occurring environmental mercury. Mercury readily forms amalgams with other metals (e.g., silver, zinc, tin), which can possibly contribute to mercury loss during analysis (ASTDR, 1999).

In spite of these complications, several methods have been developed for determining trace amount of mercury in such samples. A variety of detection methods have been used to detect mercury selectively and sensitively. Good review papers have been published for the determination of total mercury...
concentrations in environmental and biological samples. These methods can be listed as follows: gravimetry, micrometry, radiometry, titrimetry, colorimetry and fluorometry, atomic absorption spectrometry (AAS) (cold vapor, electro thermal etc.), atomic fluorescence spectrometry (AFS), atomic emission spectrometry (AES), inductively coupled plasma - atomic emission spectrometry (ICP-AES), microwave induced plasma - atomic emission spectrometry (MIP-AES), direct current plasma - atomic emission spectrometry (DCP-AES), neutron activation analysis (NAA), X-ray fluorescence (XRF), electron probe microanalysis (EPMA), proton induced X-ray emission (PIXE), mass spectrometry (MS), electrometry (polarography, amperometry, voltammetry etc.), chromatography, and other miscellaneous methods.

1.16.1. Environmental Samples

A number of analytical methods can be used to determine mercury levels in air, water, soils, sediments, pharmaceuticals, and fish and other foods. In the case of complex samples, decomposition of the matrix and reduction of the mercury to its elemental form are required.

CVAAS and CVAFS have been shown to be sensitive (detection at low- to mid-ng/m³ levels), accurate, and precise methods for monitoring mercury in air in the form of both vapors and suspended particulates (ATSDR, 1999). AFS, partially due to its low- ng/m³ sensitivity and high accuracy and precision, is gaining in popularity (Horvat, 1996). The combination of AFS, AAS, and GC has been shown to be effective in speciating different organic and inorganic forms of mercury (Bloom and Fitzgerald, 1988).
Detection and quantification of mercury in aqueous media can be accomplished through a number of analytical methods. CVAAS, ASV, ICP-MS, ICP-AES, microwave-induced plasma AES, NAA, GC/AFS, high-performance liquid chromatography (HPLC) with ultraviolet detection, HPLC with electron capture detection, and spectrophotometry have all been successfully employed to quantify mercury in drinking-water, surface water, groundwater, snow, seawater, and wastewater effluents (ATSDR, 1999). CVAAS, because of its high sensitivity (sub-ng/L) for mercury and high reliability, is the method preferred by the US Environmental Protection Agency (US EPA, 1994a; 1994b) and the Association of Official Analytical Chemists (AOAC, 1984). While water samples generally do not require predigestion, mercury is usually reduced to the elemental state and preconcentrated prior to the actual analysis. As with samples from other media, a colorimetric method based on the formation of a coloured complex in the presence of mercury (Cherian and Gupta, 1990) may be used as a quick and simple field screen to detect mercury at mid-μg/L concentrations; however, without a predigestion method, organically bound mercury might not be fully measurable.

CVAAS, a sensitive and reliable technique that requires little sample preparation beyond matrix digestion and the reduction of mercury to its elemental form, is the most commonly used method of quantifying mercury in sediment, soils, and sludge (ATSDR, 1999). CVAFS with flow injection analysis, following microwave digestion, has been shown to have good precision and sensitivity in the mid-ng/kg range (Morales-Rubio et al., 1995). CVAAS and direct current ASV (Lexa and Stulik, 1989) have been successfully used for testing organic and total mercury levels, respectively, in soil and/or sediment. For on-site screening, portable field X-ray fluorescence has been used to monitor soil contamination at
low-mg/kg levels (Grupp et al., 1989). CVAAS, with its consistent high sensitivity and reliability, is one of the most common methods used to quantify mercury in fish, shellfish, other foods, and pharmaceuticals. Other methods successfully used include flameless AAS for mercury in fish, wine, and other food (ATSDR, 1999).

1.16.2. Biological Samples

Mercury concentrations in humans and other mammals have been determined in blood, urine, body tissues, hair, breast milk, and umbilical cord blood. Most methods use atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), or neutron activation analysis (NAA), although mass spectrometry (MS), spectrophotometry, and anodic stripping voltammetry (ASV) have also been employed. The most commonly used method is cold vapor (CV) AAS (ATSDR, 1999). Through CVAAS, mercury concentrations below the μg/L or μg/kg level can be reliably (>76% recovery) measured through either direct reduction of the sample or reduction subsequent to predigestion. Electro thermal AAS has also been demonstrated to be highly sensitive and to produce excellent accuracy (ATSDR, 1999). Sub-μg/L or μg/kg range sensitivity and excellent accuracy has also been demonstrated with gas chromatography (GC)/microwave-induced plasma atomic emission detection (Bulska et al., 1992). Recovery of >90% and high precision have also been obtained with AFS when the samples were predigested in a closed container in a microwave oven (Vermeir et al., 1991a; 1991b). ASV and isotope-dilution spark source MS, which also require predigestion of the sample, have also produced high precision and accuracy (recoveries >90%). Inductively coupled plasma–atomic emission spectroscopy (ICP-AES) and ICP-MS can also be used to accurately (>90%
recovery) determine total mercury in blood and urine with sub-μg/L sensitivity, but with less precision. In the case of blood mercury analysis, methods exist for the separation of organic and inorganic mercury (ATSDR, 1999). For analysis of urine mercury levels, expression of urinary mercury in units of μg Hg/g of creatinine is useful in adjusting for the variability in urine output or urine concentration.

1.17. MERCURY EXPOSURE: TOXICOLOGICAL ASPECTS

The mercuric ion, sometimes referred to as mercuric mercury or divalent mercury, is the second oxidation state, where two electrons have been removed from the mercury atom. It is responsible for nearly all the inorganic and organic chemical compounds of mercury. It is a product of the metabolism of inhaled mercury vapor, organic compounds of mercury and mercurious ion also converted into mercuric ions. It therefore, plays a key role in the toxicology of most forms of mercury. The mechanism of disposition of mercuric mercury in the body is yet to be understood. The chemistry of mercuric mercury is dominated by its high affinity for thiol groups, specifically the thiol anion. The forward and backward reactions are very rapid so that mercury can move quickly from one thiol group to another (Rabenstein et al., 1982; Govindaswamy et al., 1992). It is assumed that mercury binds to thiols in vivo based on the chemistry of this metal, but few mercury thiol compounds have been identified in tissues. Inorganic mercury and methyl mercury complexes with reduced glutathione (Ballatori and Clarkson, 1985) have been identified in bile in animal experiments and a methyl mercury-cysteine complex in fish protein (Harris et al., 2003).
Inorganic mercury has a non-uniform distribution after absorption, being accumulated mainly in kidneys (Emanuelli et al., 1996), causing acute renal failure (Tanaka-Kagawa et al., 1998). This form of mercury has great affinity for SH groups of endogenous biomolecules, such as the enzyme δ-aminolevulinic acid dehydratase (δ-ALAD), which may contribute to its toxicity (Clarkson, 1997). Thus, it is invariably found in cells and tissues bound to endogenous thiol-containing molecules such as glutathione, cysteine, homocysteine, metallothionein, and albumin (Zalups, 2000; Augusti et al., 2008). It has been shown in animal experiments that mercuric mercury is secreted in bile as a conjugate with reduced glutathione (Ballatori and Clarkson, 1985). Presumably, thiol ligands of two glutathione molecules attach to the mercuric cation to form a structure that resembles that of oxidized glutathione and is exported from the liver cells on glutathione carriers. This process is not operative in suckling rats, but data are lacking on human infants. Mercuric mercury (Hg$^{2+}$), attaches to the thiol ligands of two reduced glutathione molecules to form a complex similar to that of oxidized glutathione. Thomas et al. (1988) reported that the elimination of mercury from neonatal rats dosed with methyl mercury increases sharply with age above 17 days.

Reduced glutathione (GSH) also plays an important role in the renal handling of mercury. Inhibition of the enzyme gammaglutamyl transpeptidase (gamma GT), which degrades GSH, causes a marked reduction in renal uptake and a large increase in urinary excretion of mercury (De Ceaurriz et al., 1994). The fact that inhibition of gamma GT is less effective when levels of GSH are reduced in renal cells has led to the idea that inorganic mercury is secreted from proximal tubular cells into the tubular lumen as a GSH complex (Tanaka et al., 1993). This complex is then degraded by gamma GT. Mercury is subsequently reabsorbed into the
renal cells as a complex with one of these products, probably the mercury-cysteine complex, possibly via the large neutral amino acid transporter (Wei et al., 1999). The mechanisms of intestinal absorption of both mercurous and mercuric mercury have not yet been identified. With respect to the mercuric species, both secretion and absorption process may be occurring at the same time (Zalups et al., 1999). It is highly likely that the complexes of mercury with small-molecule thiols such as GSH and cysteine are involved. The reason why the GI absorption of inorganic mercury in suckling animals is much higher than in adults remains unknown (Clarkson and Magos, 2006) despite the passage of more than 25 years since the first report in 1978 (Kostial et al., 1978). Alpha-lactalbumin, a major protein component in breast milk, is known to bind divalent metals and perhaps enhance their absorption (Lonnerdal, 2003). Extrapolation to human infants will remain speculative until we know more about the mechanisms involved. The formation of complexes with thiol-containing small molecules such as cysteine and glutathione plays a major role in the processes of transport and disposition of mercury in the body.

1.17.1. Mercury induced Oxidative Stress

The effects of mercury on cellular systems have received a great deal of attention in recent decades due to the increasing exposure of living organisms to the metals in the environment (Cavallini et al., 1999). Mercury is known to accumulate in living organisms (Su et al., 2005), causing serious damage. An important feature of mercury toxicity is the generation of free radicals or reactive oxygen species (ROS). Binding of mercuric ions to sulphydryl groups may result in altered glutathione levels, leading to an increase of ROS, like superoxide anion radical, hydrogen peroxide and hydroxyl radical (Diplock, 1991) that initiate the
Introduction and Review of the Literature

Oxidative stress, an important mechanism of cell injury. Oxidative stress occurs when the quantity of free radicals in the body to be coped up exceeds the availability of antioxidants (Halliwell, 1994). Membrane lipids and proteins are especially prone to attack by free radicals, considered to be reliable indicators of oxidative stress. Lipids are a heterogeneous group of compounds having several important functions in the body such as being an efficient source of energy, constituents in cell membranes and nerve tissues, thermal and electrical insulators and acting as local hormones etc (Murray et al., 2000). Lipid peroxidation (LPO) is an oxidative deterioration of lipid containing many number of the carbon-carbon bonds. The ROS, which are generated during the oxidative stress, are one of the causative agents for lipid peroxidation. Mercury produces oxidative damage via H₂O₂ generation thereby leading to lipid peroxidation (El-Demerdash, 2001). ROS bring about the peroxidation of membrane lipids, which leads to membrane damage (Scandalios, 1993). Lipid peroxidation is the symptom most often used as an indicator of increased oxidative damage (Zhang and Kirkam, 1996). Increased lipid peroxidation was reported in animals (Fukino, 1984; Lund, 1993; Huang et al., 1996; El-Demerdash, 2001) and plants (Huang et al., 1996; Cargnelutti et al., 2006) that were exposed to various Hg compounds, which correlated with the severity of hepatotoxicity and nephrotoxicity in animals (Erçal et al., 2001). Significant reduction in GSH level in tissues is also reported by Cantoni et al. (1984) which is due to an increase in reactive oxygen species production and may result in the generation of lipid peroxides (Sener et al., 2003).

Oxidative stress induced biochemical changes are crucial etiological factors of several chronic human diseases including various renal ailments (Hogg, 1998; Modlinger et al., 2004). Both nephrotoxic acute renal failure and ischemic acute
renal failure have been found to involve oxidative damage in mercury toxicity. Mercuric chloride (HgCl₂) administration to experimental animals is a well-known model of nephrotoxic acute renal failure and is widely employed to study its pathophysiology (Alam et al., 2007). In addition to inducing lipid peroxidation, HgCl₂ was also reported to alter GSH concentration and affect other antioxidant enzyme activities of cells. Both, GSH and cellular antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and various peroxidases like glutathione peroxidase (GPx) play an important role in HgCl₂ induced nephrotoxicity. Gstraunthalier et al. (1983) have shown HgCl₂ to inhibit the activities of renal SOD, CAT, GR and GPx in addition to depleting GSH content of kidney tissues. The inhibition in CAT and GPx, induced by HgCl₂ may probably be due to their inactivation during the catalytic cycle. The functional alterations, histological changes and hydrodynamic alterations induced in this model are well-understood (Stein et al., 1978; Verstrepen et al., 1995). A number of evidences advocate the role of oxidative stress in Hg²⁺ induced renal toxicity. For instance, HgCl₂ exposure augments H₂O₂ production by the mitochondria in renal epithelial cells (Nath et al., 1996). Mercuric chloride (HgCl₂) leads to attenuation of antioxidant armory depleting GSH (Alam et al., 2007). Ariza et al. (1998) showed that Hg²⁺ induces H₂O₂ formation and stimulates the activities of copper-zinc SOD and xanthine oxidase in AS52 cells. It however, did not affect other antioxidant enzymes, such as CAT, GPx, and GR. On the other hand, Hussain et al. (1999) reported increased CAT, and GPx activity, and GSH content in the livers, kidneys and brains of mice exposed to 1 mg/kg/day HgCl₂ for 14 days. The activated antioxidant defense system of cells was reported to be a compensatory mechanism for HgCl₂ induced oxidative stress. Similarly, Woods and Ellis (1995) reported that increased levels of GSH and the activities of GR
and GPx in rats exposed to MeHg in drinking water were an adaptive response to Hg exposure in renal epithelial cells.

1.17.2. Role of Metallothionein in Mercury Toxicity

Metallothioneins (MT) belong to the "stress-specific stress proteins" that participate in the detoxification of heavy metals and oxygen free radicals by binding to the metal before it can cause harm (Sato and Kondoh, 2002). It is a family of cysteine rich, low molecular weight (MW ranging from 3500 to 14000 Da) proteins found in micro-organisms, plants and all invertebrate and vertebrate animals. Unicellular eukaryotes such as yeast have a copper-MT whose synthesis is induced by a copper-activated transcription factor. Higher organisms have two major cadmium/zinc MT isoforms, whose synthesis is controlled by a zinc-activated transcription factor (Syring et al., 2000). Metallothioneins (MTs) have the capacity to bind both physiological (Zn, Cu, Se) and xenobiotic (Cd, Hg, Ag) heavy metals through the thiol group of its cysteine residues, which represents nearly the 30% of its amino acidic residues (Sato and Kondoh, 2002). They were first discovered in 1960 by Kagi & Vallee when they purified a Cd/binding protein from horse (equine) renal cortex (Kagi and Vallee, 1960). It was characterized in 1970 by Kagi and others, and has been used since. Its function is not clear, but the experimental data relates MTs with protection against metal toxicity, regulation of physiological metals (Zn and Cu) and protection against oxidative stress. There are four main isoforms of MT expressed in humans. In the human body, large quantities are synthesized primarily in the liver and kidney. Their production is dependent on availability of the dietary minerals, as zinc, copper and selenium, and the amino acids histidine and cysteine.
Metallothionein (MT) is expressed to a certain extent in almost all mammalian tissues. The biological significance of MT is related to its various forms MT-I, MT-II, MT-III and MT-IV in mammalian MT, but the positions of cysteine are the same among these isoforms. Generally, MT means MT-I and MT-II isoforms, which are expressed in nearly all organs of the body, and are induced by a wide variety of stressors including heavy metals and oxidative stress-inducing agents (Sato and Kondoh, 2002). For MT-I several isoforms of the protein exist and it is likely that these isoforms are related to various functions involved in developmental processes occurring at various stages of gestation. Toxicokinetics and biochemistry of essential and toxic metals such as cadmium, zinc, mercury and copper in organs e.g. kidney, CNS, are often related to metallothionein (Nordberg and Nordberg, 2000). The degree of affinity of MT to metals is Zn<Cd<Cu<Hg = Ag. Although all 20 thiol groups present in MT are usually coordinated with metal, the ability of MT to hydroxyl radicals, which are primarily responsible for the toxicity, is more than 300 times greater than that of glutathione (Sato and Kondoh, 2002). MT is induced in the proximal tubules of kidneys exposed to mercuric chloride (HgCl₂), and its protective role has been confirmed in MT knockout mice in HgCl₂ nephrotoxicity experiments (Satoh et al. 1997; Yoshida et al., 2004). MT is an effective scavenger of hydroxyl radicals and mammalian MTs can functionally substitute for superoxide dismutase in oxidative stress (Morales et al., 2006).

1.17.3. Role of Antioxidants in Mercury Toxicity

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E. Low levels of
antioxidants, or inhibition of the antioxidant enzymes causes oxidative stress and may damage or kill cells (Ercal et al., 2001).

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. As free radicals are potentially toxic, they are usually inactivated or scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids. Taking into account that oxidative stress and endogenous thiols depletion are involved in inorganic mercury toxicity, it has been suggested that antioxidants could help in the treatment of mercury poisoning (Patrick, 2002; Augusti et al., 2008). In fact, superoxide dismutase treatment protected from renal failure and morphological changes induced by mercuric chloride (Girardi and Elias, 1995). Besides, recent studies demonstrated that the non-enzymatic antioxidant melatonin could prevent mercuric chloride-induced changes (Sener et al., 2003), whereas another non-enzymatic antioxidant, trolox, was able to prevent changes induced by methyl mercury (Usuki et al., 2001). Furthermore, HgCl₂ associated lipid peroxidation was significantly reduced by an antioxidant, selenium dioxide pretreatment. Selenium can achieve a protective effect, either by directly binding to Hg or serving as a cofactor for GPx, and thereby facilitating its ROS scavenging activity. Another well-known antioxidant, vitamin E, was shown to protect against HgCl₂ induced hepatic lipid peroxidation in high concentration (Andersen and Andersen, 1993). However, excess dietary β-carotene (Andersen and Andersen, 1993), sodium ascorbate and promethazine (Huang et al., 1996) did not protect against HgCl₂ associated lipid peroxidation (Augusti et al., 2008). Melatonin, a known lipid peroxidation retarder, has been found to have a
protective effect against HgCl₂ induced acute renal toxicity (Nava et al., 2000; Sener et al., 2003). Likewise, a number of plant extracts with antioxidant properties have been shown to inhibit HgCl₂ induced renal toxicity (Ahn et al., 2002; Alam et al., 2005).

1.7.3.1. Selenium

Selenium is a trace element with antioxidative activity and is necessary for activities of some enzymes in the body (Rotruck et al., 1973). The principle role of selenium is associated with the control of lipid peroxidation because this trace element is a component of selenoenzymes contributing to the antioxidant system (Jendryezko and Drozdz, 1993; Posielezna et al., 1993; Danch et al., 1994; Turan et al., 1997). Under certain conditions selenium is an immuno stimulative factor (Jendryezko, 1994). Selenium is a micronutrient that has a narrow margin between beneficial and harmful levels. Both deficiency and over supply of selenium in a diet are associated with the occurrence of clinical symptoms including Kashan disease and Kashin Beck disease (Whanger, 1989). On the other hand, previous studies on the effects of selenium deficiency were reported and found it caused liver cirrhosis in rats (Al-Bader et al., 1998). Selenium is also used in hepatoprotective influence in liver cirrhosis, and it was found that it has the ability to generate free radicals (Rai et al., 2001) and also, selenium effect on phenyl mercury toxicity and mercury retention in chicken were studied and showed that the selenium increased the mercury levels in liver cells and affected on histological structure (Elena et al., 2003).
1.17.3.2. Vitamin E

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble antioxidant vitamins (Herrera and Barbas, 2001). Of these, α-tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolising this form (Brigelius-Flohe and Traber, 1999). It has been suggested that the α-tocopherol form is the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Herrera and Barbas, 2001; Traber and Atkinson, 2007). This removes the free radical intermediates and prevents the propagation reaction from continuing. The oxidized α-tocopheroxy radical radicals produced in this process may be recycled back to the active reduced form through reduction by ascorbate, retinol or ubiquinol (Wang and Quinn, 1999). The functions of the other forms of vitamin E are less well-studied (Azzi, 2007).

1.17.3.3. Curcumin

Curcumin (diferuloyl methane) is the principal curcuminoid of the Indian curry spice viz. turmeric (Curcuma longa), which is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae, the other two curcuminoids being demethoxycurcumin and bis-demethoxycurcumin. The curcuminoids are polyphenols and are responsible for the yellow color of turmeric. Curcumin can exist in at least two tautomeric forms, keto and enol. The enol form is more energetically stable in the solid phase and in solution (Kolev et al., 2005). It is also hepatoprotective (Marotta et al., 2003). Curcumin is known for its antitumor (Aggarwal and Shishodia, 2006), antioxidant, antiarthritic, anti-amyloid and anti-inflammatory properties (Stix, 2007). Anti-inflammatory properties may be due
to inhibition of eicosanoid biosynthesis (Srivastava et al., 1995). In addition it may be effective in treating malaria, prevention of cervical cancer, and may interfere with the replication of the HIV virus (Padma, 2005). In HIV, it appears to act by interfering with P300/CREB-binding protein (CBP) of its reverse transcriptase. Curcumin acts as a free radical scavenger and antioxidant, inhibiting lipid peroxidation and oxidative DNA damage. Curcuminoids induce glutathione-S-transferase and are potent inhibitors of cytochrome P450, powerful scavenger of the superoxide anions, hydroxyl radicals and nitrogen dioxide. They are strong inhibitor of the generation of superoxide, hydroxyl radical and lipid peroxidation. Prophylactic effects against toxic metals viz. Cd, Cu, Fe, Pb and Se are well documented.
In view of toxic metal mercury posing health problems due to environmental pollution and also by occupational exposure, it is often required to detect an amount of very low quantity of the metal in water samples or biological matrices including blood/urine. Mercury analysis is complicated, difficult and requires considerable skill and experience on the part of the analyst. This is true because concentrations found in the environment and biological material are normally very low (<1-200 μg/kg). Although available methods for analysis of mercury are sensitive at ppb level but the cost involved becomes too high. It was therefore, thought to develop quick and easy method of detection and also improving upon known methodology for quantifying the levels of mercury in environmental and biological samples. The monitoring of mercury levels in natural water system and as toxicity marker in human urine is very important. The analytical methods used for the detection of mercury are based on costly, sophisticated and sometimes-complicated techniques, like atomic absorption spectrophotometry coupled with vapor generation assembly (AAS-VGA), inductively coupled plasma-mass spectrometry (ICP-MS) and neutron activation analysis (NAA). Due to the highly volatile nature of mercury and interfering effects of matrices (in environmental and biological samples), the detection of mercury at very low concentration (μg/L levels) is not practically feasible with simple spectrophotometric analysis. WHO permissible limit of the mercury in drinking water is 1 μg/L (WHO, 1984). The need for development of quick and easy method for screening for mercury in the samples has been felt for long. A Thin Layer Chromatography (TLC) method for mercury analysis was attempted which could be useful for quick screening of aqueous environmental samples and urine sample. Electrochemical methods are cheap and procedure is easy for
quantitation of metal from the samples. Voltammetric technique is well known for estimation of metals. Scanty reports are available for the determination of mercury in environmental samples using voltammetric technique.

Mercury is a heavy metal of increasing concern as a global pollutant. The health effects of mercury on humans have been observed for centuries now. Mercurial compounds, organic or inorganic forms exhibit a variety of toxicological effects including neurotoxicity, nephrotoxicity and gastrointestinal toxicity with ulceration and hemorrhage (Stohs and Bagchi, 1995). In addition to explaining and exploring the effects of mercury toxicity in the human body, it is equally vital to understand possible ways to both treat and reduce exposure of mercury to vulnerable human populations.

The detoxification techniques involve removing mercury from the body using 'chelation', complexing the mercury to a bigger molecule. The three different main chelation techniques involve different molecules: DMPS, EDTA, DMSA. These detoxification techniques are also used as a diagnostic tool to determine concentration of Hg in blood and decrease overall mercury concentration. The idea behind chelation involves a molecule used as a chelator, because Hg is usually embedded in cell walls. The chelator picks up Hg by attachment to a sulfur-side group and then exchanges Hg-molecule for a metal with a higher stability constant. The chelator picks up mercury and safely takes it through intestines until excretion. For the case of DMPS and EDTA, the technique is conducted by delivering the powdered form of the molecule in solution by intravenous infusion, while for DMSA, an intravenous administration is usually not required. Use of DMPS is the common chelation technique for adults while DMSA is used to treat mercury toxicity in children. The DMPS technique appears to have been originally developed in China, and can also be used to remove
other heavy metals also from the human body. During chelation procedure, several supplements are recommended for consumption, including prochitosan which increases the rate of digestion, garlic which increases sulfur concentration, and cilantro, vitamin C, and vitamin E that decrease side effects of chelation. It is also recommended to eat a high-protein diet during chelation treatment while avoid foods high in sugar. The sulfur-side groups in the amino acids from protein help to facilitate chelation. However, side effects of chelation therapy may also include elimination of essential trace metals from the body besides load on kidney for elimination of metal chelator complex. The toxicity of metals including mercury has a role in putting oxidative stress in biological system leading thereby to toxic manifestations. Antioxidants are present in many foods and play role in ameliorating oxidative stress. Antioxidants terminate chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols. Considering oxidative stress to play a crucial role in HgCl₂ induced toxicity, compounds with antioxidant potential are expected to have a protective role against it. Hence we endeavoured to examine the role of pre and post treatment of antioxidants viz. selenium, vitamin E and curcumin in mercury intoxication.

Selenium is an essential trace element for animals and humans. It is well established that selenium deficiency causes health implications in humans and animals. Also selenium can be toxic and can cause poisoning (selenosis) in humans and animals (Tinggi, 2003). Despite the essentiality of selenium and its protective effect against mercury toxicity, selenium is not currently used in the treatment of human mercury intoxication, possibly because selenium toxicity may be manifested after ingestion of element levels only slightly higher than
those nutritionally required (Perottoni et al., 2004). There has been increased interest in the mode of action of selenium as an antioxidant, while its role in prooxidant effects is also being probed.

Vitamin E is a major lipid-soluble chain-breaking antioxidant that prevents free radical-initiated peroxidative tissue damage, and plays a central role in the overall antioxidant defense mechanism (Chow, 1991; 2001). The underlying mechanisms of the chemopreventive functions of vitamin E are still poorly understood and may include the scavenging of reactive oxygen and nitrogen species, control of tumor growth through the induction of cell differentiation, inhibition of cell cycle progression, and/or stimulation of apoptosis. Vitamin E compounds can trigger these events by the modulation of specific signaling pathways and gene patterns (Betti et al., 2006). Vitamin E has been reported to restore the stress induced oxidative derangements in rat brain and it also provides defense against peroxidation of poly unsaturated fatty acid (PUFA) present in cellular and subcellular membrane phospholipids due to oxidative stress (Zaidi and Banu, 2004). However, the mechanism by which vitamin E exerts its protective effect against oxidative tissue damage in metal toxicity is yet to be delineated (Ibrahim and Chow, 2005).

Curcumin is a natural phenolic curcuminoid, the active principle of turmeric (Curcuma longa) (Dinkova-Kostova and Talalay, 1999). It has been extensively investigated for its hepatoprotective potential (Rukkumani et al., 2004). Medicinal plants and their active principles have received great attention as potential antiperoxidative agents (Lee and Park, 2003). Curcumin, has been widely used for centuries as an indigenous medicine (Dinkova-Kostova, 2002). Curcumin exhibits a wide range of pharmacological effects such as antioxidant, antitumor, anti-inflammatory and hepatoprotective activities (Ammon and
Wahl, 1991). Curcumin by scavenging or neutralizing free radicals, interacting with oxidative cascade, quenching oxygen, inhibiting oxidative enzymes like cytochrome P450, and by chelating metal ions like Fe, inhibits peroxidation of membrane lipids and maintains cell membrane integrity and their function (Pulla and Lokesh, 1994; Balasubramanyam et al., 2003). Thus, curcumin may stabilize the cell membrane and significantly reduce the extent of lipid peroxidation in the liver, lung and kidney (Kalpana and Menon, 2004).

Further uptake of mercury in the mammalian system and studies on the role of antioxidants in rendering prophylactic/therapeutic effects may be of immense use in protecting the toxic manifestations due to mercury in real situation of occupational setting or unavoidable environmental exposure. In the present work, we have studied the above said natural antioxidants in prevention and mitigation of mercury toxicity, which was characterized in rats by examining the oxidative stress parameters in liver, kidney and brain tissues, and serum biochemical alterations as a biomarker of mercury induced hepatotoxicity and nephrotoxicity. Accumulation of mercury in liver, kidney, brain and blood tissues, histopathological evaluation (liver and kidney tissues) and metallothionein gene expression studies at mRNA level in liver and kidney tissues were further investigated.
OBJECTIVES OF THE WORK

In view of the above considerations, investigations with the following objectives were undertaken:

❖ To develop cheap, rapid, convenient and sensitive methods for qualitative and quantitative detection for mercury in environmental aqueous and biological (urine) samples.

❖ To investigate the toxicity of mercury exposure in rats and to examine the effects of natural antioxidants viz. selenium, vitamin E and curcumin during mercury intoxication.