Life of modern man has been greatly changed by the development of chemicals such as pharmaceuticals, pesticides, food additives, building materials and industrial chemicals etc. Toxic chemicals at any level of chronic exposure affect human health. Fortunately, the body has mechanisms for transforming, eliminating or compartmentalizing many toxic chemicals encountered in a lifetime. Nonetheless, these “safety” mechanisms may be inadequate or even inappropriate in our modern industrialized society, especially for susceptible people such as the elderly, individuals with specific nutritional habits and others who are physiologically stressed. Some toxic chemicals viz. fluoride, arsenic, lead, mercury, cadmium and nickel etc. are well documented examples for manifesting health hazards resulting in the decline or altered cellular functions.

The toxicity induced by these chemicals on various biological systems including human being is of two types:

**Acute toxicity** includes the adverse effects occurring within a short time of (oral) administration of a single dose of substance or multiple doses given within 24 hours (Gupta and Salunkhe, 1985).

**Chronic toxicity** where an ingestion of small quantities of the toxic substance over a prolonged period of time leads to toxic concentration and thus symptoms of poisoning occur. The symptoms in chronic toxicity may differ from those seen in acute toxicity.
In the present thesis, investigations on the effects of two toxic substances "fluoride" and "arsenic" on some organs of mice were carried out. Hence, a review is presented here.

**Fluoride** is the most active of all electrolytes found in the body and is deposited mainly in bones and teeth. The main source is drinking water. Sodium fluoride is a hazardous waste by-product from the manufacture of aluminium and is a common ingredient in rat and cockroach poisons, anesthetics, hypnotics, psychiatric drugs, military nerve gas etc.

One of the most toxic inorganic arsenic compounds is arsenic trioxide \((\text{As}_2\text{O}_3)\). Arsenic is listed as a presumed carcinogenic substance and has been found as a contaminant in such common items as wine, glues, pigments, certain water supplies and sea-food (WHO, 1981).

**PHYSICAL AND CHEMICAL PROPERTIES**

**FLUORIDE**

It is a solid, white substance with a molecular weight of 41.988. It has place VII A in the periodic table with an atomic number 9, atomic mass of 19 and one valence state (WHO, 1970).

**ARSENIC**

It is an odorless, white solid with a molecular weight of 197.84. Arsenic displays variable valencies (-3, +3 and +5) and has both cationic and anionic forms (WHO, 1981). Elemental arsenic exists at room temperature as metallic gray arsenic and yellow arsenic.
Grey arsenic is the common stable form, whereas, yellow arsenic is unstable (WHO, 1981).

**LD₅₀ VALUE**

The statistically derived single dose of a chemical that can be expected to cause death in 50% of a given population of organisms under a defined set of experimental conditions is known as Lethal Dose (LD₅₀) value.

**FLUORIDE**

The LD₅₀ values for male and female rats are 250 mg and 180 mg F/kg body weight respectively and for male mice is 54.4 mg F/kg body weight, while, the females have LD₅₀ value of 51.6 mg F/kg body weight (Pillai et al., 1987; 1988).

**ARSENIC**

The LD₅₀ of arsenic trioxide in Swiss mice is 39.4 mg/kg (U.S.NAS, 1997; USEPA, 1980). Human oral LD₅₀ for As₂O₃ (arsenic trioxide) is 1.4 mg/kg (Hallenbeck and Cunnigham, 1986).

**HALF LIFE**

The half-life of a substance is the amount of time taken for one-half of the chemical to be degraded.
FLUORIDE

The half life of fluoride in blood and soft tissues has been reported to be a few hours, while in skeleton, it has a longer life of about 8 years (WHO, 1984).

ARSENIC

The half-life of inorganic-arsenic in blood is two hours and that of methylated metabolites 5 to 20 hours (WHO, 1981).

RECOMMENDED SAFE LEVELS

FLUORIDE

The 1989 report "Diet and Health" issued by the National Research Council stresses that fluoridation of drinking water supplies at a level of 1 ppm protects against dental caries and in such concentrations is not associated with any known adverse health effects. A review of drinking water standards has confirmed optimal levels between 0.7 and 1.2 ppm dependent on an average daily air temperature (NHMRC, 1991). A dietary F intake below 1.0 mg/day is safe and does not lead to dental fluorosis (Desai et al., 1998).

ARSENIC

The maximum permissible limit of arsenic (0.01 mg/litre) was recommended in drinking water by WHO (1996) and according to them drinking water containing more than 0.05 milligram per litre is harmful to health.
OCCURRENCE AND SOURCES

FLUORIDE

Fluoride is a trace element, which is found in our water, food, vegetation, soil, air, environment and body. Potential sources of fluoride emission include industrial plants concerned with phosphoric acid and superphosphate productions, aluminium smelters, foundries, glass, brick and tile works, plastic and fluorinated hydrocarbon production and coal burning (Smith, 1985).

ARSENIC

Arsenic exists in the metallic state in nature in water supplies and seafood. Today, arsenic poisoning is encountered through industrial exposure.

IN AIR

FLUORIDE

Air is invariably considered as the major carrier of fluoride where it exists as HF and SiF4. Traces of fluoride in the air of rural communities and cities arise from both natural sources and human activities. Fluoride compounds reach the air from forest fires, man's industrial activities, from the volcanoes, dust generated by the weathering and outcropping of fluoride containing soils and minerals and smokes from burning coal (WHO, 1984) (Please see Flow Chart).
Fluoride in the environment through industrial and other man-made sources

Adapted from Miller (1993) with slight modification
ARSENIC

The most recent information about occurrence of arsenic in air is from US Environmental Protection Agency (USEPA, 1980), which revealed that the atmosphere is a significant channel for the recycling of arsenic to water and soil via fallout or precipitation (Please see Flow Chart).

The air borne arsenic is generally due to contribution from industrial contamination and may range from a few nanograms to a few tenths of a microgram per cubic meter. Air-borne arsenic is usually trivalent arsenic oxide (WHO, 1981).

Occupational exposure to arsenic compounds takes place mainly among workers, especially those involved in the processing of copper, gold, and lead ores, cutting and sawing wood treated with arsenic containing preservatives and production of arsenic-containing pesticides. The recommended air-borne exposure limit is 0.002 mg/m³, which should not be exceeded during any 15-minute work period for inorganic arsenic (US DHHS, 1998).

IN WATER

FLUORIDE

Water is main source of fluoride. The natural fluoride content of water in different areas vary according to its geological formation, the amount of rainfall and the quality of water lost by evaporation. All drinking water contains some fluoride naturally ranging from 0.1 to more than 20 ppm. Surface water from river and lakes generally contain lower concentration of fluoride than well water and the highest amount of fluoride was detected in water from bore wells (WHO, 1984). High fluoride content has been found in
Exposure to arsenic and various modes of toxicity,

Adapted from Roy and Saha (2002) with slight modification.
many parts of Asia, Europe, Australia, North and South America (WHO, 1984). The water samples collected from endemic villages of North Gujarat revealed high fluoride content ranging from 1.0 to 6.53 ppm (Chinoy, 1991a,b; Chinoy and Narayana, 1992; Chinoy et al., 1992a; 1994a). The US DHHS (1998) has established a maximum contamination level of 0.05 mg/litre for arsenic in drinking water, while the World Health Organization (WHO, 1996) has established a value of 0.1 mg/L for arsenic in drinking water.

ARSENIC

Arsenic is mainly transported in the environment by water and occurs in both inorganic and organic forms. In oxygenated water the two inorganic arsenic species usually occur as arsenate As(V) but under reducing condition for instance, in deep well water, arsenite As(III) predominates. Methylation of inorganic arsenic to organic arsenic monomethylarsonic acid (MMA) and finally to dimethyl arsenic acid (DMA) is associated with biological activity in water (Roy and Saha, 2002). Wine and mineral water can sometimes contain several hundreds of micrograms of arsenic per litre, probably as a result of arsenic containing pesticides.

The natural concentration of total arsenic in drinking water varies in different parts of the world and could be severely contaminated through industrial operations. Leaching of arsenic from coal preparation wastes and fly ash from coal-fired power plants may also result in the contamination of water (WHO, 1981).

Arsenic has been found in the ground water above the maximum permissible limit (0.05 mg/litre) in seven districts (560 villages) of West Bengal (India) covering an area of
37,493 km$^2$ having about 34 million population. This water is used by the villagers for drinking, cooking and other household purposes. People have skin manifestations and high arsenic in hair, nail, urine and skin scales (Mandal et al., 1996).

IN ROCKS AND SOIL

**FLUORIDE**

Fluorine is the seventeenth most abundant element and represents about 0.06-0.9% of the Earth's crust (WHO, 1984). The main primary fluoride-containing mineral is fluorospar or fluorite (CaF$_2$), which has been used as flux in several countries. The fluoride content of topsoil may be increased by the addition of fluoride containing phosphate fertilizers, pesticides, and irrigation water or by deposition of gaseous and particulate emissions. To a large extent, soil fluoride determines its level in water, vegetation, domestic and wild animals and indirectly in humans whose food is derived from the above sources.

**ARSENIC**

The arsenic content of the Earth's crust is 1.5-2 mg/kg; it ranks 20th in abundance in relation to other elements (USNAS, 1977).

The level of arsenic in soil is about 7 mg/kg, but could be as high as 1000 mg/kg in the agricultural soils where extensive use is made of pesticides, herbicides and defoliants (Merian, 1984). Arsenic compounds tend to form insoluble complexes with soils and sediments (Merian, 1991). Uncontaminated soils were found to contain arsenic levels between 0.2 and 40 mg/kg, while arsenic-treated soil contained upto 550 mg/kg.
Arsenic is of environmental concern because it is much more toxic than arsenate and is more mobile in soil system. Most arsenic (about 80% of the total) that is released to the environment from human activities is released to soil (USEPA, 1982).

Arsenic is widely distributed in a large number of minerals. The highest mineral concentration generally occurs as arsenides of copper, lead, silver or gold or as the sulfide. In soil, arsenic can also be volatilized by microbial functioning to gaseous arsines, which may travel in air for long distances or they are oxidated rapidly depending on environmental conditions. Oxidation returns arsenic back to inorganic species and the cycle of arsenic is completed because atmospheric inorganic arsenic deposits back into soil by rain or by dry deposition (Turpeinen et al., 2000).

**TOTAL HUMAN INTAKE**

**FLUORIDE**

The fluoride contents in air, water and food determine the human intake of fluoride. It is reported that persons living near industrial sources of fluoride could inhale 0.06 mg during a day of maximum pollution. Intake of fluoride will be more in persons living in endemic areas through drinking water. Most plants contain between 0.1 and 10 ppm of fluoride on dry-weight basis and because of this high level (beyond 1 ppm) in most plants, it is directly related to the vegetable, fruits, cereals, citrus fruits, tubers etc. Fluoride is also found in fluoride containing dentrifices, chewing gum, mouthwashes, toothpastes, anesthetic agents, etc. (WHO, 1984).
ARSENIC

Daily human intake is between 0.01 to 0.3 mg arsenic depending on the diet. The arsenic concentration in normal human organs and body fluids is between 0.02 and 0.06 ppm. Higher arsenic levels are found in muscle, lungs, femurs, skin, teeth, nails and especially hair. Arsenic levels in hair are generally higher than 0.4 mg/kg (Merian, 1991). In normal persons, arsenic concentration in the blood is about 0.004 mg/kg. Cebrian (1985) found arsenic concentration of 0.002 mg/L in blood and 0.001 mg/L in urine in unexposed Mexicans. There are many species and strain differences in the toxicity of arsenical compounds. The purity, physical form and solubility of the compounds also influence toxicity. Arsenic has been found in meat, fish and poultry.

ABSORPTION

FLUORIDE

In the industrial environment, the respiratory tract is the major route of absorption of both gaseous and particulate fluoride. Solutions of fluoride salts are rapidly and almost completely absorbed from the gastrointestinal tract, probably by simple diffusion (WHO, 1984).

Dermal absorption of fluoride has only been reported in the case of burns resulting from exposure to hydrofluoric acid (WHO, 1984).

ARSENIC

Absorption of inorganic arsenic from the gastrointestinal tract can occur following the ingestion of food, water, beverages or drugs, containing arsenic or as a result of
inhalation and subsequent mucociliary clearance. The absorption of ingested arsenic will
depend on the solubility of the compound in question and whether the arsenic compound
is given in solution or as undissolved particles (WHO, 1981). Inorganic arsenic absorbed
from the gastrointestinal tract, undergoes rapid distribution to nearly all the organs (US
DHHS, 1998).

**DISTRIBUTION**

**FLUORIDE**

Absorbed fluoride is distributed throughout the body as the fluoride ion. Observations show that after absorption from the gut, fluoride enters the circulation (WHO, 1984). Three-fourth of the total fluoride of food is found in the plasma, which exists in free and bound forms, the latter is bound to the serum albumin (WHO, 1984). Fluoride content in blood was 0.1 ppm when the water contained 1.0 ppm fluoride (Underwood, 1977). The ingested fluoride enters the circulation mixed with large volume of extra-cellular fluid. The sequestration of fluoride from circulation into the skeleton, urinary excretion and loss through sweat help in the regulation of plasma levels of fluoride and is a protective measure of nature (US DHHS, 1998).

The levels of fluoride in most of the soft tissues of the body are lower than 1 ppm, but are higher than those of plasma (Eichler et al., 1966). The only exception being tendons and placenta where the fluoride content appears to be related to their calcium content (WHO, 1984). The fluoride content of the brain is 0.4 to 0.68 ppm and the cerebro-spinal fluid is 0.1 ppm (WHO, 1984). The total fluoride of muscle normally is 0.2 ppm (WHO, 1984). Under normal conditions, soft tissues, organs contain little or no
fluoride. However, with impaired kidney function or prolonged fluoride exposure, relatively large amounts of fluoride can accumulate in soft tissues.

ARSENIC

Once absorbed, arsenic is reported to get distributed in the liver, kidney, skin, lungs and spleen (WHO, 1981). After absorption by the lungs and gastrointestinal tract, 90 to 95% of arsenic is located in erythrocytes bound to hemoglobin. It is transported through the blood to other parts of the body. Arsenic is normally found in higher concentrations in human hair and nails than in other parts of the body. This has been explained by the high content of keratin in these tissues (WHO, 1981). The –SH groups of keratin may bind trivalent arsenic. Arsenic has a predilection for skin and is excreted by desquamation of skin.

EXCRETION

The urinary excretion of fluoride or arsenic is utilized to determine the degree to which man is being exposed. Therefore, it is considered as principal route of excretion, despite several other sources of elimination such as sweat, faeces, and saliva and to a significant extent, milk.
FLUORIDE

URINE

Renal fluoride excretion involves glomerular filtration followed by pH dependent tubular reabsorption. Fluoride appears rapidly in the urine after absorption.

SWEAT

Usually only a low percent of fluoride intake is excreted in the sweat.

FAECES

About 10% of the total daily fluoride excretion takes place through faeces. If the fluoride is ingested as relatively insoluble compounds such as bone meal, cryolite and calcium salts or if such precipitants as aluminium and calcium compounds are present, larger proportion of the fluoride are unabsorbed in the intestinal tract and appear in the faeces. This may amount to as much as 30% of ingested fluoride (WHO, 1984).

MILK

The concentration of fluoride in human milk is quite similar to that in plasma (WHO, 1984). In non-fluoridated areas, fluoride intake of infants from the different sources e.g. breast milk, several milk formulations, cow's milk and yogurt shake is not very high (Koparal et al., 2000).
SALIVA

Salivary fluoride levels were found to be about 65% of plasma levels (WHO, 1984).

ARSENIC

URINE

Arsenic is cleared from the body relatively rapidly and primarily in the urine. About 70% of arsenic is excreted mainly through urine (Ishinishi et al., 1986). Arsenic is also lost from the body in the hair and nail (WHO, 1981).

METABOLISM

FLUORIDE

The toxicity of fluoride is aggravated mainly through its adverse effect on general body and tissue metabolism.

The presence of high amount of fluoride in the body would interfere with the vitally important hydrogen bond between biomolecules. Fluoride is known to form hydrofluoric acid (HF) in the stomach (Gharzouli et al., 1995). The strong hydrogen binding of fluoride to amides amounts for interference of fluoride with many functions of the body particularly enzyme activity, carbohydrate metabolism (Underwood, 1997), lipid metabolism (Singh et al., 1985) and protein metabolism (WHO, 1984). It is a general inhibitor of oxidative metabolism (Chinoy and Sequeira, 1989a).

Calcium has strong affinity to fluoride ion, dietary calcium can protect the organism of fluoride intoxication by reducing absorption of fluoride through the gut wall.
vice formation of calcium fluoride (CaF$_2$) which is an insoluble compound (Susheela, 2001).

**ARSENIC**

The metabolism of inorganic arsenic (As) in children in villages in northern Argentina was studied by Concha et al. (1998). The major human metabolic pathway for inorganic arsenic is methylation of the inorganic arsenic, As (III) and As (V), which are metabolized to dimethylarsenic acid [DMA (V)] and monomethy arsenic acid [MMA (V)] before excretion in the urine (Turpeinen et al., 2000).

The putative pathway for the biomethylation of arsenic involves enzymatically catalyzed oxidative methylation reactions yielding methylated arsenicals that contain, As in the pentavalent state As$^V$. Since As$^{III}$ is favored as the substrate for the enzyme, methyltransferases (which catalyses the methylation of arsenicals), methylated arsenicals containing As$^V$ are chemically or enzymatically reduced to trivalent form in the cellular environment (Del-Razo et al., 2000). Notably methylated arsenicals that contain As$^{III}$ have been shown to be extremely potent inhibitors of NADPH dependent flavoprotein oxidoreductases and are also potent cytotoxins in a variety of human cell types.

**ACUTE FLUORIDE TOXICITY**

Acutely toxic doses cause gastroenteritis, muscular weakness, followed by depression, pulmonary congestion as well as respiratory and cardiac failure. Fluoride is a fairly effective inhibitor of cholinesterase.

The symptoms of acute fluoride poisoning usually include nausea, vomiting,
excessive salivation, cramps in the abdomen and diarrhea. A fall in blood pressure due to a direct toxic effect on cardiac muscle may subsequently occur (WHO, 1984).

**CHRONIC FLUORIDE TOXICITY**

Chronic manifestations of excess fluoride include mottled and brittle teeth, anorexia, dense bones, and loss of weight, strength, pain in back, and legs. Sensitive individuals have eczema, atopic dermatitis and urticaria. Prolonged ingestion of water with high fluorine content causes skeletal fluorosis in adults. The bony and cartilaginous skeleton of the thorax is markedly affected. The vertebral column becomes rigid and patient develops a 'pokar-back'. Bony exostoses can easily be seen or felt (WHO, 1984).

**ACUTE AND SUB-ACUTE EFFECTS AFTER SHORT-TERM EXPOSURE OF ARSENIC**

Acute effects caused by the ingestion of inorganic arsenic compounds mainly arsenic (III) oxide, are well documented in the literature. The major lesions are profound gastrointestinal damage, resulting in severe vomiting and diarrhea, often with blood-tinged stools. Other acute symptoms and signs include muscular cramps, facial oedema, and cardiac abnormalities. Shock can develop rapidly as a result of dehydration (WHO, 1981).

Sub-acute effects mainly involve the respiratory, gastrointestinal, cardiovascular, nervous, and hematopoietic systems. 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 conjunctivitis and dermatitis (WHO, 1981). Nasal septum perforation may appear within two weeks.

The hematopoietic system may also show effects characterized by anemia and leucopenia, especially granulocytopenia (WHO, 1981). These effects are usually reversible within 2-3 weeks. Acute renal damage was indicated by a high incidence of microscopic hematuria.

**FLUOROSIS**

Fluorosis is a crippling disease, widespread in India and abroad. In India, one of the serious health problems today is prevalence of dental, skeletal and/or non-skeletal fluorosis (Susheela, 2001; Chinoy, 2002). Endemic fluorosis has also been reported in Hohhot region of Inner Mongolia (Xie et al., 1999) and Kenya (Kahama et al., 1997). Fluoride toxicity is increasingly becoming a matter of grave concern as many countries have been declared endemic for fluorosis. People suffer from paralysis and some have developed permanent skeletal deformities and damage to spinal cord. Excessive fluoride intake affects the kidney, liver and nervous system.

**DENTAL FLUOROSIS**

Dental fluorosis is a disturbance affecting the enamel. The brownish-black discoloration of the more severe fluorotic defects is the sign of dental fluorosis (WHO, 1984). Enamel mottling has been reported from Rajasthan in India (Choubisa et al., 1997) and other states of India (Susheela, 2001).
**SKELETAL FLUOROSIS**

Fluoride is a powerful stimulator of bone formation, but the use of fluoride salts as a treatment for osteoporosis remains controversial (Franke, 1994). Histological observation of bone tissue shows osteosclerosis, mottled bone, formation defects and often hyperosteoidosis (Teotia et al., 1971). F accelerates bone resorption (Nishino et al., 2001). The symptoms/complaints include severe pain and stiffness in the backbone, joints, and rigidity in the hip region. The X-ray reveals increased girth/thickening and density of bone and in certain patients, osteomalacia type changes are seen due to calcium deficiency, constriction of vertebral and intervertebral foramen occurs and pressure on nerves lead to paralysis. The long term fluoride exposure from drinking water containing $\geq 4.32$ ppm increases the risk of overall fractures as well as hip fractures (Li et al., 2001).

Fluoride can also damage the foetal skeleton if the mother consumes water/food with high concentration of fluoride during pregnancy/breast feeding (Susheela and Kumar, 1991).

**ARSENICOSIS**

The symptoms of arsenic toxicity may develop over a prolonged period of time often taking upto 8-14 years from the initial days of contamination. This period differs from individual to individual depending on the quality of arsenic ingested, immunity level of the individual and the total time period of the actual arsenic ingestion.
The results of arsenic contamination results in the following diseases:

1. **MELANOSIS**

   Melanosis results in a gradual change of complexion towards blackishness. Generally, the limbs are first affected and subsequently the change is seen all over the body. In the process of this change, white and black spots are often seen across the body, termed as “spotted melanosis” which is a dangerous precursor to cancer.

2. **KERATOSIS**

   Initial stages of Keratosis witnesses the hardening of palms and soles. In medical terms, this hardening is referred to as ‘diffuse Keratosis’ and may lead to gangrenous ulcer, which has the potential of turning into squamous cell and basal cell carcinoma of the skin (Liu et al., 2002).

**BOWEN’S DISEASE**

Bowen’s disease is an intraepidermal squamous-cell-carcinoma (SCC) of the skin or mucous membrane that pursues a slow and relatively benign course over a period of years, but may progress to invasive SCC. Bowen’s disease lesions may be found in areas of the skin and mucous membranes including the nail beds, palms, and soles (Col et al., 1999). The biggest arsenic calamity in the world is in West Bengal, India and in Bangladesh. People of this area have higher arsenic content in nail, hair, urine and skin. Many people have skin lesions such as melanosis, leucomelanosis, keratosis, hyperkeratosis especially on palms and soles forming corns or warts which may lead to squamous cell carcinoma of skin, oedema and gangrene (Chakraborty and Shah, 1987;
Arsenic produces Mee's lines or transverse white bands across finger nails appearing about six weeks after onset of symptoms of toxicity. Arsenic can also produce mixed pigmentary changes including circumscribed area of pigment loss together with generalized increase in pigmentation (Klaassen et al., 1986).

**DETECTION**

**FLUORIDE**

Fluorosis has no treatment, but it can be easily prevented provided the disease is recognized/detected at an early stage.

The conventional method of detection of the disease until recent times is through X-rays or radiographs which can be helpful for detection of the disease only in later stages i.e. when the bone density and bone mass are increased and ligaments are calcified. Serum sialic acid levels in fluorotic human subjects were significantly low with respect to control population residing in fluoride free region which indicated that the individuals have been affected by the disease. Hence, sialic acid has been suggested as a prognostic test (Susheela and Jha, 1982; Chinoy et al., 1992a).

It is also a fact that male infertility with abnormality in sperm morphology, oligospermia, azoospermia and low testosterone levels is very common in those residing in endemic areas for fluorosis and consuming high fluoride contaminated water. It is not necessary that all male members would be infertile (Susheela, 2001).
converted in the liver to the less toxic methylated form that is excreted via the urine and hence the enzyme could be used for detection of toxicity (Roy and Saha, 2002).

REMOVAL OF FLUORIDE FROM DRINKING WATER

Defluoridation is removal of excess fluoride from water. There are a number of methods available for defluoridation, e.g. Nalgonda technique, domestic defluoridation, fill and draw defluoridation plant for small community and for rural water supply.

The recommended defluoridation method is Nalgonda technique which is a combination of several unit operations and processes incorporating rapid mixing, chemical interaction, flocculation, sedimentation, filtration, disinfection and sludge concentration to recover water and aluminium salts (Rajiv Gandhi National Drinking Water Mission, 1993; Nawlakhe and Paramasivam, 1993).

The silty clay was found to be a potent fluoride-sorbing agent, which could be used for the defluoridation of water (Agarwal et al., 2001). Trejo-Vazquez and Bonilla-Petriciolet (2001) used an activated boehmite, a new adsorbant which maximizes the fluoride removal from water.

REMOVAL OF ARSENIC FROM DRINKING WATER

Inorganic arsenic compounds are most commonly found in water. There are very few methods for removing the arsenic and they have some side effects.

The arsenic removing technology (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 through an as yet undefined
Recent studies have revealed that fluoride and traces of aluminium form a complex, fluoroaluminate which stimulates cellular heterotrimeric G-proteins in osteoblastic cells (Susa, 1999).

**ARSENIC**

The high retention of arsenic in the skin seen in the animals receiving arsenic (III) may be explained by its reaction with sulphydryl groups of proteins which are abundant in the skin (WHO, 1981). As (III) and As (V) inhibit protein synthesis and replace phosphate in the nucleotides during DNA synthesis (WHO, 1981; Merian, 1991). Arsenite when given to guinea pigs, chimpanzees and baboons, the bulk of the tissue arsenic was shown to be in the protein fraction and minor amounts in the acid soluble and lipid fractions (WHO, 1981).

**EFFECT ON CARBOHYDRATE METABOLISM**

**FLUORIDE**

Fluoride has been traditionally known as an inhibitor of glycolysis. In rabbits treated with fluoride, a decline in glycogen concentration occurred in spleen, lens, liver and skeletal muscle (Shashi et al., 1987). On the contrary, glycogen accumulation occurred in fluoride treated fishes (Shaikh and Hiradhar, 1988; Chinoy et al., 1994c) and in liver, muscle, vas deferens and uterus of fluorotic rats and mice (Chinoy, 2002; Chinoy and Sequeira, 1989a; Chinoy and Patel, D., 1996; Chinoy and Sharma, 1998; Chinoy and Mehta, 1999a; Chinoy and Patel, T., 1999; Chinoy et al., 1991c; 1993b; 1994b; 1995) which was correlated with the decrease in the activity of phosphorylase.
animals with chronic fluorosis (Guan et al., 1999). Fluoride inhibits many enzymes involved in lipid metabolism. Lipidosis is a disorder of lipid metabolism leading to abnormal fat accumulation in body tissues particularly in the brain and liver. Hyperlipidemia may occur due to enzymatic defect, the inability of brain to degrade the lipid in the body (Marks et al., 1996). From the above findings it is evident that fluoride may interfere with lipid metabolism.

Fatty acids in the human atheromatous plaque were degraded in the presence of fluoride ions. The accumulation of fluoride in plaque appears to alter its physicochemical properties, e.g. resistance to blood pressure and shear forces, facilitating the formation of emboli (Chlubek et al., 2001).

Fluoride treatment to male and female mice, rats revealed a decline in the activities of 3β and 17β hydroxysteroid dehydrogenases in testis and ovary suggesting a block in the steroidogenic pathway. These results were correlated with accumulation of cholesterol in the ovaries, testis and muscle of treated mice and rats, which suggests that its metabolism might be altered (Chinoy, 1999a; 2002; Chinoy and Patel, D., 1996; Chinoy and Sharma, 1998; Chinoy and Mehta, 1999b; Chinoy and Patel, T., 2001; Mathews et al., 1996; Narayan and Chinoy, 1994a). This led to a decline in circulating testosterone and estradiol levels in mice, rats and human populations in endemic areas of North Gujarat (Chinoy et al., 1992a).

An abnormal composition of fatty acids from phospholipids in liver and kidney was observed in the rats with chronic fluorosis. The unsaturated fatty acids were decreased and the saturated fatty acids increased which might be the result from oxidative
stress and an increased level of lipid peroxidation (Shao et al., 2000).

ARSENIC

Chinoy et al. (2001) reported that the NaF + As$_2$O$_3$ treatment increased the cholesterol level in ovary and testis of treated mice, similar to those found after fluoride treatment.

EFFECTS ON NUCLEIC ACID METABOLISM

FLUORIDE

Fluoride has been reported to cause a depression in DNA and RNA synthesis in cultured cells (Strochkova et al., 1984). Similarly, other authors (Patel, D. and Chinoy, 1997; 1998; Memon and Chinoy, 2000a) have also reported a decrease in DNA and RNA levels in ovary, uterus, liver and gastrocnemius muscle of fluoride treated mice. The DNA/protein ratio, RNA/Protein and DNA/RNA ratio were also altered indicating disturbances in the process of transcription and translation by NaF in mice ((Patel, D. and Chinoy, 1998).

ARSENIC

As (III) and As (V) inhibit DNA and RNA synthesis (Nakamuro and Sayato, 1981). Lynn (1999) reported that arsenite at low concentration could cause oxidative DNA damage in human vascular smooth muscle cells which may be important in arsenic induced atherosclerosis. The arsenic could induce DNA single strand breaks in human blood cells (Zhang et al., 1999). Arsenite and arsenate both stimulated the transformation
and DNA synthesis of human lymphocytes. Effects of inorganic arsenicals on DNA synthesis in unsensitized human blood lymphocytes were biphasic, the chemicals at very low concentrations enhanced DNA synthesis, whereas, higher concentrations inhibited the DNA synthesis (Meng and Meng, 2000). High dose (12.5 mg/L)arsenic (As$_2$O$_3$) has damaging effects on DNA of bone marrow cells of mice in vitro and induces DNA protein cross-link at the same time. However, lower doses (2.5, 0.5 mg/L) of arsenic could affect the sensitivity of DNA to other carcinogens (Zhtgong and Xuemlng, 2000). DNA single-strand breaks and DNA-protein cross-link were induced by the treatment of dimethylarsenic acid(DMAA) (Kato et al., 1994).

**FLUORIDE AND FREE RADICALS**

Free radicals are highly reactive species that have an unpaired electron, e.g. the hydroxy (OH$^-$) and superoxide radicals (O$_2^-$). Cellular damage caused by these oxygen derived species (ROS) has been implicated in the aetiology of a range of diseases such as atherosclerosis, cancer, Parkinson's disease and other neurodegenerative disorders. The production of superoxide radicals is caused by incomplete oxygen oxidation and are liable to react with several molecules, provoking their destabilisation.

The high reactivity of superoxide radicals may lead to chemical modification and impairment of proteins, lipids, carbohydrates and nucleotides in living cells as well as the peroxidation of membrane lipids with an increase in the permeability of the cell membrane (Subramaniam et al., 1994; Marks et al., 1996; Rzeuski et al., 1998). Fluoride is known to stimulate the production of superoxide radicals in humans and animals.
Free radicals and lipid peroxidation play an important role in fluorosis (Sun et al., 1994) and the high fluoride concentrations are likely to inhibit superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, resulting in the accumulation of large amounts of free radicals and peroxides causing cell damage in people living in areas endemic to fluorosis (Li and Cao, 1994). Epidemiological studies of patients with dental and skeletal fluorosis from endemic regions in China also revealed an inhibition of GSH-Px and SOD activities and reduced glutathione levels in blood with increased lipid peroxide levels in serum (Bian et al., 1994; Dai et al., 1999). The depleted GSH by NaF treatment strongly suggests that, like several compounds, fluoride might also be largely dependent on GSH for detoxification (Li et al., 1999). The fluoride induced lipid peroxides could also be reduced by oral intake of glutathione and selenium in rats (Liang et al., 1999; Li et al., 1999). Moreover, some herbal antioxidants have also been used in reducing free radical damage in blood, brain, liver, kidney in cases of chronic fluorosis in China (Liu, 1999).

Animal studies from our laboratory have revealed that fluoride administration in different doses to rodents for 30, 45, 60 days inhibited the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase and glutathione (GSH) levels but increased the lipid peroxides in their ovary, testis, brain, kidney, liver (Chinoy, 2002; Chinoy and Patel, D., 1998a; Chinoy and Sharma, 1998; Chinoy and Patel, T., 2000; Chinoy and Mehta, 1999c: 2000; Chinoy et al., 1995: 1997a,b), thus rendering these tissues susceptible to injury.

Cell damage and cell death have been induced by the generation of reactive oxygen species (ROS) through the administration of a wide variety of chemical
compounds including fluoride. ROS contribute to neurotoxicity. The external granular layer of the mouse cerebellum was destroyed (Trabelsi et al., 2001). On the other hand, free radical scavengers such as GSH, GSH-Px and SOD decreased in erythrocytes of rats exposed to 100 ppm fluoride in drinking water for four months (Shivarajashankara et al., 2001).

**ARSENIC AND FREE RADICALS**

Dong et al. (1999) showed that arsenic could induce lipid peroxidation and decrease the capacity of SOD and GSH-Px in heart of mice.

The studies of Hirata et al. (1988) on Syrian Golden Hamsters have suggested that GSH is needed to protect the cells from damage by arsenite (As III) and agents that reduce GSH levels may increase the toxic effects of arsenite. Low levels of GSH prevent the efficient methylation of arsenite. The fundamental role of GSH may be the protection of thiol groups especially dithiol groups of proteins (Aposhian, 1989) present in tissues from oxidative stress by xenobiotics (Larsson et al., 1983). The dimethyl-arsenic peroxyl radical is assumed to play a dominant role in causing the DNA damage (Yamanaka and Okada, 1994).

Arsenic can induce oxidative damage in human fibroblast cell (Lee and Ho, 1994). Methylated arsenic forms and endogenous ascorbic acid cause the release of Fe from ferritin and Fe-dependent formation of reactive oxygen species (ROS) which lead to arsenic carcinogenesis in man (Ahmad et al., 2000).
Association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity in a human population of Northeastern Taiwan was reported (US DHHS, 1998).

CENTRAL NERVOUS SYSTEM - BRAIN

FLUORIDE

Lu et al. (1961) found stimulation of central nervous system (CNS) by intraperitoneal injection of NaF to rats. A diet of 70 ppm of NaF caused an increase in sensitivity to the paralytic effects in rats, which is attributed to the inhibition of cholinesterase activity. Shashi et al. (1994) reported that fluoride treatment to rabbits resulted in decrease in RNA and protein in cerebellar cortex which caused degenerative changes in Purkinje cells. High fluoride concentration in drinking water can decrease the cerebral functions of mice as fluoride is a neurotoxicant (Sun et al., 2001).

The effects of fluoride were the partial and complete paralysis of arms and legs in advanced fluorosis due to pressure upon the spinal cord by newly formed bone protruding into the spinal canal and muscular damage in patients suffering from occupational fluorosis. This is the result of a direct action of the fluoride ion on the ganglion (Mansour and Kruger, 1985).
FLUORIDE

Guan et al. (1999) demonstrated that the contents of phospholipid and ubiquinone are modified in brains affected by chronic fluorosis and the changes in membrane lipids could be involved in the pathogenesis of this disease. The metabolism of brain phospholipids might also be altered by fluoride accumulation in brain tissue, which is related with the degeneration of the neuron (Guan et al., 1997). A neuropathological and computerized morphometric analysis by Chlubek et al. (1998) revealed a marked shrinkage of cerebellar, granular and Purkinje cells, perivascular myelin swelling and astroglia reaction, especially in the white matter of brain in NaF treated rats. Ubiquinone content was increased in the brains of rats treated with higher concentrations of fluoride after seven months (Guan et al., 1999). Spittle (1994) reported impaired functioning of central nervous system in rats with NaF suggesting the entry of fluoride in brain. Chronic exposure to fluoride may be associated with cerebral impairment affecting particularly the concentration and memory in some individuals (Spittle, 1994). Chen et al. (1999) found that the high fluoride might directly enter into brain and lead to its damage due to the deposition of fluoride and calcium complex. According to Griffiths and Bryson (1997), fluoride is a powerful central nervous system (CNS) toxin, and might adversely affect human brain functioning even at low doses.

The fluoride induced free radical toxicity has been reported recently in cerebral hemisphere of male and female mice (Chinoy and Patel, T., 2000; Memon and Chinoy, 2000b). The extent of alterations in neuronal density and cerebrovasculature was greater
in rats in the NaF group than in controls (Jensen et al., 1998).

**ARSENIC**

A large number of epidemiological studies and case reports indicate that digestion of inorganic arsenic can cause injury to the nervous system. Acute high dose exposures (1 mg As/kg body weight or above) often lead to encephalopathy, with signs and symptoms such as headache, lethargy, mental confusion, seizures, hallucination and coma (USDHHS, 1998). Intermediate and chronic duration exposures to lower levels (0.05 - 0.5 mg As/kg/day) are typically characterized by a symmetrical peripheral neuropathy (Franzblau and Lilis, 1989) which usually begins as a numbness in the hands and feet, but later may develop into a painful "pins and needles" sensation. The sensory and motor nerves are both affected, muscle weakness often develops, sometimes leading to wrist drop or ankle drop (Chhuttani et al., 1967). Histological examination of nerves from affected individuals revealed axonopathy with demyelination (Goebel et al., 1990). Sensory loss in the peripheral nervous system is the most common neurological effect, consisting of Wallerian degeneration of axons but is reversible if exposure is stopped (USDHHS, 1998). Heyman et al. (1956) reported 41 cases of suspected arsenic neuropathy in the USA. The symptoms and signs included peripheral nervous disturbances. Dysfunction of the blood-brain barrier was indicated in rats fed arsenite at a concentration of 500 mg/kg in a cereal diet for 35 days (WHO, 1981). Disturbances in the functional state of CNS occurred due to pericellular oedema, plasmolysis, and karyolysis of the neurons.

Few animal studies have reported neurotoxic effects due to arsenic exposure. Arsenic administered as arsenite or arsenic (III) oxide passes the blood-brain barrier in
mice, guinea pig, rabbits, hamsters and monkeys, although the levels found in the brain are low as compared with those in other tissues (Vahter and Norin, 1980).

Inhibition of cholinesterase activity was observed in rats exposed for 3 months to arsenic (III) oxide (WHO, 1981). Central nervous system disorders were observed in rats exposed to 2 to 10 mg arsenic trioxide via stomach intubation (USEPA, 1984). Arsenic patients were diagnosed of neurologic disorders that ranged from sleep disorder and memory impairment to paralysis (Abou-Donia et al., 2000).

Central nervous system deficits (hearing loss, eye damage, abnormal EEGs, mental retardation, epilepsy), electrocardiographic changes (elevated ST wave and extended QT interval) occurred in infants who had been fed arsenic contaminated milk for 1-2 months (WHO, 1981).

**CARDIOVASCULAR SYSTEM - HEART**

**FLUORIDE**

The existing studies have reported conflicting results between fluoride and the incidence of cardio-vascular disease. Some studies have shown apparent decrease in the prevalence of cardiovascular disease in areas with higher fluoride levels, while others have revealed higher incidence (Xu and Xu, 1997). Caruso et al. (1970) observed a direct vasodilatory effect by fluoride. Vascular changes characterized by microvascular injury, perivascular disintegration of tissue cells, and vascular proliferation were predominated by fluoride ingestion (WHO, 1981). Singh et al. (1998) suggests that patients with endemic skeletal fluorosis should be regularly assessed for cardiovascular abnormalities.
HEART

FLUORIDE

The mice maintained on 100 ppm NaF/day from birth until their sacrifice at the age of 6 to 8 weeks showed an adverse effect on the level of different enzymes in the heart (WHO, 1981). In human, the increase in abnormal heart rhythm with fluoride intoxication is clearly demonstrated (Xu and Xu, 1997). High dose of fluoride causes severe damage leading to cardiac irregularities in humans (Zhiliang et al., 1987). Several workers believe that calcification of arteries is an integral feature of skeletal fluorosis. Aortic calcification and degeneration of smooth muscle fibers in the tunica media of the aorta were reported in fluoride intoxicated rabbits (Susheela and Kharb, 1990).

The experimental animals given massive doses of fluoride cardiac irregularities and low blood pressure have been reported. Fluoride caused degenerative changes in the myocardium of rabbits administered 10-30 mg of NaF/kg/day orally for 15-169 days (Shashi and Thapar, 2001). Adverse effects of fluoride on cultured myocardial cells of mice were reported (Xu and Xu, 1997) which included alterations in the rate of throbbing.

ARSENIC

A number of studies in humans indicate that arsenic ingestion may lead to serious effects on the cardiovascular system. Characteristic effects on the heart from both acute and long-term exposure include altered myocardial depolarization and cardiac arrhythmias (Little et al., 1990). Long-term arsenic contaminated drinking water intake
causes peripheral vascular disease, ischemic heart disease and neuropathy and/or cancer (Pi et al., 1999; Wu et al., 2001).

A high prevalence of a peripheral vascular disease called "black foot disease" was found in China (Province of Taiwan) (Tseng et al., 1995). The most common peripheral vascular phenomena, Raynaud's syndrome was attributed to arsenic exposure in which the persons had abnormal skin pigmentation. This was observed in swelter workers who are exposed to chronic inhalation of arsenic trioxide (Lagerkvist et al., 1988). High oral doses of inorganic arsenic can lead to premature ventricular contractions and ventricular tachycardia that require medical intervention or may even result in death (US DHHS, 1998).

Peripheral vascular lesions in 23% of 180 vintorers with chronic intoxication have been reported (WHO, 1981). In six cases, the inadequate peripheral circulation caused gangrene, chronic nephritis and cardiac failure.

EFFECTS ON HEMATOLOGICAL PARAMETERS

FLUORIDE

BLOOD

The blood acts as a transport medium for fluoride. About 75% of blood fluoride is present in the plasma, the rest is mainly in or on the red blood cells (WHO, 1984). Red cells from humans exposed chronically to toxic level of fluoride through drinking water showed significant increase in lipid peroxidation, membrane cholesterol and phospholipids (Saralakumari and Ramakrishna Rao, 1991).
Fluoride will accumulate on the erythrocyte membrane, besides other cells, tissues and organs. The erythrocyte membrane in turn loses calcium content, the RBCs attain the shape of an amoeba with pseudopodia like folds projecting in different directions. Such RBCs are termed as 'echinocytes' (Rajiv Gandhi National Drinking Water Mission, 1993).

Majority of fluorotic human cases in Mehsana district of North Gujarat, India, exhibited reduced haemoglobin levels. Thus fluoride might lead to a state of mild anemia. However, this aspect could also be related to malnutrition (Chinoy, 1996; Mathews et al., 1996).

Mishra and Mohapatra (1998) found that the average haemoglobin content, total RBC count and hematocrit (%) in blood samples were significantly reduced, while mean corpuscular concentration and volume were significantly elevated in individuals from the contaminated areas in comparison to those from the uncontaminated areas. Patients with fluorosis had decreased haemoglobin, but increase in number of erythrocytes and abnormal lymphocyte counts in children living near aluminium plants (Mucuch et al., 1963). Fluoride depletes the energy reserves and the ability of white blood cells to properly destroy foreign agents by the process of phagocytosis. As little as 0.2 ppm fluoride stimulates superoxide production in resting white blood cells. Even micro-molar amounts of fluoride, below 1 ppm, may seriously depress the ability of white blood cells to destroy pathogenic agents. Greenberg (1982) observed morphological abnormalities in cell structure and mitotic figure formation in immature leucocytes of mice given NaF in drinking water.
ARSENIC

BLOOD

The reported figures on arsenic levels in the blood vary. Bergstrom and Wester (1966) noted a mean level of 0.002 mg/kg in whole blood (3 samples), of which 0.0011 mg/litre was present in the serum, while, a mean arsenic concentration of 0.004 mg/kg was found in the whole blood of 8 normal subjects (WHO, 1981). Black foot disease patients and members of their families living in the endemic areas of China (Province of Taiwan) showed mean values of about 0.03 mg/litre in plasma and 0.093 mg/litre in whole blood (Tseng et al., 1995; 2000).

The major part of inorganic and organic arsenic in blood is cleared fairly rapidly in man. Blood arsenic will therefore reflect exposure for only a short period following absorption and will be very time dependent (WHO, 1981).

Although anemia is often noted in humans exposed to arsenic by the oral route (WHO, 1981), these effects may be due to a suppression of erythropoiesis (WHO, 1981) or a direct cytotoxic or hemolytic effect on the blood cells (Fincher and Koesker, 1987). Chronic exposure to 0.007 mg/kg/day in drinking water also resulted in anemia (Mazumder et al., 1988).

Leukopenia is also common in cases of oral exposure to inorganic arsenicals (Franzblau and Lilis, 1989). Arsenic exposure may have a significant effect on carbohydrate metabolism resulting in increased concentration of glycosylated hemoglobin in workers exposed to arsenic as compared to the control group (US DHHS, 1998). Reduction of inorganic arsenic by nascent hydrogen may also result in arsine
(AsH₃) which is taken up by the erythrocytes and cause hemolysis, leading to arsenic acid which also damages the kidney (Savory and Wills, 1984). 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 haemoglobin and acute uremia may lead to death (Arnold, 1988).

**EFFECTS ON THE HEMATOPOIETIC SYSTEM**

**FLUORIDE**

The relationship between fluoride and human hematopoiesis is of interest for several reasons. It has given an insight into the potential toxicity of fluoride to normal hematopoietic cell growth, viability, differentiation and apoptosis. Sodium fluoride is potentially harmful to human cells involved in hematopoiesis (Machalinski et al., 2000; 2001).

**ARSENIC**

Long-term exposure to inorganic arsenic has resulted in disturbances of the hematopoietic system. Bone marrow examination shows disturbed erythropoiesis and occasionally megaloblastic changes (WHO, 1981). A decrease in hematocrit and in haemoglobin has been observed in female rats exposed to arsenite in the feed (250 mg As/kg diet) for 2 years and in rats given sodium arsenate in the feed (50 mg As/kg diet) for 10 weeks (WHO, 1981). The same effects were observed in cats given arsenite or arsenate in the diet in doses of 1.5 mg As/kg body weight (WHO, 1981).

The effect of inorganic arsenic compounds on hematopoietic function in adult
mammalian liver were reported by Woods and Fowler (1977). The effects of ingested arsenic (As\(^{3+}\)) on hepatic heme biosynthetic capability and hemoprotein function in adult male rats exposed for 6 weeks to 0, 20, 40 or 85 ppm sodium arsenate in the drinking water revealed selective inhibition of mitochondrial bound heme biosynthetic pathway enzymes (WHO, 1981).

EXCRETORY SYSTEM AND KIDNEY

FLUORIDE

Kidney is a site for potential toxicity because this organ is exposed to relatively high concentrations of fluoride as approximately 50% of fluoride is cleared from the body by it. Fluoride elimination depends mainly on kidney function and acute renal failure would further contribute to accumulation of fluoride (Borysewics et al., 2000). Kono et al. (1984) found that in adults exposed to fluoride for a longer period, the kidney gets damaged and therefore the excretion of fluoride gets significantly diminished. The activity of \(\alpha\)-glutathione S-transferase, a marker of tubular damage, particularly in the S3 segment of proximal tubule and of N-acetyl \(\beta\)-D-glucuronidase in rats were increased by fluoride (Usuda et al., 1998).

NaF treatment caused severe alteration in the structure of mice kidney as compared to control, viz. disorganization of glomeruli with increased space between glomeruli and capsule. The renal tubules showed vacuolization and distortion and the number and size of mitochondria were reduced with decrease in activities of marker enzymes (Chinoy et al., 2000; Sharma and Chinoy, 1998).

According to Suketa and Mikami (1977), there is a close relationship between
polyurea and changes in excretion of certain urinary ions in fluorosis. The kidney tubules are damaged due to continuous filtration of the ingested fluoride. In cases of prolonged exposure, accumulation of fluoride and its combination with calcium frequently result in formation of kidney stones (Chinoy, 1995). Evidence suggests that high intake of fluoride causes nephrolithiasis in tribal populations (Singh et al., 2001). Nishiura et al. (2001) studied the renal damage by continuous intravenous administration of fluoride.

ARSENIC

Studies by Ginsburg (1965) showed that arsenate (As⁺³) is actively transported by the kidney tubules which is reduced to As⁺³, the more acutely toxic chemical form. Arsenite induced marked morphological and physiological changes in the kidney. Inorganic arsenic and its metabolites are thought to be excreted by a complex process which includes glomerular filtration, secretion and active reabsorption in the proximal tubule. Fast elimination of arsenic in urine relieves the kidney of the high arsenic burden possibly minimizing the potential adverse effects on the renal system (US DHHS, 1998). Bouletreau et al. (1977) stated that direct chemical nephro-toxicity in acute intoxication causes tubular impairment in the proximal part with mild alteration of glomeruli. The studies of Hirata et al. (1990) corroborate with the above observations.

Rats given arsenic in the drinking water for 6 weeks, showed increased kidney weights in relation to body weight (WHO, 1981). Arsenic concentration categories 0.1-0.5 and ≥ 0.5 µg/L are responsible for bladder and kidney cancer in Finland (Kurtio et al., 1999).
**FLUORIDE AND APOPTOSIS**

Enhancement of fluoride cytotoxicity at acidic pH was observed in rat alveolar macrophage cell line (Hirano and Ando, 1997). Li et al. (2000) reported that apoptosis in hepatocytes and neurons can be induced in vivo by chronic fluoride poisoning. Their data suggest that oxidative stress may play some role in the inducement of hepatocyte apoptosis. Sodium fluoride could potentially damage human hematopoietic cells inducing apoptosis in bone marrow as well as cord blood cells (Machalinski et al., 2001).

**ARSENIC AND APOPTOSIS**

Park et al. (2000) investigated the in vitro effect of As$_2$O$_3$ in cell cycle regulation, and apoptosis in human myeloma cell lines and reported that it has an antiproliferative effect on cell cycle related proteins in cancer cells. This process was associated with loss of mitochondrial transmembrane potential and an increase of caspase-3 activity. Di Noto et al. (1999) reported the arsenic trioxide (As$_2$O$_3$) is a useful drug for the treatment of acute promyelocytic leukemia (APL), acting through a complex mechanism involving the induction of apoptosis.

Recently, it was reported that arsenic trioxide (As$_2$O$_3$) and As$_2$O$_3$ plus DTT (Dithiothreitol) induced apoptosis in NB4 cells was modulated by oxidant modifiers (Gurr et al., 1999). Arsenic trioxide and interferon alpha synergize to induce cell cycle arrest and apoptosis in human T-cell lymphotropic virus type I-transformed cells (HTLV-I transformed cell) (Bazarbachi et al., 1999). Arsenic trioxide(As$_2$O$_3$) induces clinical remission in acute promyelocytic leukemia (APL) with minimal toxicity and apoptosis in
AIM: derive NIH-3T3 cells at low (1 to 2 micromol/L) concentration (Dai et al., 1998).

FLUORIDE AND CANCER

Fluoride confuses the immune system and causes it to attack the body's own tissues, and increases the tumour growth rate in cancer prone individuals (WHO, 1984). Increased incidence of melanotic tumours with sodium fluoride have been reported (WHO, 1984).

The fluoridation of water has been linked to increased risk of osteosarcoma, thyroid follicular cell adenoma, hepatocellular carcinoma etc., in rats (Lee, 1993) as well as osteosarcoma and bladder cancer (Grandjean et al., 1992) in male populations living in fluoridated areas (Yiamouyiannis, 1993). The various surveys and laboratory investigations support water fluoridation as being causal for cancer in the oral cavity and pharynx (Takahashi et al., 2001).

ARSENIC AND CANCER

Exposure to arsenic has been associated with the induction of cancer for nearly a century. There is sufficient evidence that inorganic arsenic compounds are skin and lung carcinogens in man, but that the data for other sites were inadequate (IARC, 1980). An excess of deaths due to respiratory cancer has been observed among workers exposed to inorganic arsenic in the production and use of pesticides, gold mining and in the smelting of non ferrous metals especially copper (WHO, 1981; Liu and Chen, 1996).

A large number of epidemiological studies and case reports suggest that ingestion of inorganic arsenic increased the risk of developing skin cancer (Hsueh et al., 1995).
Arsenic trioxide causes selective necrosis in solid murine tumours by vascular shut down (Lew et al., 1999). Arsenic concentrations and daily dose are associated with bladder and kidney cancers in Finland (Kurttio et al., 1999). Zhang et al. (2000) reported that death rate due to malignancy in arsenic exposure group was high than in the unexposed persons in endemic villages in Mongolia.

**MECHANISM OF ACTION**

**FLUORIDE**

Fluoride has been found to accumulate in nearly every organ of the body (Makhni et al., 1980).

Fluoride may be slowly released from skeleton and this fluoride may add to the levels in blood and urine. Fluoride ions are taken up rapidly by bone by replacing hydroxyl ions in bone apatite. The consensus is that fluoride is incorporated into the hard tissues largely by a process of exchanges and by incorporation into the apatite lattice during mineralization (WHO, 1984).

Fluoride is known to combine with hydrochloric acid of the stomach and is converted to hydrofluoric acid which has high corrosive property (Susheela et al., 1992). The most important effect of fluorides is on soft tissues viz, intestine, liver, lung, heart, muscle and brain and is known to cause a series of disorders and aggravate many others.

**ARSENIC**

Inorganic arsenic has been shown to impair tissue respiration *in vivo* in the liver and kidney of mice and rats (WHO, 1981). Arsenic has to pass the mitochondrial
membranes and its membrane damage appears to play a prominent role in the emergence of some of the observed effects. In vitro studies have shown that rat liver mitochondria can accumulate arsenite and arsenate through energy-dependent processes (WHO, 1981).

It was recognized many years ago that trivalent inorganic arsenic reacts with the sulphhydryl groups of proteins. Many enzymes containing such groups have been shown to be affected by arsenite (WHO, 1981). In particular, the marked inhibitory effects of As (III) on mitochondrial respiration mediated NAD-linked substrates, appear to play a critical role in the toxicity of this agent. A depression in the activity of succinate dehydrogenase in various tissues has also been demonstrated (WHO, 1981). Arsenite [As(III)] can inhibit more than 200 enzymes.

Arsenic can affect DNA repair, methylation of DNA and increase radical formation. Inorganic arsenic can often be methylated by cells, which appears to reduce its acute toxic effects in many cases. Such tissue specificity in methylation may have important implications in toxicity and carcinogenesis (Abernathy et al., 1999).

**REVERSAL OF TOXICITY AND AMELIORATIVE EFFECTS OF VITAMINS C, E, D AND CALCIUM PHOSPHATE ON FLUORIDE AND ARSENIC INDUCED EFFECTS**

Fluoride and arsenic are potent health hazards and affect virtually every phase of body metabolism. In view of the millions of people affected with fluoride and 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 endemic populations. Chinoy and co-workers (Chinoy, 2002; Chinoy
and Patel, D., 1998a,b; Chinoy and Patel, T., 2000; Chinoy and Sequeira, 1989b; 1992; Chinoy et al., 1991b; 1994b, 1995; 2001; Chinoy and Sharma, 1998; Chinoy and Mehta, 1999a; Chinoy and Memon, 2001; Chinoy and Nair, 2001; Narayana and Chinoy, 1994b) have reported partial or incomplete recovery in several biochemical parameters in various organs of mice and rats, after the withdrawal of NaF and/or As$_2$O$_3$ treatment for one or two months. However, after withdrawal of treatment for one to two months and simultaneous ingestion of therapeutic agents viz., ascorbic acid, calcium phosphate, vitamins E and D and amino acids (glycine, glutamine) were more effective in reducing the fluoride or arsenic induced toxicity and resulted in recovery of all tissues to almost normal status. The consumption of a protein supplemented diet also reduced the fluoride toxicity, whereas, a protein deficient diet aggravated it (Chinoy and Mehta, 1999b). The impact of protein deficiency on arsenic methylation may be a factor in explaining differential susceptibility to arsenic across populations (Mushak and Crocetti, 1995).

**ASCORBIC ACID (AA)**

The absorption of vitamin C in human occurs in buccal mucosa, stomach and small intestine. Gastrointestinal absorption of vitamin C is rapid and effective with an active absorption mechanism. Vitamin C rapidly equilibrates in intra and extra cellular compartments. It is a strong reducing agent and hence has a general importance as an antioxidant affecting the body's redox potential. Ascorbic acid (AA) is oxidised to dehydroascorbic acid through monodehydroascorbic acid which is an ascorbic acid free radical having stronger reducing properties than AA (Chinoy, 1978). AA forms part of the body's antioxidant defence against reactive oxygen species and other free radicals and
thereby prevents tissue damage (Marks et al., 1996) and is involved in detoxification of many foreign compounds. 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 oxidoreduction reaction in various tissues (Chinoy, 1978). The therapeutic role of ascorbic acid in alleviation of fluoride or arsenic induced toxicity in several tissues of treated mice, rat and guinea pig has been reported earlier (Yu and Hwang, 1993; Chinoy, 2002; Chinoy and Patel, D. 1998a,b; Chinoy and Mehta, 2000; Chinoy and Memon, 2001; Chinoy and Patel, T., 2000; Chinoy and Sharma, 1998; 2000; Chinoy et al., 1994b; 1995; 1997a,b).

**CALCIUM (Ca^{2+})**

Calcium has strong affinity to fluoride ion. Dietary calcium can protect the organism from fluoride intoxication by reducing its absorption through the gut wall, viz. formation of calcium fluoride (CaF$_2$) which is an insoluble compound. Calcium rich drinking water protects against absorption of fluoride (Hillier et al., 2000).

Calcium activates several enzymes, whereas, both calcium and ascorbate are known as inhibitors of phosphodiesterase (PDE) and enhance C-AMP levels. Ameliorative role of Ca$^{2+}$ for mitigation of fluoride, aluminium and arsenic induced toxicity in some tissues in mice, rats, rabbits has been reported (Chinoy, 2002). The
results revealed a synergistic or additive action of calcium and ascorbic acid.

VITAMIN E (Vit. E)

The basic function of vitamin E in living organisms is its role as an antioxidant protecting all membrane lipids and polyunsaturated fatty acids (PUFA) against oxidative degradation (Marks et al., 1996). Vitamin E (α-tocopherol) has therapeutic roles in numerous disease states especially those involving oxidation related events (Phelps, 1987). Isomers of tocopherol function as biological antioxidants and free radical scavengers (Burton, 1990; Basu and Dickerson, 1996; Chinoy and Sharma, 1998). It is further known that vitamin E deficiency causes central nervous system disturbances (Bender, 1992), reproductive failure (Nelson, 1980), necrotizing myopathy, liver and kidney damage and neurological abnormalities. The severity of damage to human is increased where dietary intake of protein, calcium, magnesium and vitamin C are low (Kennedy, 1999; Chinoy and Mehta, 1999a). α-tocopherol interacts enzymatically with vitamin C which enhances its radical scavenging action (Marks et al., 1996).

VITAMIN D

Administration of vitamin D during NaF withdrawal period to mice was found to be effective in recovery of NaF induced effects (Chinoy, 2002; Chinoy and Patel, 1998a; Chinoy and Sharma, 1998; Sharma and Chinoy, 1998; Chinoy et al., 2000).

Gupta et al. (1991) have also reported reversal of fluorosis in children from an area consuming water containing 4.5 ppm of fluoride in Rajasthan by a combination of
vitamins C, D and calcium. Hence the fluoride and arsenic induced toxicity was transient and reversible (Chinoy, 2002).

MISCELLANEOUS THERAPEUTIC AGENTS

Hsu et al. (1999) and Xia et al. (1999) have reported that selenium can alleviate the symptoms of arsenic poisoning in exposed persons. Similarly, prevention by antifluorosis preparations containing zinc, salt, boron or selenium compounds have also been used by Sun et al. (1999) in China. Antioxidative preparations containing glutathione, B carotene and superoxide dismutase have been used for cases of fluorosis and arsenism (Qiu and Sun, 1999). The antioxidants function in cooperation in various combinations, especially with vitamin C, E, selenium and GSH on fluoride toxicity (Sun et al., 2001).

CHELATING AGENTS

Chelating therapy with dimercarol (BAL), dimaval (DMPS) or meso-2.3-dimercaptosuccinate acid (DMSA) is effective (Roy and Saha, 2002). In case of severe intoxication by arsenicals, BAL is the only arsenic antidote and these chelating agents are effective in reducing arsenic burden (Roy and Saha, 2001).

In the light of the above data, the present work was undertaken to investigate the effects of fluoride and arsenic on some soft tissues, when administered alone or in combination. The amelioration of toxicity by some antidotes viz., vitamins (C and E) and calcium phosphate were also studied which were fed (orally) individually together.