CHAPTER I

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INTRODUCTION

The advancement of science and development of modern technology has resulted in augmentation of production of certain chemicals/compounds etc., to meet the ever increasing demand in industry, agriculture and medicine. A large part of the population is exposed to a variety of such chemicals in their daily life, many of which are hazardous and have also been involved in numerous industrial disasters. The improper and extensive use of chemicals and pesticides in the present decade are the major culprits of environmental pollution.

Escalation in the amounts of hazardous and toxic pollutants in the biosphere and their entry into the biological system have serious repercussions on our natural resources and ecological balance. Consequently, mankind has a grim future if the same trend continues.

While an adequate water supply is one of the basic needs of all human beings, there are many barriers to fulfilling this need in various parts of the world. The pollution of ground water by arsenic and fluoride are some such barriers and have been identified in many developing and some developed countries. Hence it is essential to evaluate the effects of environmental toxicants on animal and human beings.

The enormous and increasing number of man-made chemicals in the environment to which we may be potentially exposed has thrust the science of toxicology into the limelight. The ultimate objective of the discipline of toxicology is to study the effects of
various toxicants on animal and human health.

Although different toxicants are known, emphasis has been laid on fluoride and arsenic toxicity in the present thesis. Their occurrence, distribution, properties, biological role and effects have been reviewed.

PHYSICAL AND CHEMICAL PROPERTIES

FLUORIDE

The atomic number of fluoride is 9 and atomic mass is 18.998403 and it belongs to the sub-group VII-A of the periodic system, where it is placed above chlorine. The mass number of its isotope are 18 and 19 but only the natural isotope 19 is stable. The outermost orbital contains 7 electrons and its valence is 1 (Underwood, 1977). There are various inorganic and organic forms of fluoride. The inorganic forms of fluoride are gaseous elemental form fluorine (F₂), hydrogen fluoride, alkali fluorides, fluorospar, cryolite, fluoroapatite and various other compounds. There are also many organic forms of fluoride viz. fluorocarbons, methoxy-fluorene, enflurane, and isoflurane etc. Natural organic fluorides are rare, the most well-known being fluoroacetic acid and fluoro-oleic acid (WHO, 1984)

ARSENIC

The atomic number of arsenic is 33 and atomic mass is 74.9216. Arsenic belongs to the sub-group Va of the periodic system, where it is placed below phosphorus and above antimony. The mass number of its isotopes range from 68 to 80, but only the
natural isotope 75 is stable. Elemental arsenic exists at room temperature as metallic or gray arsenic and yellow arsenic. Gray arsenic represents the common stable form. Its density is 5.73 g/cm³ and melting point 814°C. Density of yellow arsenic is 2.03 g/cm³ at 18°C. Arsenic displays variable valencies (-3, +3 and +5). Arsenic trioxide (As₂O₃) or white arsenic, exists in different forms such as arsenolite and clandetite (WHO, 1981). Various inorganic and organic arsenic forms are known like arsenic (III) oxide, arsenous acid, arsenic trichloride, arsenic (V) oxide, arsenic acid, methylarsonic acid, arsanilic acid, arsphenamine, arsenecholine etc. Among the forms of arsenic, arsenous acid or arsente (As³⁺) is more toxic in vivo than arsenic acid or arsenate (As⁵⁺) and also more inhibitory in vitro (Roy and Saha, 2002).

LD₅₀ AND HALF LIFE

FLUORIDE

The LD₅₀ value for female mice is 51.6 mg/kg body weight respectively (Pillai et al., 1987). The toxicokinetic studies revealed that the absorbed fluoride in the human body is distributed between blood, soft organs and the skeleton. The short half life of fluoride in blood and soft organs is few hours and skeleton has a relatively longer half life of mostly about eight years (WHO, 1970, 1984).

ARSENIC

The LD₅₀ values in mice is 39.4 mg/kg body weight (Harrison et al., 1958). Arsenic has a biological half-life of 30-60 hours. Arsenic which enters the blood stream
is excreted mainly in the urine in several forms, including arsenite (As$^{3+}$), arsenate (As$^{5+}$), methylarsonic acid (MAA), dimethylarsinic acid (DMAA) and other organically bound arsenic compounds. As$^{3+}$ is oxidized to As$^{5+}$ in the body and then excreted As$^{3+}$ and/or As$^{5+}$ are methylated in the body (WHO, 1981).

**DISTRIBUTION IN AIR, WATER AND SOIL**

**FLUORIDE**

Fluoride is widely distributed in the earth’s crust and is released into air, soil and water by a great variety of industrial, agricultural and other activities in a wide spread number of geographical locations. Food, water and air are the three principle fluoride sources to human. It is the most electronegative and reactive of all elements and thus in nature, is rarely found in its elemental state (WHO, 1984).

**FLUORIDE IN WATER**

Most of the surface water contains less than 0.1 ppm of fluoride, whereas, water penetrating the soil and coming into contact with fluoride containing minerals may take up more fluoride. The occurrence also depends on the porosity of the rocks or soil (through which the water passes), residence time, temperature, pH and presence of other elements such as calcium which may complex with fluoride (WHO, 1984). Fluoride compounds are fairly soluble and hence it is present in both surface and ground waters. Surface waters obtained from river and lakes generally contain lower concentrations of fluoride than spring and well waters (WHO, 1984). The ground water concentration
Fluctuates within wide limits from <1 to 25 mg/litre. In fresh water the content is 0.01-0.3 mg/litre and in sea water is high, averaging 1.3 mg/litre (WHO, 1984). Usually the population is supplied with drinking water having 1 mg/litre or more of fluoride content. In many parts of Asia, Europe, Australia, North and South America, the levels can be much higher than permissible limits (WHO, 1970). If fluoride exceeds the permissible limit in drinking water, such populations are at a risk of getting over exposed to fluoride and a few instances of control system failure have resulted in acute intoxications (WHO, 1984).

FLUORIDE IN ROCKS AND SOIL

Fluoride represents about 0.06-0.09% of the earth’s crust and fluoride content of soil usually increases with depth (WHO, 1984). The main fluoride containing mineral is fluorospar or fluorspar (CaF₂) which has been used as flux in several countries. The fluoride content of soil vary from under 20 to several thousand ppm, the higher records being mostly from areas with bedded phosphate or fluoride deposits. To a larger extent, soil fluoride determines its levels in water, vegetation, domestic and wild animals and indirectly in humans, whose food is derived from the above sources.

FLUORIDE IN AIR

Fluoride is known to be the most serious air pollutant. Traces of fluoride in the air of rural communities and cities arise from both natural sources and human activities. The natural source include effluents from volcanoes, dust generated by the weathering of
fluoride containing soils, outcropping of fluoride containing minerals and ocean spray. Besides the natural sources, industries are major culprits of fluoride emission into the environment to which man is constantly exposed. It includes steel production, ceramic factories, coal-burning power plants, brick works, glass works and oil refineries (WHO, 1984).

ARSENIC

Arsenic is the 20th most abundant element in the earth’s crust, but 12th most common in the human body. The major sources of air-borne arsenic emissions are the smelting of metals (mainly nickel-copper smelters) burning of coal, pesticide use, coal-fired, geothermal power plants and volcanoes. Global man-made releases have been estimated at 24,000 tons per year, compared with natural releases of about 8,000 tons per year. Volcanoes and coal contribute 7,000 and 550 tons, respectively (Merian, 1991).

ARSENIC IN WATER

Arsenic occurs in both inorganic and organic forms in water. Inorganic arsenic can exist in several oxidation states and forms in water depending on the pH of the water. The main organic arsenic species, methylarsonic acid and dimethylarsinic acid, are generally present in smaller amounts than the inorganic forms, arsenite and arsenate. The absorption of arsenate by Fe and Al oxides in sediments and formation of As$_2$S$_3$ removes arsenic from solution and prevents large arsenic concentrations from being present in water (WHO, 1981; 1996). Penrose et al. (1977) reported that sea water ordinarily contains
arsenic concentrations ranging from 0.001-0.008 mg/litre. High levels of arsenic have been found in waters from areas of thermal activity.

ARSENIC IN ROCKS, SOIL AND SEDIMENTS

Arsenic is a labile element and is subject to several processes in the soil. Under various conditions, it is oxidized, reduced, methylated, volatized and adsorbed. Arsenic is widely distributed in a large number of minerals. The highest mineral concentrations generally occur as arsenides of copper, lead, silver, or gold or as the sulfide. The arsenic content of the earth’s crust is 1.5-2 mg/kg (US NAS, 1977). Uncontaminated soils were found to contain arsenic levels between 0.2 to 40 mg/kg, while arsenic treated soils contained up to 550 mg/kg (WHO, 1981).

ARSENIC IN AIR

According to WHO (1981) airborne particulate matter has been shown to contain both inorganic and organic arsenic compounds. In air, arsenic is present mainly in particulate form as arsenic trioxide, with background levels of 1 to 10 ng/m$^3$ in rural areas and 20 mg/m$^3$ in urban areas (US NAS, 1977). Near smelters or coal-burning plants, the levels can reach 1000 mg/m$^3$ or more (WHO, 1981).

IMPORTANCE AND USES

FLUORIDE

A beneficial function of fluoride has been claimed since the late 1930s in the
prevention of human dental caries. It was also claimed that fluoride is beneficial for the maintenance of a normal skeleton in the adults. Fluoride may be necessary for normal hematocrit levels, fertility and growth. However, later studies have revealed that these are myths (Susheela, 2001). Considerable controversy also exists regarding benefits of fluoridation of water for the prevention of caries (WHO, 1984).

USE OF ARSENIC COMPOUNDS

Arsenic compounds are mainly used in agriculture and forestry (US NAS, 1977; US EPA, 1984) and industry. The arsenic trioxide is commercially obtained as a smelter product of various arsenic containing ores. Arsenical pesticides were used extensively, however, these uses sharply declined after the 1960s. Arsenicals are also used in pharmaceuticals for both human e.g. Fowler’s solution (potassium arsenite) and animals.

SOURCE

FLUORIDE

Traces of fluoride have been found to enter the human body every day regardless of the site of habitation through air, water and food. Human beings ingest fluoride mainly through drinking water, food, beverages etc., which contribute to 0.2-1.5 mg/day of fluoride (WHO, 1984). Occupational exposure may add considerably to the total intake of fluoride.
ARSENIC

Arsenic is a natural part of the environment and hence, low levels of arsenic are present in soil, water, food and air. Normally about 50 μg of arsenic is taken, each day from these sources. Of these, food is usually the largest source. The highest levels are detected in sea food, meats and grains. Mean levels in fish and sea food are usually about 4-5 ppm (US DHHS, 1998). In many people intake of arsenic is through occupational sources, e.g. copper or lead smelting, wood treating, pesticide application, etc. Arsenic is frequently found in plants, often as a result of pesticide treatment. The concentration typically vary from 0.01 to 5 ppm (US NAS, 1977). It is also inhaled through saw dust and smoke of wood treatment plant by workers and carpenters (WHO, 1981).

ABSORPTION

The process of absorption of fluoride and arsenic appears to be governed by an interplay of anatomical, physiological and biochemical factors.

FLUORIDE

GASTROINTESTINAL ABSORPTION

Absorption of fluoride entering the gastrointestinal tract is effected by a number of factors such as the chemical and physical nature of the ingested fluoride and the characteristics and amount of other components of the ingesta (US NAS, 1971). Solutions of fluoride salts are rapidly and almost completely absorbed from the gastrointestinal tract, probably by simple diffusion. Fluoride from insoluble or sparingly soluble
substances such as calcium fluoride and cryolite, is less efficiently absorbed. However, some fluorides may be more easily dissolved in the stomach because of the low pH, and hydrogen fluoride will then be formed. This compound may easily penetrate biological membranes, and its chemical reactivity is the probable cause of the resulting gastrointestinal symptoms when large amounts have been ingested (WHO, 1984).

**RESPIRATORY ABSORPTION**

In the industrial environment, the respiratory tract is the major route of absorption of both gaseous and particulate fluoride. Hydrogen fluoride being highly soluble in water is rapidly taken up in the upper respiratory tract (WHO, 1984).

**DERMAL ABSORPTION**

Data is lacking regarding dermal absorption of fluoride and has only been reported in the case of burns resulting from exposure to hydrofluoric acid (WHO, 1984).

**PLACENTAL TRANSFER**

Fluoride crosses the placenta. The fluoride content of the fetal skeleton and teeth increases with the age of the fetus and with the fluoride concentration of the drinking water used by the mother (WHO, 1984).
GASTROINTESTINAL ABSORPTION

Absorption of inorganic arsenic from the gastrointestinal tract can occur following the ingestion of food, water, beverages or drugs containing arsenic. The absorption of ingested arsenic will depend on the solubility of the compound in question, as well as whether the arsenic compound is given in solution or as undissolved particles. Several studies in human indicate that arsenates and arsenites are well absorbed across the gastrointestinal tract (US DHHS, 1998).

RESPIRATORY ABSORPTION

Human exposure to inorganic arsenic through inhalation usually occurs occupationally or during cigarette smoking. Since arsenic exists in air as particulate matter, absorption across the lung involves two processes, deposition of the particles onto the lung surface, and absorption of arsenic from the deposited material. In many work places, the particles containing arsenic are of relatively large size (WHO, 1981), resulting in deposition primarily in the upper respiratory passages (i.e. nasal cavity, nasopharynx, larynx, trachea, and bronchus). Subsequent absorption can then take place either directly from the respiratory tract or gastrointestinally after mucociliary clearance in the airways.

In workers exposed to arsenic trioxide dusts in smelters, the amount of arsenic excreted in the urine (the main route of excretion) was about 40-60% of the estimated inhaled dose (US DHHS, 1998).
DERMAL ABSORPTION

Human data containing the uptake of arsenic through the skin are extremely limited (US DHHS, 1998). The dermal exposure leads initially to arsenic binding to skin which may slowly be taken up into the blood, even after the exposure ends.

PLACENTAL TRANSFER

Placental transfer of arsenic was presented in a case of arsenic (III) oxide ingestion during the third trimester of pregnancy (WHO, 1981). A total of about 400 mg arsenic was taken in a liquid preparation causing the death of the foetus. In studies on 101 women in 2 southern cities in the USA (WHO, 1981), cord blood levels of arsenic were about as high as maternal blood levels.

DISTRIBUTION IN THE BODY

FLUORIDE

The fluoride absorbed by the human body will circulate in the body and then be retained in the tissues, predominantly the skeleton from where it is slowly released and may add to the levels in blood and urine. Studies in a number of subjects over several weeks of observation suggest that retention may be 35-48%. Approximately 99% of the fluoride in the body is localized in the skeleton. The rest is distributed between blood and soft tissues (WHO, 1984). Fluoride ions are taken up rapidly by bone by replacing hydroxyl ions in bone apatite lattice during mineralization (WHO, 1984) Fluoride is also distributed in hair, bone marrow cavities, and in breast milk (WHO, 1984).
ARSENIC

After absorption by the lung and through the gastrointestinal tract, 95 to 99% of the arsenic is located in the erythrocytes bound to the globin of hemoglobin. It is transported by the blood to other parts of the body within 24 hours (Merian, 1991).

Analysis of tissues taken at autopsy from people who were exposed to background levels of arsenic in food and water, revealed that arsenic was present in all tissues of the body (US DHHS, 1998). Most tissues had about the same concentration level (0.905 - 0.15 ppm), while the levels in hair (0.65 ppm) and nails (0.36 ppm) were somewhat higher. Following injection of radio labelled arsenite in patients terminally ill with malignant disease, the isotope was found to be widely distributed in the body and the highest concentrations were in the liver and kidney (WHO, 1981; US DHHS, 1998).

METABOLISM

FLUORIDE

Fluoride is incorporated into bones and teeth, where, as a result of similarities in size and charge, it replaces the hydroxyl ion in the crystal lattice of apatite. It can be released from bone by ion exchange at the crystal surface and by the dissolution of bone crystals through osteoclastic activity. Fluoroapatite is less soluble, more compact and slower to undergo remodeling in bone (Urbanska et al., 2001). A large fraction of ingested fluoride is excreted in the urine, and urinary fluoride levels provide a relative index of current fluoride intake. Human urinary fluoride concentration depend upon and in fact, are nearly equal to the drinking water concentration (WHO, 1984) and also useful
to diagnose fluorosis (Susheela, 2001).

ARSENIC

The metabolism of inorganic arsenic has been extensively studied in humans and animals. Two processes are involved (1) reduction/oxidation reactions that interconvert arsenate and arsenite, and (2) methylation reactions, which convert arsenite to monomethyl arsine acid (MMA) and dimethyl arsine acid (DMA). These processes appear to be similar whether exposure is by the inhalation, oral, and, or parenteral route. The human body has the ability to detoxify arsenic by changing inorganic arsenic to less toxic organic forms (by methylation) that are more readily excreted in urine. In addition, inorganic arsenic is also directly excreted in the urine. It is estimated that by means of these two processes, more than 75% of the absorbed arsenic is excreted in the urine (US DHHS, 1998).

The relative proportions of As (+3), As (+5), MMA and DMA in urine can vary depending upon the chemical administered, the time after exposure, the route of exposure, the dose level, and the exposed species. In general, however, DMA is the principal metabolite, with lower levels of inorganic arsenic (As^+3 and As^+5) and MMA. In humans, the relative proportions are usually about 40-60% DMA, 20-25% inorganic arsenic, and 15-25% MMA (US DHHS, 1998).

Studies in vitro indicate that the substrate for methylation is As (+3) and that As (+5) is not methylated unless it is first reduced to As (+3). The main site of methylation appears to be the liver, where the process is mediated by enzymes that utilize S-
adenosylmethionine as cosubstrate (US DHHS, 1998). The arsenic methyltransferase and monomethylarsonic acid (MMA) methyl transferase are involved in the transfer of a methyl group from S-adenosylmethionine to As (+3) yielding MMA, which is then further methylated to DMA (dimethylarsinic acid). Humans who ingested a dose of MMA converted a small amount (about 13%) to DMA (US DHHS, 1998). Reduced glutathione probably acts as a co-factor \textit{in vivo} but other thiols (L-cysteine, dithiotheretol) can substitute \textit{in vitro} (Roy and Saha, 2002).

Since the methyl derivatives of arsenic appear to be less toxic than inorganic arsenic and since methylation tends to result in lower tissue retention of inorganic arsenic (US DHHS, 1998), the biomethylation process is usually viewed as a detoxification mechanism.

\textbf{ELIMINATION}

\textbf{FLUORIDE}

In adults, approximately half of the absorbed fluoride is excreted \textit{via} the urine which is the principal route of its excretion. Some excretion takes place through sweat and faeces, and fluoride also appears in saliva (less than 1%) (WHO, 1984).

The proportion of ingested fluoride that is eliminated in the faeces varies depending on circumstances (US EPA, 1980). Fluoride present in faeces results from two sources: the ingested fluoride that is not absorbed and the absorbed fluoride that is re-excreted into the gastrointestinal tract.

Usually, only a small percent of the fluoride intake is excreted in the sweat.
However, under excessive sweating as much as 50% of the total fluoride excreted may be lost via perspiration (WHO, 1984).

The concentration of fluoride in human milk is quite similar to that in plasma (WHO, 1984)

**ARSENIC**

The urinary excretion of arsenic appears to account for 30-60% of the inhaled dose. Nearly all arsenic that is deposited in the lung is excreted in the urine (US EPA, 1989). Studies in humans indicate that ingested MMA and DMA are excreted mainly in the urine (75-85%) and this occurs mostly within 1 day (US DHHS, 1998). The urinary arsenic levels were higher for men than for women and increased with age up to 60 years and then decreased (Roy and Saha, 2002).

Direct measurement of arsenic excretion in humans who ingested known amounts of arsenite or arsenate indicate that very little is excreted in the faeces (US DHHS, 1998).

**DETECTION**

**DETECTION OF FLUORIDE**

Fluoride is detected by the fluoride ion-specific electrodes. There are several fluids (urine, plasma, saliva) that may be used to determine the amount of fluoride in the various compartments of the body. Enamel is not the tissue of choice because most of its fluoride is taken up during tooth formation. Bone fluoride levels are much better indicators of long term fluoride exposure and body burden, though fluoride is not
uniformly distributed throughout the bone. The concentration of fluoride in nails and hair appear to be proportional to intake over longer periods of time (WHO, 1984).

Identifying parameters for early detection of fluoride toxicity/fluorosis are necessary because the disease can be reversed, provided it is detected at an early stage. The sialic acid/ glycosaminoglycan ratio (SA/GAG test) in serum thus emerged for early detection of skeletal fluorosis and for differentiating skeletal fluorosis from ankylosing spondylitis (Susheela et al., 1988). Haptoglobin (Hp) and C-reactive protein (CRP) are markers for identifying other biochemical changes, if any, closely associated with fluoride toxicity (Susheela and Jethanandani, 1994).

Complaints of male infertility with abnormality in sperm morphology, oligospermia (deficiency of spermatozoa in the semen), azoospermia (absence of spermatozoa in the semen) and low testosterone levels in the case of patients hailing from an endemic area should lead one to suspect fluoride toxicity besides other reasons (Susheela, 2001).

Methods for determination of fluoride in air, water, soil or any biological sample are available. It can be detected by electro analysis, spectral analysis, chromatography technique, photon-tagged nuclear reaction analysis, X-ray photoelectron spectrometry and thermogravimetric analysis and differential scanning colorimetry (Yin et al., 2001).

DETECTION OF ARSENIC

Atomic absorption, spectrophotometry (AAS) is the most common analytical procedure for detecting arsenic in biological materials like blood, hair, serum, urine, nails,
marine biota or soft tissues. Detection limits in blood and urine are about 0.1-1 ppb for most techniques; limits for hair and tissues are usually somewhat higher. A normal person has an average concentration of 0.05 mg arsenic/100 mg hair and a concentration higher than 0.1 mg/100 mg hair indicates arsenic poisoning (US DHHS, 1998).

The arsenic concentration in biological fluids and tissues may also be determined by neutron activation analysis (NAA). In this approach, the sample is irradiated with a source of neutrons which converts a portion of the arsenic atoms to radioactive isotopes which can be quantified after separation from radioisotopes of other chemicals. X-ray fluorescence is also capable of measuring arsenic in biological materials (US DHHS, 1998). Inductively-coupled plasma (ICP) atomic emission spectrometry (ICP-AES) and ICP mass spectrometry (ICP-MS) are increasingly common techniques for the analysis of arsenic, and both methods can generally provide lower detection limits than absorbance detection methods. Various types of chromatography or chelation extraction techniques are most commonly used in combination with AAS, ICP-AES or ICP-MS for detection of arsenic species (i.e. analysis of organo-arsenicals or different inorganic species, rather than total) (US DHHS, 1998). Another approach involves selective reduction of arsenate and arsenite (permitting quantification of individual inorganic arsenic species), and selective distillation of methyl arsines to quantify MMA and DMA (US DHHS, 1998).

Presence of arsenic is also detected by some symptoms developed in the body like typical pigmentation in the nails about five weeks after exposure to arsenic, a transverse white stria, 1-2 mm in width appears above the lunule of each fingernail i.e. Mees line.
REMOVAL OF FLUORIDE FROM DRINKING WATER

The drinking water source requires to be tested for fluoride, using an Ion Selective Electrode technology. If the drinking water source is contaminated with fluoride more than 1.0 mg/litre of water, the people need to be advised to collect water from another source with less amount of fluoride, i.e. less than 1.0 mg/litre for cooking and drinking purposes. The different approaches for getting safe water in India are as follows. Community installations for water treatment, using either the Nalgonda technology or Activated alumina technology are effective. Another approach is treating the water at home in buckets or in earthenware pots, using the Nalgonda technology, where alum and lime in certain proportions are mixed (depending upon the fluoride content and alkalinity of the raw water). Occasionally, the treated water needs to be tested for fluoride to ensure safe levels. There is a community installation commercially available for removal of fluoride, based on the principles of reverse osmosis (Kent-RO water purifying system) which is a viable proposition. However, it is not cost effective. In the rural areas, the most viable proposition, if water treatment is inevitable, is the use of a domestic filter, using activated alumina technology. The system has been standardized by IIT, Kanpur, India, with assistance from UNICEF. Nearly ten thousand families are using the filter in Rajasthan and Andhra Pradesh in India (Susheela, 2001).

REMOVAL OF ARSENIC FROM DRINKING WATER

Several methods such as coagulation and precipitation by ferric chloride or alum or adsorption onto activated carbon and alumina, or use of ferric hydroxide impregnated
adsorbents are effective for the partial removal of soluble arsenic from water (Roy and Saha, 2002). However, it is not feasible to implement such a chemical treatment or process in situ to stop arsenic contamination of underground aquifer. A natural process of mitigation of aquifer was recommended by recharging rainwater to the shallow aquifer and simultaneously restricting the future tube well to a depth of 50 m (Roy and Saha, 2002).

The appropriate treatment approach for removal of arsenic will depend on a particular water system’s needs, resources, location and other factors. Many existing technologies can effectively treat arsenic in surface water with some limitations. Coagulation/filtration, the standard treatment for remediating arsenic and other contaminants from surface water, uses iron, which reacts with arsenic to create a solid that precipitates from the water. However, this treatment system produces an arsenic contaminated sludge that might need to be disposed off in a hazardous waste landfill. Other common water treatment approaches, including reverse osmosis, also perform well in removing arsenic, but produce waste streams. Reverse osmosis involves pushing water through a membrane that captures contaminants. But this method produces a larger waste stream than other treatment methods, which may make the method impractical where water is scarce. Anion exchange technology is also effective for removing arsenic, but offers its own disadvantages. This method involves passing water with anions of arsenate through a column of resin beads containing exchangeable, innocuous ions such as chloride, resulting in a swap that leaves the arsenate in the water column and the chloride in the water. The least expensive treatment option is activated alumina adsorption which
involves passing acidified water through columns of activated alumina that adsorbs the arsenic. Regenerating the column, however, requires running hazardous chemicals viz., sodium hydroxide and sulfuric acid through the system. Although existing technologies can effectively treat arsenic in surface water, arsenic removal technology (AsRT) (as called by its developers) seems to be more cost-effective than traditional methods at removing arsenic to below a detection limit of 1 μg/l. The AsRT involves pumping arsenic contaminated water through a bed of sand and iron filings. As the water passes through the iron filter, arsenic is removed from the solution.

Recently, the All India Institute of Hygiene and Public Health in the West Bengal, India, has developed and installed several hand pump - attached arsenic removal plants, made on the principle of oxidation - coagulation - flocculation - sedimentation - filtration, in arsenic affected villages of West Bengal, to provide arsenic-free drinking water (Roy and Saha, 2002). Recently, another method applied in Bangladesh is Solar Oxidation and Removal of Arsenic (SORAS) method. SORAS could immediately lead to a four-fold reduction of the arsenic intake in a large fraction of the population until better methods are available (Hug, 2001)

**ACUTE EFFECT**

**FLUORIDE**

In acute poisoning, practically all the organs and systems are affected. Symptoms include vomiting, diffuse abdominal pain of spasmodic type, diarrhea, severe weakness, muscle spasms, partial or total paralysis, cardiovascular system disorders, convulsions and
coma (WHO, 1984). In acute poisoning, fluoride kills by blocking normal cellular metabolism, by inhibiting enzymes particularly, metalloenzymes involved in several essential processes. The strong affinity for calcium results in hypocalcaemia (WHO, 1984). Results of acute poisoning also indicate increased deamination of aminoacids in the liver, lesions in the kidney (Birkner et al., 2000) and renal dysfunction which is dose related (Dote et al., 2000).

All inorganic compounds of fluorine are not equally toxic. The toxicity depends on the mode of entry into the body and the physical and chemical properties of the compound. There is no specific treatment in fluoride poisoning except for the administration of calcium salts, e.g., calcium gluconate intravenously (WHO, 1984).

**ARSENIC**

Arsenic affects tissues rich in oxidative enzyme systems and is a capillary poison, resulting in hypovolemia, shock, and circulatory failure. In humans, acute symptoms may occur within minutes or hours of ingestion, depending upon the vehicle, solubility, and particle size (WHO, 1981; Merian, 1991).

Acute effects caused by the ingestion of inorganic arsenic compounds mainly arsenic (III) oxide, are gastrointestinal damage, severe vomiting and diarrhoea often with blood-tinged stools, weakness, staggering gait, hypothermia, and death. Acute poisoning may also lead to necrosis and perforation of the stomach or intestine. If the individual survives, exfoliative dermatitis and peripheral neuritis may subsequently develop. Other symptoms include leg cramps, shock, stupor, paralysis, and coma. Cardiac abnormalities
and reversible anemia and leukopenia have also been reported.

Subacute effects mainly involve the respiratory, gastrointestinal, cardiovascular, nervous, and hematopoietic systems. Reduction of inorganic arsenic by nascent hydrogen may also result in arsine which is taken up by the erythrocytes, causing hemolysis, leading to arsenic acid which also damages the kidneys (Menan, 1991). As a result of the rapid destruction of the red blood cells, jaundice occurs, and the urine turns a reddish-violet colour due to hemoglobinuria. Blockage of free hemoglobin and acute uremia may lead to death (Arnold, 1988).

Exposure to irritant arsenic compounds such as arsenic (III) oxide, in air can acutely damage the mucous membranes of the respiratory system and exposed skin. This can result in severe irritation of the nasal mucosa, larynx, bronchi and ear canal, as well as in conjunctivitis and dermatitis. Arsenicals may also act as skin contact allergens (US NAS, 1977; IARC, 1980; US EPA, 1981, Menan, 1991).

**CHRONIC TOXICITY**

**FLUORIDE**

A prolonged intake of large amounts of fluoride through natural water or fluoride containing dust causes a crippling disease called fluorosis which could be skeletal, dental or non-skeletal (affecting soft tissues) fluorosis (WHO, 1984; Das et al., 2000, Susheela, 2001). The study of Rwenyonyi et al. (2000) showed a significant increase in the severity of fluorosis with increasing age in a community exposed to high fluoride, whereas, no change in severity with age was observed in another community exposed to low fluoride.
DENTAL FLUOROSIS

The very first sign of chronic fluoride toxicity is exhibited by teeth. Fluoride in tooth paste, mouth rinses and sodium fluoride tablets administered as prescription can contribute to the fluoride burden of the body leading to dental fluorosis which occurs in children during the developmental stages when the teeth are exposed to fluoride (Susheela, 2001). One of the earliest symptoms of dental fluorosis is mottled enamel which is characterized by minute abnormal white flakes, yellow or brown spots or striations scattered irregularly over the tooth surface. Dental mottling is confined more frequently to the permanent teeth. Dental fluorosis is not only a cosmetic problem but is also known to cause social problems (Susheela, 2001). Dental fluorosis and related studies have been described by several workers (Fomon et al., 2000; Tsutsui et al., 2000; Cao et al., 2001).

SKELETAL FLUOROSIS

One of the most severe effects of fluoride in human beings is "skeletal fluorosis" due to elevated intake over prolonged period of time. The accumulation of fluoride in the skeletal tissues is associated with pathological bone formation. The symptoms range from histological changes, increase in bone density, bone morphometric changes and crippling skeletal fluorosis. The effects of fluoride on the bone depend on the type of bone and its inorganic and organic constituents. A fluoresced bone shows characteristic structural changes, viz., increased bone mass and density, exostosis (bony outgrowth) at bone surfaces, increased trabecular bone volume, cortical porosity and periosteocytic lacunar
surface, increased osteon diameter and mottling of the osteons, formation of unmineralized cartilaginous loci within the trabeculae of the cancellous bone but not in the cortical bone (Susheela, 2001). It has also characteristic biochemical changes, viz., reduction in collagen content and in hydroxylation of proline and lysine in bone collagen, reduction in collagen cross-link precursors, altered glycoaminoglycans and proteoglycan contents in cancellous but not in cortical bone (Susheela, 2001).

In some endemic areas of India, "genuvalgum" was described as manifestation of fluoride toxicity among population groups in whom dietary calcium was low (Krishnamachari and Krishnaswamy, 1973). Genuvalgum is a crippling form of fluoride toxicity which occurs in relatively younger children around 8-10 years. It is a peculiar skeletal deformity characterized by knock knee or pain and deformity in the lower limb mostly among children (Chakma et al., 2000). Various studies related to skeletal fluorosis have been carried out all over the world (Choubisa, 2001; Alarco-Herrera et al., 2001; Li et al., 2001a; Susheela, 2001).

NON-SKELETAL FLUOROSIS

In recent years, the conventional belief that fluoride affects only bone and tooth has been negated as the evidences of the involvement of soft tissues, organs or systems has been brought to light and is dealt with as non-skeletal fluorosis. Investigations on soft tissue involvement in fluorosis have attracted attention in the recent past, and convincing evidence from fluorosis patients is now available to demonstrate the damage/involvement of skeletal muscle, ligaments, thyroid and parathyroid glands and spermatozoa in human
fluoresced patients. There are evidences for involvement of other organs and systems viz. kidney, liver, adrenal gland and reproductive organs (Chinoy, 1991a,b; 1992; 1995; 1996; 2002; Susheela, 2001).

Detection of fluorosis at early stages has become possible because of the understanding of soft tissue manifestations in fluorosis. Studies on skeletal muscle biopsies in endemic skeletal fluorosis by Sesikeran et al. (2000) showed the primary changes are related to the nerve, with muscle being affected secondarily in skeletal fluorosis. On the other hand, recent studies have shown that skeletal muscle is directly involved in fluorosis (Susheela, 2001).

In Gujarat, several districts are endemic to fluorosis, where a large population is under the influence of the disease. Chinoy and co-workers (Chinoy, 1996; Chinoy and Narayana, 1992; Chinoy et al., 1992a; 1994a; Mathews et al., 1996) carried out a survey study in more than 100 villages and 1000 individuals. Their results deal with the interrelationship of fluoride and soft tissue functions.

ARSENIC

In animals

Non-carcinogenic effects after long-term exposure are very common in animals. In rats, the glossy appearance of their pelage is lost, especially on the back and nape of the neck. Histopathological changes included severe skin ulcerations, crust formations, scarring of epidermis and subcutaneous tissues and hyperkeratosis (Ishinishi et al., 1976).

Liver lesions have frequently been observed with cirrhosis, necrosis, bile duct
proliferation, and fatty changes in rabbits (WHO, 1981).

Chronic effect of arsenic (III) on liver of mice caused decreased concentration of free SH- groups. On the other hand, glutathione reductase showed a tendency to increase during the treatment period (Benko et al., 1978).

Ultrastructural changes in the hepatocytes of mice after arsenic exposure via drinking water (50 mg As (III)/litre) showed invagination of the nuclear membrane, undulation of the mitochondrial structures, disappearance of glycogen, as well as appearance of dense lamellar structures in the peroxisomes (Mohelska et al., 1980).

No marked changes were found in histology of the heart in cats after chronic exposure of arsenic (Massmann and Opitz, 1954). However, dysfunction of the blood-brain barrier in rats (Tamura and Nozaki, 1972) and diminished acetylcholinesterase activity in the temporal region and in blood of arsenic exposed animals (Aly et al., 1975, Rozenstein, 1970) were observed. Histological changes in the brain included pericellular oedema, plasmatic impregnation of the vascular walls, plasmolysis and karyolysis of the neurons (Rozenshtein, 1970).

Rats given arsenic in the drinking water showed increased kidney weights in relation to body weights (Brown et al., 1976). The proximal tubular cells contained electron dense lysosome-like bodies and swollen mitochondria. Impaired kidney function, including decreased urea clearance and increased serum creatinine have been reported in rabbits given intravenous injections of arsemous acid (Shibuya, 1971).

Studies on laboratory animals indicate that arsenic can impair resistance to viral infections. Increased mortality from viral infections among mice exposed to arsenic was
reported by Gainer and Pry (1972).

In humans

Effects of arsenic on the respiratory system has been reported primarily as a result of occupational exposure. The symptoms include lesions of the mucous membranes in the respiratory system and perforation of the nasal septum (WHO, 1981).

Exposure to inorganic arsenic compounds has been associated with the development of chronic pathological liver changes. Several authors have reported liver damage following treatment with arsenic in the trivalent inorganic form (WHO, 1981). A common finding in these reports was portal hypertension without signs of liver cirrhosis. All patients had been on the arsenic medication, mostly Fowler’s solution for several years.

Changes in the electrocardiogram have rarely been reported after chronic exposure (Butzengeiger, 1949). Inorganic arsenic has effects on hematopoietic system alongwith disturbed erythropoiesis and occasionally megaloblastic changes (WHO, 1981). Hopenhayn-Rich et al. (2000) showed that arsenic exposure increased the risk of late fetal and infant mortality. Some studies reported that long-term exposure to arsenic in the workplace and through drugs resulted in peripheral neuropathy (WHO, 1981)

GLOBAL SCENARIO AND RELATED DISEASES

FLUORIDE

Fluorosis is an endemic public health problem in 23 nations around the globe
including India. Senegal is the first nation to reduce the upper permissible limit of fluoride in drinking water from 1.5 mg/litre to 0.6 mg/litre. The reason for such drastic change is due to the high prevalence of dental fluorosis in children with 1.5 mg/litre of fluoride in drinking water. India reduced the upper limit of fluoride in drinking water from 1.5 mg/litre to 1.0 mg/litre. In Mexico, where drinking water fluoride level ranges from 2.6-2.7 mg/litre, dental fluorosis is reported. African nations and China are facing serious health problems due to very high and widespread occurrence of fluoride in drinking water. Fluorosis appears in Sosnivka (a Ukranian Town), U.K, Poland, New Zealand, Finland, Thailand, China, Mexico, Tanzania, and India (Susheela, 2001; Takahashi et al., 2001; Tsutsui et al., 2000; Seppa et al., 2000). The classical features of endemic fluorosis are dental and skeletal changes. Relation between environment and endemic fluorosis have also been reported in Hohhot region of inner Mongolia (Xu et al., 1997) and Kenya (Kahama et al., 1997).

In India, the disease, fluorosis is known to occur for the past 6 decades. The number of states where endemicity is known to occur has increased to 17 out of the 32 states and union territories (Susheela, 2001). Among them Andhra Pradesh, Gujarat, Rajasthan, Karnataka and Orissa are more endemic. In endemic areas ‘echiocyte’ formation in RBC is common. Their membrane which is deficient in calcium content is pliable and is thrown into folds. The RBCs attain the shape of an amoeba with pseudopodia like folds projecting into different directions and such RBCs are termed as Echinocytes (Susheela, 2001). There is also relationship between hyperparathyroidism and endemic fluorosis (Gupta et al., 2001).
ARSENIC

It is well known that hyperpigmentation, keratosis, and cancer are the major manifestations of chronic arsenism from any source, but peripheral circulatory disorders have also been reported occasionally in chronic arsenicism. It seems reasonable to assume that arsenic may be the common etiological factor for skin cancer and Blackfoot disease involving peripheral vascular disorder resulting in gangrene. The prevalence rates for skin cancer, Bowen's and Blackfoot diseases increased with the arsenic content of well water, i.e. the higher the arsenic content, more patients suffered from skin cancer, Bowen's and Blackfoot diseases (Tseng, 1977; Col et al., 1999). A positive association between the arsenic levels of drinking water and the prevalence of skin cancer in endemic areas of chronic arsenicism has been reported from the district of Reichenstein, Silesia in Poland, Cordoba Province in Argentina, Antofagasta in Chile and Taiwan (Tseng, 1977). Several findings of Ishinishi et al. (1977) suggest that arsenodermaatitis, depigmentation, perforation of nasal septum, hyposmia, anosmia, and peripheral nervous disturbance attributed to exposure to arsenic were observed in workers of a mine at Japan. Apart from this, arsenic contents in surface or river water of many areas have been reported from several parts of the world like Germany (Quentin and Winkler, 1974) and Norway (Lenvik et al., 1978). Well water samples from an area in Alaska (Harrington et al., 1978) and thermal waters in New Zealand and Japan (Ritchie, 1961; Nakahare et al., 1978) contained high levels of arsenic.

In seven districts of West Bengal, India, 560 villages have more than a million people drinking arsenic contaminated water and about 200,000 people are suffering from
arsenic related diseases (WHO, 1981; Chowdhury et al., 2000; Akhtar Ahmad et al., 2001a; Acharyya, 2002). Arsenic has been found in groundwater above the maximum permissible limit of 0.5 μg/l as recommended by WHO (1981). Many people have arsenical skin lesions as: melanosis, leucomelanosis, keratosis, hyperkeratosis, oedema, gangrene, skin cancer (Mandal et al., 1996). In China also, arsenic toxicity is prevalent and together with fluoride is responsible for causing various health hazards (Wu et al., 1999; Yoshida et al., 1999; Sun et al., 2001a).

EFFECT ON GENERAL BODY METABOLISM

The toxicity of fluoride and arsenic is aggravated mainly through their adverse effects on general body and tissue metabolism. Therefore, the earlier findings on the role of fluoride and arsenic on general body metabolism is presented here.

PROTEIN METABOLISM

FLUORIDE

Fluoride is known to reduce protein synthesis (Hongslo and Holland, 1979) and inhibit growth of cell cultures (Holland, 1979). Impairment of the polypeptide chain initiation is believed to be the main reason for the inhibition of protein synthesis (Hoerz and McCarty, 1971). Decreased protein levels were reported in serum, various tissues and organs of mice and rats intoxicated with NaF (Chinoy and Sequeira, 1989a; Chinoy, 1991a,b; 1992, 2002; Chinoy et al., 1991a,b,c; 1992b, 1993a,b; Patel et al., 1994). Chinoy et al. (1997a) also reported that the testis and cauda epididymal proteins of mice were
altered with disappearance of some proteins and induction of some new ones. Fluoride also inhibits many enzymes (Chinoy, 2002; Chinoy and Memon, 2001). Birkner et al. (2000) found that acute poisoning in rats is due to disruption of cell metabolism since fluoride inhibits enzymatic processes, particularly metalloenzymes responsible for important vital processes.

ARSENIC

A number of sulfhydryl containing proteins and enzymes have been found to be altered by exposure to arsenic (Klassen et al., 1986). Arsenite (As^3+) having a high affinity for thiol groups in proteins, can form complexes with vicinal thiols and inhibit more than 200 enzymes (Roy and Saha, 2002). Recent studies by Chinoy and co-workers (Chinoy, 1999a; Chinoy and Nair, 2001; Chinoy and Shah, 2001; Chinoy et al., 2001a) also revealed decline in total proteins after treatment with arsenic alone or in combination in muscle, kidney, testis and ovary of mice.

CARBOHYDRATE METABOLISM

FLUORIDE

Fluoride is known as an inhibitor of glycolysis and affects the utilization or storage of carbohydrates. Shashi et al. (1988) have reported decline in glycogen concentration in spleen, lens, liver and skeletal muscle of rabbits treated with fluoride. On the contrary, accumulation of glycogen occurred in liver, gastrocnemius, muscle, vas deferens and uterus of fluoride treated rats, mice and fishes (Shaikh and Hiradhar, 1985;
The accumulation of glycogen was accompanied by a decrease in the activity of phosphorylase. This might be related to less utilization of glycogen which led to hypoglycemia in sodium fluoride treated mice and rats (Chinoy, 1992; Chinoy and Patel, D., 1996, Chinoy and Sharma, 1998; Chinoy and Memon, 2001; Chinoy et al., 1994c; Patel et al., 1994).

Catecholamines are known to regulate the carbohydrate metabolism. The serum of fluoride treated mice showed an enhancement in the levels of catecholamines (Chinoy and Patel, D., 1996; Patel et al., 1994). However, the urinary catecholamines were not influenced by fluoride intake (Chinoy, 1996).

**ARSENIC**

Alteration in carbohydrate metabolism is a major problem in poisoning with trivalent arsenicals (WHO, 1981). The prevalence of diabetes in arseniasis-hyperendemic villages in Taiwan was reported to be significantly higher than the general population since ingested inorganic arsenic is diabetogenic in human beings (Tseng et al., 1999). Decreased activity of phosphorylase and accumulation of glycogen after treatment with arsenic alone or combined with NaF in liver, muscle and uterus of mice were reported by Chinoy and associates (Chinoy, 1999a; Chinoy and Nair, 2001).
LIPID METABOLISM

FLUORIDE

The interrelationship of fluoride and lipid metabolism assumes considerable importance in view of the implication that fluoride plays a role in the incidence of arteriosclerosis. In rats supplemented with 100 ppm of fluoride resulted in marked reduction in plasma free fatty acids, either due to partial inhibition of lipolysis or to the lesser availability of depot fat in the extra hepatic tissues. The liver and serum lipid fractions were also affected (Saralakumari et al., 1988). However, the treatment brought about a noticeable increase in total lipids, triglycerides and phospholipids in the serum which point to the formation of a fatty liver. In liver of rabbits treated with NaF, triglycerides were decreased with a concomitant inhibition of lipase activity (Singh et al., 1985). Shashi (1992a) reported altered lipid metabolism in the reproductve organs of male rabbits. Subcutaneous injection of NaF (5, 10, 20 and 50 mg/kg body weight) to rabbits for 100 days revealed hyperlipidemia, hyperphospholipidemia in the brain (Shashi, 1992b)

Treatment with fluoride in rats and mice resulted in an accumulation of cholesterol in testis and ovary concomitant with a decrease in activity of 3β and 17β hydroxysteroid dehydrogenases (HSDs) as well as circulating testosterone and estradiol levels (Chinoy, 1992; 2002, Narayana and Chinoy, 1994a; Chinoy and Mehta, 1999a,b; Chinoy and Patel, T., 2001). From the above findings it is evident that fluoride might interfere with lipid metabolism and the gonadal steroidogenesis.
ARSENIC

Schiller et al. (1977) described blockage in conversion of pyruvate to citrate which would decrease the availability of citrate for the synthesis of fatty acids which in turn would result in less storage triglycerides. The effects of arsenic on mitochondrial pyruvate utilization results from the inhibition of the pyruvate dehydrogenase by arsenic (Schiller et al., 1977). Decline in 3β and 17β HSD alongwith accumulation of cholesterol and hence altered steroidogenesis, after As₂O₃ or NaF + As₂O₃ administration to mice was also reported by Chinoy (1999b) and Chinoy et al. (2001a) in ovary and testis. Hence the effects on gonadal steroidogenesis were similar by both arsenic and fluoride.

FREE RADICAL TOXICITY

Although oxygen is essential for life, it can also provoke damaging oxidative events within cells (Srivastava, 1998) as it has a tendency to form highly reactive oxygen species (ROS) or free radicals such as the hydroxyl radical (OH·), superoxide (O₂⁻), and hydrogen peroxide (H₂O₂) during various metabolic reactions like transformation of substrates for energy production, oxidation of endogenous compounds and detoxification of xenobiotics. Environmental pollutants such as insecticides, chemicals used in processing food etc., and radiation are also responsible for free radical formation.

Cellular damage by free radicals (FRs) or ROS toxicity include lipid peroxidation of membranes, cross linking of proteins, depolymerisation of polysaccharides, non peroxidative damage to mitochondria and DNA. Normally the oxidative events are kept under control by innate antioxidants within the living cells which counteract the effect of
free radicals in the form of free radical scavengers. A variety of enzymes help to maintain cells in a reduced state despite the presence of aerobic environment (Srivastava, 1998). The activity of antioxidative enzymes in cells such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase play a decisive role in the maintenance of cellular integrity. Their concerted metabolic action protects the cells against free radicals, most notably $O_2^-$ (Zawierta et al., 2000).

Studies from our laboratory (Chinoy and Mehta, 1999c; Chinoy and Patel, D., 1998a; Chinoy and Shah, 2002; Sharma and Chinoy, 1998; Memon and Chinoy, 2000) have revealed inhibition in the activities of SOD, GSH-Px and catalase after fluoride administration in testis, ovary, liver and brain along with increased levels of lipid peroxides, thus rendering the tissues susceptible to injury. Increased lipid peroxidation in RBCs, brain, liver and modification of fatty acid composition of phospholipids in liver and kidney due to oxidative stress in rats were reported by Shivarajashankara et al. (2001a) and Shao et al. (2000). Modification of membrane lipids in rats after administration of 30 or 100 ppm fluoride in drinking water for seven months was also reported by Wang et al. (2000), who suggested that it might be induced by oxidative stress.

High fluoride concentrations in endemic regions in China also inhibited the serum SOD and GSH-Px activities in exposed population but increased blood lipid peroxide levels resulting in the accumulation of large amounts of free radicals and peroxides causing cell damage in these people (Li and Cao, 1994; Pi et al., 2002). Increased lipid peroxidation associated with free radical mediated oxidative stress was also demonstrated by increased levels of malondialdehyde in the blood of children who suffered chronic
fluoride toxicity (Shivarajashankara et al., 2001b).

The depleted glutathione (GSH) levels by NaF treatment in animal studies strongly suggests that, like several compounds, fluoride might also be largely dependent on GSH for detoxication (Li et al., 1999). Similar studies from our laboratory (Chinoy and Narayana, 1994; Chinoy et al., 1995; 1997a,b; Chinoy and Patel, D., 1998a; Sharma and Chinoy, 1998; Chinoy and Mehta, 1999c) have also revealed reduced GSH levels in several organs of mice, rats and guinea pigs treated with fluoride. Studies by Dai et al. (1999) on endemic fluorotic patients have indicated that prolonged drinking of water containing high fluoride concentration caused reduced levels of GSH and GSH-Px. On the other hand, fluoride induced lipid peroxidation could be reduced by oral intake of glutathione and selenium in rats (Liang et al., 1999; Li et al., 1999). Similarly, some herbal antioxidants have also been used in reducing free radical damage in case of chronic fluorosis in China (Liu, 1999).

ARSENIC

Arsenic can participate in oxidation reduction reactions with species of oxygen like \(O_2\), \(O_2^-\) and \(H_2O_2\) and hence these reactions may be modulated by endogenous reducing agents such as glutathione, ascorbate and tocopherol (Roy and Saha, 2002). Wu et al. (2001) suggested that ingestion of arsenic contaminated well water may cause deleterious effects by increasing the level of reactive oxidants and decreasing the level of antioxidant capacity in plasma of individuals. Moreover, persistent oxidative stress in peripheral blood may be a mechanism underlying the carcinogenesis and atherosclerosis induced by long
term arsenic exposure (Wu et al., 2001). Studies indicate that arsenic might inhibit the activities of catalase and glutathione peroxidase, leading to accumulation of $\text{H}_2\text{O}_2$ (Lee and Ho, 1995). Li et al. (2001b) suggested that arsenic may trigger oxidative stress through multiple pathways, but $\text{H}_2\text{O}_2$ and $\text{O}_2^*$ are the main ROS involved in arsenic induced DNA damage. However, the exact mechanisms are still not clear. Arsenic is a pro-oxidant and thus may cause lipid peroxidation (Roy and Saha, 2002). Chinoy (2002) also showed decline in the activity of SOD, GSH-Px and catalase, levels of GSH, total and reduced ascorbic acids along with increase in lipid peroxides and dehydroascorbic acid in brain and ovary of mice after treatment with arsenic alone or in combination with fluoride.

**NUCLEIC ACIDS**

**FLUORIDE**

Fluoride has been reported to cause decrease in DNA and RNA synthesis in cultured cells (Strochkova et al., 1984), in rabbit ovary (Shashi, 1994) and in the ovary and uterus of fluorotic mice which could affect their metabolism (Patel, D. and Chinoy, 1998). The inhibition of DNA and RNA synthesis may result in delayed mitotic and meiotic cycles including chromosomal breakages (Vorishilin et al., 1973). The DNA/RNA and RNA/protein ratios were also altered significantly which suggested that fluoride might affect the translation and transcription processes (Patel, D. and Chinoy, 1997; 1998).
ARSENIC

Several studies have indicated that inorganic arsenic affects DNA repair mechanisms. Inorganic arsenic compounds could inhibit the incorporation of radioactive labelled phosphorus and nucleotides into the nucleic acids in lymphocytes (Sibatini, 1959; Petres et al., 1977). It is likely that the messenger RNA is already altered during its formation and delivers faulty information to the sites of protein synthesis (Petres et al., 1977). Arsenic either incorrectly builds into the nucleotide chains during nucleic acid synthesis or in higher concentrations, competitively inhibits the incorporation of phosphorus (Petres et al., 1977). Arsenic also induced DNA-base modification, DNA methylation and aberrant expression of genes through generation of reactive oxygen species (Roy and Saha, 2002). Arsenic could also induce DNA amplification (Lee et al., 1988).

REPRODUCTIVE EFFECTS

FLUORIDE

The interrelationship of fluoride and reproductive functions were unknown until 1970. Messer et al. (1973) found that fluoride plays an important role in reproduction. Tao and Suttie (1976) contradicted these reports and reported that fluoride does not have any essential role in reproduction. The work carried out by Chinoy and associates has clearly revealed that fluoride treatment affected the structure and functions of several reproductive organs and fertility rate of treated male and female mice, rats, rabbits and guinea pigs (Chinoy, 1991a,b; 1995; 1999b; 2002; Chinoy and Sequeira, 1989a,b; 1992;
NaF treatment significantly reduced the DNA and RNA levels in ovary of rabbits (Shashi, 1994) and mice (Patel, D. and Chinoy, 1998). The accumulation of cholesterol, concomitant with decline in 3β and 17β hydroxysteroid dehydrogenase activities and serum estradiol or testosterone levels in female and male animals suggested that fluoride interferes with cholesterol metabolism and steroidogenesis in testis and ovary (Chinoy, 1992; 1995; 1999b; Chinoy and Sequeira, 1989a; Chinoy and Mehta, 1999a,b; Chinoy and Patel, T., 2001; Chinoy et al., 2001a). Studies carried out by Patel, D. and Chinoy (1998) revealed that the oestrus cycle was irregular with prolonged duration of the diestrus stage which in turn severely affected the fertility rate in mice treated with 5 mg NaF/kg body weight for 45 days. Fluoride also induced lipid peroxidation and impaired the production of free radical scavengers such as glutathione and the function of the protective enzymes viz., glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase, which might render these tissues susceptible to injury (Chinoy and Patel, D., 1998a).

Further reports from our laboratory have revealed that NaF caused histological changes in ovary and uterus of mice (Chinoy and Patel, D., 1998b). The ovary showed vacuolization in the stromal region and in corpora lutea as well as necrotic follicles with pyknotic cell nuclei. In the uterus, decrease in thickness of myometrium, atrophy and confluence of endometrial glands with nuclear pyknosis of epithelial cells were observed.
Fluoride treatment caused a significant decrease in total protein levels in ovary and uterus of NaF treated mice (Chinoy and Patel, D., 1996; Chinoy et al., 2001a) which would affect the activities of their various enzymes. There was an increase in glycogen in uterus which might also be related to the decrease in the activity of phosphorylase in NaF treated mice (Patel et al., 1994; Chinoy and Patel, T., 2001). NaF also caused decline in uterine weight and the levels of DNA and RNA (Patel, D. and Chinoy, 1997; 1998). The structural and biochemical changes in ovary would affect its folliculogenesis and in uterus, the secretions and internal milieu would be altered.

ARSENIC

The possible impact of arsenic on reproductive functions has been given less attention, but the collective evidence from human and laboratory studies suggest adverse effects on several reproductive end points. Adverse reproductive impact among the offspring of employees and nearby residents exposed to arsenic from Swedish copper smelters were reported (Hopenhayn-Rich et al., 2000), wherein female workers gave birth to infants with lower weights than women who resided outside the smelter area, and the difference was greater if the mothers worked in highly exposed jobs. An increased trend in the rates of spontaneous abortions and congenital malformations was observed with increasing occupational and residential exposure (Hopenhayn-Rich et al., 2000) A study in Texas also found an increase in the rates of still births in relation to residential exposure from an arsenic pesticide factory (Ihrig et al., 1998). In Bulgaria, the incidence
of toxemia of pregnancy and the mortality from congenital malformations were significantly higher than the national rates in an area near a smelter with environmental contamination from various metals (Hopenhayn-Rich et al., 2000).

**COMBINED TOXICITY OF FLUORIDE AND ARSENIC**

Since the 1960s a large number of wells have been dug due to lack of water in many parts of the world. Unfortunately in many of them fluoride as well as arsenic are abundant like in China. So, combined arsenic-fluoride poisoning is an exceptional disease in the world. Liu et al. (1999) demonstrated that the toxicity of arsenic and fluoride has two aspects, namely the direct and indirect actions. A clinical study on syndrome of endemic arsenism and fluorosis (SEAF) on sixty five patients in Xinjiang, China, has been reported by Huang et al. (1992) which revealed that it is a kind of chronic syndrome resulting from the combined, harmful effects of arsenic and fluorine. Peripheral neuritis and cardiovascular changes were observed in this syndrome more often than in simple arsenism or simple fluorosis. The excessive quantities of these two trace elements in blood might have a synergistic, harmful effect on the nervous and circulatory systems. Studies from our laboratory on fluoride, arsenic combined treatment (Chinoy, 1999a; Chinoy and Nair, 2001; Chinoy and Shah, 2001; 2002; Nair et al., 2002) revealed many histological and histocytometric changes in soft tissues along with alterations in some specific biochemical parameters and genotoxic effect in human lymphocyte cultures.
Wu et al. (1999) reported that fluoride and arsenic seemed to be unilateral in action in humans.

**COMBINED TOXIC EFFECT ON OVARY AND UTERUS**

Only limited information exists on the reproductive effects of fluoride and arsenic combined toxicity. The histological studies on ovary of mice revealed structural alterations (Chinoy, 1999b; Chinoy et al., 2001a). The treatment brought about disintegration, necrosis and dense vacuolization in the stromal tissue, follicular atresia, pyknosis in the follicular cell nuclei and hemorrhage. Further, the corpus luteum diameter and number of primary and secondary follicles were decreased. The above data clearly elucidates alterations in ovarian structure and folliculogenesis, which would influence its functions.

The combined treatment also resulted in changes in histology of the uterus. Vacolization in the myometrium and endometrium occurred in treated group of animals as compared to control. Atrophy and confluence of the endometrial gland with pyknosis of their cell nuclei was also observed. These changes would influence the growth of the organ as well as its enzymes, secretion, metabolism and ultimately change its internal milieu which is so necessary for nidation and implantation. Reports from our laboratory by Chinoy and co-workers (Chinoy, 1999b; 2002; Chinoy et al., 2001a) on biochemical parameters of ovary and uterus after combined treatment of fluoride and arsenic revealed that the treatment also affected protein metabolism in ovary and uterus, carbohydrate metabolism in uterus, altered steroidogenesis and caused free radical toxicity in ovary of mice.
TERATOGENICITY

FLUORIDE

Studies carried out by Glenn et al. (1982) suggested that fluoride may exert effects on fetal growth. Babies, whose mothers had received fluoride tablets during pregnancy were somewhat heavier and slightly longer at birth.

ARSENIC

Inorganic arsenic enters the mammalian fetus easily, as was shown by experiments on mice, rats, hamster and man. Development abnormalities, low birth weight, malformations, and fetal death have been observed (Merian, 1991).

Lugo et al. (1969) reported a case of fetal death after maternal inorganic arsenic poisoning during pregnancy, but no unusual pathological changes were observed in the foetus. Offspring of smelter employees showed reductions in birth weight and malformations (IARC, 1980). Babies born to women exposed to arsenic dusts during pregnancy had a higher than expected incidence of congenital malformations and average birth weight was slightly below average (Hopenhayn-Rich et al., 2000). Adverse pregnancy outcomes in terms of spontaneous abortion, stillbirth, and preterm birth rates were significantly higher in the exposed group than those in the nonexposed group (Akhtar Ahmed et al., 2001b).
Conflicting reports are available in the literature regarding the genotoxic effects of fluoride (Li et al., 1988). Jachimczak and Skotarczak (1978) have reported that sodium fluoride induces chromosome aberrations in cultured human leucocytes, whereas, Kram et al. (1978) observed no significant changes in SCE in bone marrow cells, after ingestion of fluoride diets to mice. Thompson et al. (1985) found that fluoride did not induce an increase in the frequencies of chromosomal aberrations or sister chromatid exchanges (SCEs) in human lymphocyte cultures. However, studies carried out by Sheth et al. (1994) have reported an increase in the frequency of SCEs in endemic human population of North Gujarat, India, as compared to control for the first time in the affected fluorotic population. Gadhia and Joseph (1997) reported an increase in the frequency of chromosome aberrations but no appreciable increase of SCEs in human lymphocytes of normal individuals cultured with different doses (10, 20, 30 µg/ml) of fluoride. This discrepancy might be related to the fact that Gadhia and Joseph (1997) have used normal individuals and cultured their lymphocytes with different concentrations of fluoride added in the medium, whereas, Sheth et al. (1994) have used blood of fluorotic individuals. In a later study however, Joseph and Gadhia (2000) also demonstrated that the rates of SCEs and chromosome aberrations in persons of one of the endemic villages with high fluoride, were significantly higher than in another with low water borne fluoride in South Gujarat, India. Li et al. (2000) found that fluoride increase the micronucleus formation in mammals and could damage the chromosomes.
The above information clearly demonstrates that at present there is no established opinion regarding the genotoxic effects of fluoride and its potential as a mutagenic agent. It is apparent that further investigations are necessary in order to clarify this important issue.

An augmented frequency of Down’s Syndrome with increasing water fluoride concentration has been reported (Erickson, 1980; WHO, 1984). Similarly, Takahashi (1998) has also reported fluoride related incidence of Down’s Syndrome birth in young mothers in several region of USA with fluoridated water.

ARSENIC

Investigations of genotoxic effects of ingested arsenic have yielded mixed results. In humans exposed to Fowler’s solution, increased sister chromatid exchange, but no increase in chromosomal aberrations was reported in one study (Burgdorf et al., 1977), while just the reverse (increased aberrations but no increase in sister chromatid exchange) was reported in another (US DHHS, 1998). As (III) and As (V) inhibit DNA, RNA and protein synthesis in leucocytes (Sibatam, 1959; Nakamura and Sayato, 1981) and replace phosphate in the nucleotides during DNA synthesis (Merian, 1991). Further, arsenic generates reactive oxygen species which are known to induce poly ADP-ribosylation which is implicated in DNA repair, signal transduction and apoptosis. As a result, arsenite may induce DNA strand breaks and NAD depletion. Hence the genotoxic effects of arsenic compounds may be connected with an inhibition of DNA repair or the induction of oxidative stress (Roy and Saha, 2002). In fact, metabolic methylation of inorganic...
arsenic to dimethyl arsenic acid is involved in induction of DNA damage and DNA single strand breaks. It is thus likely that arsenic mediated DNA protein interactions may play a major role in DNA strand breaks and chromosome aberrations (Roy and Saha, 2002).

Induction of cancer due to DNA damage appears to be the most striking long term effect of chronic exposure to inorganic arsenic. Epidemiological studies have demonstrated relationship between environmental, occupational and medicinal exposure of man to inorganic arsenic and cancer of the skin and lungs (WHO, 1981). Few recent reports on arsenic induced DNA methylation have reinforced its carcinogenic potential since both hypo- and hyper-methylation of DNA could cause aberrant expression of genes (such as oncogenes or tumour suppressor genes) which in turn cause abnormality in cell proliferation leading to carcinogenesis (Roy and Saha, 2002). Recent report from our laboratory has revealed an increase in SCEs and decline in cell cycle proliferative index in human lymphocytes exposed to fluoride, arsenic or fluoride and arsenic in vitro (Nair et al, 2002).

MECHANISM OF TOXICITY

FLUORIDE

Grucka-Mamezar et al. (2000) suggested that fluorine, due to its high chemical and biological activity as well as its small size, could easily penetrate hard tissues (bones, teeth). Fluoride ions are taken up rapidly by bone by replacing hydroxyl ions or are attracted by positively charged ions like calcium (Ca++) in bone apatite. The consensus is that fluoride is incorporated into the hard tissues largely by a process of exchange and
by incorporation into the apatite lattice during mineralization (US NAS, 1971; Das et al., 2000). Fluoride also causes biochemical changes in bone and reduces the collagen content (Susheela, 2001).

Fluoride is known to stimulate the so called respiratory burst and the production of superoxide radicals in neutrophils of humans, rabbits and guinea pigs. The high reactivity of superoxide radicals may lead to chemical modification and impairment of proteins, lipids, carbohydrates and nucleotides in living cells (Rzeuski et al., 1998). As mentioned earlier, free radical injury in several reproductive organs and soft tissues was also reported by Chinoy and co-workers (Chinoy, 1995, 2002; Chinoy and Patel, D., 1998a; Chinoy and Mehta, 1999c; Chinoy and Shah, 2002; Sharma and Chinoy, 1998; Memon and Chinoy, 2000).

Fluoride ion forms a strong hydrogen bond (NH—F) with purine and pyrimidine bases (Clark and Taylor, 1981; Caspary et al., 1987) and thereby affects nucleic acids.

ARSENIC

The mechanism of arsenic toxicity is not yet fully understood, but the possibilities are: Reduced inorganic arsenic (As³⁺) reacts strongly with sulphhydryl groups in proteins and inactivates many enzymes. A particular target in the cell is the mitochondria, which accumulates arsenic. Arsenic inhibits succinate dehydrogenase activity and can uncouple oxidative phosphorylation, the resulting fall in ATP levels affect virtually all cellular functions (Na⁺/K⁺ balance, protein synthesis, etc.). The genotoxic database for arsenic indicates that it does not induce point mutations or DNA adducts, but chromosomal
aberrations and sister chromatid exchanges. Arsenic can also potentiate mutagenicity observed with other chemicals. This potentiation may be the result of direct interference by arsenic with DNA repair processes, perhaps by inhibiting DNA ligase (Li and Rossman, 1989). Arsenic also induces DNA amplification (Lee et al., 1988). Zhao et al. (1997) have reported hypomethylation of DNA in rat liver cell line (TRL 1215) by arsenic whereas, Mass and Wang (1997) have shown hypermethylation in the human lung adenocarcinoma cell line (A549). Change in methylation of DNA can affect its structural stability and result in chromosomal damage leading directly to transformation. Germolec et al. (1997) have suggested that chronic low level exposure to arsenic stimulates secretion of growth factors by keratinocytes and the resulting increased cellular division (and concomitant DNA replication) allows greater opportunities for genetic damage to occur.

REVERSAL OF TOXICITY

Chinoy and co-workers (Chinoy and Sequeira, 1989a,b; 1992; Chinoy and Patel, D., 1998a; Chinoy and Mehta, 1999a; Chinoy and Sharma, 1998; 2000; Narayana and Chinoy, 1994b; Patel and Chinoy, 1997; Chinoy et al., 1991a; 1994c; 1995; 2001a), have reported partial or incomplete recovery in several biochemical parameters in various organs of mice and rats, after the withdrawal of NaF treatment for one or two months. But after two months, Chinoy and Sequeira (1989b) found marked recovery in the histoarchitecture of reproductive organs of male mouse. Therefore, the toxic effects induced by fluoride were found to be partially reversible after cessation of fluoride
There is hardly any data regarding withdrawal studies on arsenic toxicity alone. But reversible studies were carried out in our laboratory (Chinoy, 1999a,b; Chinoy and Nair, 2001; Chinoy and Shah, 2001; 2002; Chinoy et al., 2001a; Tewari and Chinoy, 2002) after combination treatment of NaF and As$_2$O$_3$ for 30 days in mice and the treatment was withdrawn for a further period of 30 days afterwards. These studies elucidated that toxic affects were partially recovered and a longer period of withdrawal would be needed for a better recovery.

**USE OF ANTIDOTES FOR REVERSAL OF TOXICITY**

In view of millions of people afflicted with fluoride and/or arsenic toxicity which induced a variety of pathological manifestations in soft tissues of both animals and human beings, necessitates the investigation of therapeutic agents which are easily available, cheap and have promising results in mitigation of fluoride and arsenic induced health hazards in endemic populations.

**REVERSAL OF FLUORIDE TOXICITY**

Earlier studies from our laboratory have reported the beneficial effects of some antioxidant vitamins, C and E as well as calcium, vitamin D either alone or in combination and a protein rich diet for reversal or mitigation of the toxicity. In the present study, vitamins C, E and calcium have been used. Hence their mechanisms are described here.
Ascorbic acid functions as a powerful anti-oxidant and it is also known to bind with macromolecules like nucleic acid and protein (Chinoy, 1978) by change transfer complex formation, which appears to be a very active source of energy for biological processes (Chinoy, 1978). A number of studies have demonstrated mitigation of fluorosis in experimental animals and fluorotic human populations by the ingestion of ascorbic acid (Wadhwani, 1954; Yu and Hwang, 1985; Chinoy, 1991a,b; 2002; Chinoy et al., 1991a; 1994d; 1995; 1997a,b; Narayana and Chinoy, 1994b; Patel, D. and Chinoy, 1997; Chinoy and Patel, D., 1998a; Chinoy and Sharma, 1998; 2000). Ascorbic acid is known to inhibit phosphodiesterase (PDE) (Pasternak, 1979) and thereby increase C-AMP levels. The increase in C-AMP, a "second messenger" might have resulted in the recovery in the activities of several enzymes in different tissues. Ascorbic acid itself is known to activate several hydroxylating enzymes and those involved in the oxido-reduction reactions in various tissues. Ascorbic acid depletion in ovary is considered as an index for steroidogenesis and it is also involved in overcoming stress (Chinoy, 1978).

Vitamin E is one of the most active and major lipid soluble antioxidant in tissues (Chinoy and Sharma, 1998; Sharma and Chinoy, 2000; Chinoy and Patel, T., 2001; Chinoy and Memon, 2001) which reduces cell injury by preventing oxidation in vivo of polyunsaturated fatty acids (PUFAs) to hydroperoxides and thus protects structural integrity of membranes and prevents lipid peroxidation as well as atherosclerosis. Vitamin E has also been related to changes in calcium homeostasis in tissues (Meerson et al., 1982). Sharma and Chinoy (1998) have reported that ingestion of vitamin E to fluorotic male mice brought about a significant recovery in NaF induced reproductive failure. It
also resulted in significant recovery in the activities of SOD, glutathione peroxidase, catalase and levels of glutathione, lipid peroxides in ovary as well as serum calcium and potassium levels. The antioxidant properties of vit. E are enhanced in the presence of ascorbic acid. Hence these vitamins (C and E) act synergistically for recovery of the toxicity.

It is well known that calcium (Ca²⁺) combines with fluoride to form an insoluble compound CaF₂ thereby reducing its absorption. Calcium activates several enzymes, whereas, both calcium and ascorbate are known as inhibitors of phosphodiesterase (PDE) and enhance C-AMP levels (Rasmussen, 1989). Calcium chloride and calcium gluconate have been used in acute fluoride poisoning (Yolken et al., 1976). Ameliorative role of calcium for mitigation of fluoride toxicity in mice and rats has been reported (Chinoy, 2002; Mehta and Chinoy, 2000; Ekambaram and Paul, 2001).

Vitamin D is also useful as a therapeutic agent against fluoride toxicity in male mouse (Chinoy and Sharma, 1998; Sharma and Chinoy, 2000) and in female mouse (Chinoy and Patel; D., 1998a).

Some other dietary factors besides vitamins and calcium also help in mitigation of induced toxicity. Studies conducted in our laboratory by Chinoy and co-workers (Chinoy and Patel, D., 1996; Chinoy and Mehta, 1999b; Patel, D. and Chinoy, 1998) revealed that amino acids (glycine and/or glutamine) were beneficial in promoting the recovery from fluoride induced toxicity. Feeding a protein supplemented diet to mice given alongwith sodium fluoride in different doses helped in suppressing the toxicity, whereas, a protein deficient diet aggravated it (Chinoy and Mehta, 1999a).
Intravenous administration of magnesium compounds (MgO or Mg(OH)$_2$) have been reported to increase excretion of fluoride in urine and faeces (Rao et al., 1975; Raja Reddy et al., 1985) and decrease the amount retained in bones. Aluminium sulphate and boron have also been tried for this purpose (Franke et al., 1985). Tamarind ingestion also decreased fluoride retention in bones of dogs (Khandare et al., 2000). Antioxidative preparations containing glutathione, β carotene and superoxide dismutase have been used for the mitigation of fluoride toxicity (Qiu and Sun, 1999). Similarly, preventive antifluorosis preparations containing zinc salt, boron or selenium compounds have also been used by Sun et al. (1999) in China. Recent studies by Sun et al. (2001b) revealed that low molecule antioxidants like vitamin C, E and β-carotene inhibit the fluoride induced oxidative damage and moreover, they could accelerate the excretion of fluoride.

REVERSAL OF ARSENIC TOXICITY

There is very limited information regarding reversal of arsenic toxicity. Knowledge of the physiological processes of arsenic metabolism and the biochemical pathways involved in it coupled with the knowledge of arsenic chemistry can be used to device treatments of arsenic toxicity. Increasing the intracellular antioxidant level may have preventive or therapeutic effects in arsenic poisoning (Roy and Saha, 2002). Acute arsenic intoxication may require treatment with chelating agents such as dimercaprol (BAL) and D-penicillamine. Chelating therapy is most effective when instituted within a few hours after exposure, and efficacy decreases as time after exposure increases (US DHHS, 1998). Some water soluble and less toxic analogues of BAL such as dimercaptosuccinic acid
(DMSA), dimercaptopropylphthalamadic acid (DMPA), and dimercaptopropane sulfonic acid (DMPS) are currently under investigation and may prove to be promising treatments for arsenic poisoning (US DHHS, 1998). N-acetylcysteine has been used in animals to chelate arsenic (Haddad and Winchester, 1990) and a human case study reported N-acetylcysteine to be successful in treating a case of arsenic poisoning that was not responding well to BAL treatment (Martin et al., 1990). Many of the chelating agents discussed above (BAL, DMSA, DMPA, DMPS, N-acetylcysteine), contain sulfhydryl groups. Therefore, administration of sulfhydryl-containing compounds soon after exposure could provide alternative target molecules for arsenic and prevent inhibition of enzyme functions as arsenic interacts with thiol groups of proteins as mentioned earlier. Hsu et al. (1999) and Xia et al. (1999) have reported that selenium can alleviate the symptoms of arsenic poisoning in arsenic exposed persons. Treatment of arsenic related skin cancer with recombinant interferon Alfa-2b have been attempted by Liang et al. (1999).

REVERSAL OF FLUORIDE AND ARSENIC COMBINED TOXICITY

There is hardly any data regarding reversal of combined toxicity of fluoride and arsenic. After combined treatment of NaF and As2O3 for 30 days in mice, reversible studies with the help of therapeutic agents viz. ascorbic acid (AA), Vit. E and calcium administered alone or in combination were carried out for another 30 days during withdrawal period in our laboratory (Chinoy, 1999a,b). These studies elucidated recovery in many biochemical parameters in several tissues of mice and helped to restore normal status.
Human and animal model studies on metabolism and detoxification have provided an insight into how nutritional status may ameliorate or aggravate fluoride and arsenic toxicity. Extensive work carried out on reproductive system of various animal models by Chinoy and co-workers have elucidated reversal of fluoride and/or arsenic toxicity by supplementation of vitamins viz. Vitamin C, D, E, amino acids and calcium as mentioned earlier (Chinoy, 1991a,b; 1992; 1999b; 2002; Chinoy and Patel, D., 1996, 1998a; Chinoy and Patel, T., 2001; Chinoy and Sharma, 1998; 2000; Narayana and Chinoy, 1994b; Patel, D. and Chinoy, 1997; 1998; Tewari and Chinoy, 2002; Chinoy et al., 1991a; 1992; 1994d; 1995; 1997a,b; 2001a; Patel et al., 1994).

The typical remedy for toxic metal exposure has been to remove the exposure source. However, in some countries like India and China today, millions of people are affected and it is impossible to remove everyone from exposure sites. In these cases, dietary manipulations may provide the best defence and awareness programmes need to be undertaken for the population especially in endemic areas.

In the light of the above, the present work was undertaken to investigate the possible protective effects if any, of vitamin C, vitamin E and calcium administered alone or in combination in mitigating fluoride and arsenic toxicity in mice with special reference to the ovary and uterus.

The genotoxic effects of fluoride are still controversial, while there is paucity of data regarding genotoxic effects of arsenic alone and in combination with fluoride and its mitigation. The protective effects of ascorbic acid on genotoxicity of fluoride and arsenic treated cultures were also investigated since there are some reports suggesting its
ameliorative effects on different chemical toxicity in lymphocyte cultures (Chakravarty et al., 1997; Rao et al., 1999; 2001). Ascorbic acid is a known antioxidant and has a role to play in human leucocytes. Hence, ascorbic acid has been used for our studies.

With a view to find out the possible in vivo and in vitro effects of fluoride and/or arsenic the present study was carried out in two parts:

PART I : IN VIVO STUDY

1. To evaluate effects of fluoride and/or arsenic on reproductive tissues viz. ovary and uterus of female mice.

2. To study the reversibility of toxic effects by withdrawal of treatment.

3. The role of antidotes viz., ascorbic acid, vitamin E and calcium administered alone and in combination during the withdrawal periods to find out the protective effects, if any.

PART II . IN VITRO STUDY

1. To evaluate the SCE, chromosome aberrations, aneuploidy and micronuclei analysis to find out DNA damage, if any in human (male) leucocyte cultures.

2. To find out the effects of fluoride and/or arsenic on the acrocentric and telomeric associations if any.

3. The protective effect of ascorbic acid was also studied.
Source of human exposure of fluoride and various modes of fluoride toxicity.
Adapted with slight modification from Miller, 1993
Source of human exposure to arsenic and various modes of arsenic toxicity. Adapted with slight modifications from Roy and Saha, 2002.