CHAPTER I
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In today's industrialised world, in the name of development, too little thought has been given to the effects of waste products on the environment and to the various life forms existing on this planet, including man. The socio-economic realities force individuals to opt for hazardous professions in which he/she becomes knowingly or unknowingly the victim. It is not only the man-made substances but also the great variety of naturally occurring substances which under certain conditions cause adverse health effects ranging from subtle to severe biological changes and even death.

The ever increasing quest of society to identify and prevent these ill-effects has prompted the dramatic evolution of toxicology. Toxicology is defined as the study of the adverse effects of chemicals on biological systems. The study of the adverse effects of chemicals on biologic systems is a quantitative assessment of the severity and frequency of these effects in relation to the exposure of the organisms.

The assessment of health hazards of industrial chemicals, environmental pollutants and other substances represent an important element in the protection of the health of the worker and the other members of the community. Indepth studies of the nature and mechanism of the effects of toxicants are invaluable in the invention of specific antidotes and other ameliorative measures. Along with other sciences, toxicology contributes to the development of safer chemicals used as drugs, food additives, pesticides and many useful industrial chemicals.

One of the most critical environmental issues today is ground water contamination. About 70% of all the water available in our country is polluted. In
India, more than 76% of the population who live in several rural settlements of varying populations are dependent on ground water as source of drinking water (Kumara Swamy, 1990). Therefore, the preservation, protection and management of the quality of life is dependent on the environmental components, apart from industrial, agricultural and other economic parameters. The extensive use of pesticides in the present decade are also major culprits of environmental pollution. Apart from these, trace elements are often considered to be toxic to man, despite their active role as cofactors in many metabolic pathways. The trace elements are frequently encountered by human beings in several ways either through drinking water, food or inhalation. Modern man lives in an environment which contains many controlled components, but some uncontrolled ones have the power to produce untoward and adverse effects on reproductive processes. These components include sound, atomic reactors, space research flights, malnutrition, chemicals causing environmental impairment and health hazards.

One of the problems which faced researchers include the possible impact of environmental chemical factors on the reproductive functions. These have become increasingly urgent because of the significant rise in the relative number of unfavourable outcomes of pregnancy, subfertile marriages and various malformations in new born babies. The etiological factors responsible for such reproductive dysfunctions are numerous. Despite the fact that mutagenesis and teratogenesis may be components of reproduction failure, the reproductive system has unique, if obvious additional factors of a physiological nature that separate it from the pathological processes. These include state of somatic development, sexual maturation (reproductive capacity) and state of functional activity (Dixon, 1978). However, alterations in the
degree of injury by any chemical is also influenced by species differences in endocrinological regulation of reproduction and reproductive system (Hilscher and Hilscher, 1981).

EFFECTS ON REPRODUCTION

In essence, reproductive toxicology encompasses two facets: qualitative evaluation and quantitative assessment of toxicity. Quantitative evaluation is described by Paracelsus who enunciated a dictum: "All substances are poisons: there is none which is not a poison. The right dose differentiates a poison and a remedy". In a more modern parlance, the statement is that the dose makes the poison. The relationship between the dose of a compound and the response it elicits is a fundamental concept in toxicology (WHO, 1984).

The reproductive functions may be affected by toxicants through their effects on the reproductive system of either sex. In the male, the formation, development, storage and delivery of spermatozoa may be adversely affected. The oocytes, in the females, are also susceptible to various toxicants. In addition, the implantation of the fertilized ovum as well as the growth and development of the conceptus may be affected. Some such environmental toxicants are fluoride, aluminium, mercury, lead, zinc, arsenic etc., to which human beings are exposed through food, drinking water and the environment.

Heavy metal ions are known to induce a variety of histopathological changes in gonadal tissues. Reports from our laboratory have revealed that aluminium intoxication to mice resulted in alterations in the metabolism of the testis, cauda epididymis and vas deferens whose homeostasis was disturbed which in turn had
serious repercussion on the motility and count of spermatozoa. Hence the fertility rate was altered (Chinoy and Bhattacharya, 1996; 1997).

Toxic effects of methyl mercury on reproductive functions of different adult mammalian species have been observed (Rao, 1989a). In rats, guinea pigs and mice, methyl mercury impaired testosterone production and secretion contributing to loss in fertility potential (Burton and Meikle, 1980; Rao, 1989b). Methyl mercury also had adverse effects on follicular growth, ovulation and corpus luteum (Rao, 1997).

Severe disturbances were reported to occur during gametogenesis in fishes exposed to differential doses of lead for an extended period of time (Eisler, 1988). A comprehensive list of toxicants that affect the female reproductive capacity has been provided by Thomas (1993).

Throughout the reproductive cycle, toxicants can interfere either directly on the reproductive system or the conceptus or indirectly via certain endocrine organs. Before chemicals act directly, they must reach the target organs in sufficiently high concentration. This concentration may be higher or lower than that in the blood. For example, with DDT, the concentration is almost 80 times higher in the ovary than in the plasma. A number of other substances have also been shown to penetrate the oocyte, oviduct, uterine fluid and blastocyst (Fabro, 1978).

Of the various toxicants, fluoride illustrates strikingly the classical medical concept described earlier. While a continuous daily intake of small quantities of fluoride has been found to be beneficial in the prevention of dental caries, a long-term exposure to higher quantities might have serious effects on the enamel and bone. Single gram doses cause acute toxic effects or may even be lethal (WHO, 1984). Although, fluorine to some extent has been known to be involved in the inhibition of
caries, its intake by human beings through drinking water or foods or occupational exposure for a continuous period is known to cause a hazardous crippling disease ‘Fluorosis’. The affliction is mainly of the skeletal and dental systems. Therefore, elaborate work on the role of fluoride on these systems has been carried out (Susheela, 1985; Krishnamachari, 1986; Teotia and Teotia, 1991). Eventhough the effects of fluoride on dental and skeletal systems have been well documented, its effects on the reproductive system in general and fertility in particular, has received scant attention. Therefore, the present study was undertaken to investigate the effects of fluoride on the structure and functions of reproductive organs of female mice and their fertility with special reference to protein, carbohydrate, nucleic acid, oxidative metabolisms, ovarian steroidogenesis and hormonal status.

HALF LIFE:

The toxicokinetic studies revealed that the absorbed fluoride is distributed between blood, soft tissues and the skeleton. The half life of fluoride in blood and of tissues has been reported to be few hours, while in skeleton, it has a longer half life of about 8 years (WHO, 1984).

LETHAL DOSE$_{50}$ OF FLUORIDE IN ANIMALS

The LD$_{50}$ value for male and female rats are 250 mg and 180 mg F/kg body weight respectively and for male mice is 54.41 mg F/kg body weight while the females have LD$_{50}$ value of 51.6 mg F/kg body weight (Pillai et al., 1987; 1988).
OCCURRENCE OF FLUORIDE

Fluorine is the most electronegative and reactive of all elements and thus, in nature, is rarely found in its elemental state. The toxic potential of inorganic fluorides is mainly associated with this behaviour and the formation of insoluble fluorides. Fluorine also reacts with metallic elements to form compounds that are usually ionic, both in the crystalline state and in solution. Most of these fluorides are readily soluble in water.

Fluoride in the lithosphere

Fluoride has an atomic weight of 19.0 and an atomic number of 9. It chemically combines to form fluorides as fluorspar (CaF₂), fluorapatite (Ca₁₀[PO₄]₆F₂) or cryolite (Na₃AlF₆). It is seventeenth in the order of abundance of elements in the earth's crust (Barth, 1947). The fluorine concentration in soils vary enormously from place to place with average values between 200 and 300 ppm. It was found that the fluoride concentration is lowest on the ground surface when different levels have been studied (WHO, 1984).

Fluoride in water

Fluoride occurs in sea water in concentration ranging from 0.8 to 1.4 ppm (Kappana et al., 1962). It is present in nearly all fresh ground waters, although the concentration in some water supplies may be too low to be detectable by routine methods. The range of fluoride levels in drinking water varies in different parts of the world. In Africa, areas have been reported with as much as 95 ppm in the drinking waters, the range in USA is given as 0-16 ppm, whereas, in England most supplies are
below 1 ppm (WHO, 1984).

**Fluorides in Air**

Fluorides are widely distributed in the atmosphere originating from the dusts of fluoride-containing soils, from gaseous industrial wastes, from the burning of coal fires in populated areas and from gases emitted in areas of volcanic activity. Thus fluoride in varying concentrations is freely available in nature (Cholak, 1959).

**Fluoride in Food and beverages**

Various values for fluoride concentrations in vegetables have been reported. Occasional values in the range of 1-7 mg/kg fresh weight have been reported for spinach, cabbage, lettuce and parsley, while values for other vegetables have seldom exceeded 0.2-0.3 mg/kg. Probably, in some cases, the high fluoride values have been caused by contamination from air, soil, pesticides etc. It also seems probable that some kind of contamination is responsible for the very high values of 10.7 mg/kg and 11 mg/kg, respectively for polished rice as given by Oelschlaeger (1970), but more recent confirmation of the results is lacking. According to McClure (1949), the fluoride contents of fresh pork and fresh beef varied within the range of 0.2-2 mg/kg and range for salted beef was 1.3-3.3 mg/kg wet weight. WHO (1984) reported 0.9 mg of fluoride/kg wet weight in beef from cattle with symptoms of fluorosis. The fluoride content of processed foods and beverages prepared with water containing a fluoride level of 1 mg/litre will contain about 0.5 mg/kg more fluoride than those prepared with non-fluoridated water (Anermann, 1973). Substitutes for human milk like Infant Formulae, infant gruel, syrups and juices have a relatively high fluoride content as
compared to human milk. Duckworth and Duckworth (1978) reported that the fluoride concentrations in tea infusions prepared from 12 different brands of tea, varied from 0.4 to 2.8 mg/litre. About 40-90% of the fluoride in tea leaves is eluted by brewing.

**CHEMOBIOKINETICS AND METABOLISM**

After ingestion, most fluorides are absorbed in the stomach and intestine. The gastrointestinal absorption of fluorides is markedly influenced by dietary composition. The presence of large amount of certain ions in the diet, particularly calcium, aluminium and magnesium, which form less soluble complexes with fluoride, decrease the amount of absorbable fluoride. Following absorption, fluorides are distributed throughout the body, the concentration in most soft tissues being roughly equal to that in the plasma (WHO, 1984). Fluorides are selectively accumulated in skeletal, dental and mineralizing tissues. Deposition of fluorides in the bone serves as an excellent example of an accumulation of an element in the body. Approximately 98% of the fluoride in the body is deposited in bones and such accumulation normally continues throughout life. However, an individual on a long time, constant, elevated fluoride intake does tend to reach a state of equilibrium in which the amount of fluoride in the body is deposited in bones and such accumulation normally continues throughout life. In these individuals the fluoride retained by the body is reduced and the amount excreted in the urine is increased (Zipkin and Leone, 1957). In both dental and skeletal tissues, the deposition of fluoride is related to the metabolism of the tissue; for example, the forming teeth and bone will accumulate the fluoride ion more readily (WHO, 1984). Fluoride is incorporated into the hard tissues largely by a process of exchange and by incorporation into the apatite lattice during mineralization (US NAS,
Fluoride ions are also found in fetal tissues. Fetal blood fluoride concentration is approximately equal to that of the maternal blood. In the fetus, fluoride is selectively deposited in the teeth and bones, but not in high enough levels to cause pathological changes in cattle (Shupe et al., 1963).

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 concentrations depend upon and in fact, are nearly equal to the drinking water concentration (WHO, 1984).

**FLUORIDE INTAKE IN HUMAN BEINGS**

The fluoride contents of air, water and food determine the human intake of fluoride.

The average respiration rate in an adult person is about 20 m$^3$ per day. Bierstekar et al. (1977) estimated that persons living near industrial sources of fluoride could inhale 0.06 mg fluoride during a day of maximal pollution. As only a proportion of inhaled fluoride is retained, actual uptake will be less than the above estimate. Occupational exposure may add considerably to the total intake of fluoride. Such exposure occurs in the mining and processing of fluorspar, cryolite and apatite. Assuming a total respiration rate of 10 m$^3$ during a working day, the daily amount of fluoride inhaled could be as high as 10-25 mg, when the air concentration is at the most frequent exposure limits of 1-2.5 mg/m$^3$ (ILO, 1980).

In communities, where the water is fluoridated, people would consume a mean of 2.7 mg fluoride/day as compared with 0.9 mg/day, where the water is not...
fluoridated (Kumpulainen and Koivistoinen, 1977). Accidental intake of sodium fluoride tablets has occasionally resulted in fluoride intoxication in children (Duxbury et al., 1982). Intake of fluoride as a remedy for osteoporosis as well as dental caries, when exceeds its actual requirement will result in adverse effects on skeletal system. Close to a fluoride-emitting industry, contamination of leafy vegetables may increase the total fluoride intake of local residents by about 1.7% (Jones et al., 1971). The fluoride intake from animal products is practically unaffected by industrial air pollution (US EPA, 1980).

TOXIC EFFECTS OF FLUORIDE ON EXPERIMENTAL ANIMALS AND LIVE-STOCK

Plants obtain fluoride through water and soil in endemic areas as well as from air in the vicinity of industries. Once fluoride has entered a plant, it moves in the transpirational stream. Thus, fluoride taken up by the roots passed to the stem and leaves. The fluoride in a leaf is generally immobile. The animals grazing on such vegetation have been found to be affected adversely. There have been reports concerning the fluorosis in cattle reared in a polluted area, where the animals were fed on vegetation contaminated by fluoride (Chinoy, 1995). The manifestation of chronic effects of fluoride on cattle have been found to be more or less similar to those found in man i.e. dental and osteofluorosis. Animals afflicted with fluoride exhibited a non-specific and typical lameness or stiffness associated with calcification of periarticular structures and tendon insertions, exostotic lesions, thickening of bones and mineralization of the tendons (Shupe et al., 1963). Thus, lameness is often found to be transitory in nature and limits feeding or grazing time, thereby impairing
Ruminants are the animals most often associated with a practical fluoride pollution problem. Perkinson et al. (1955) demonstrated rapid absorption of fluoride by ruminants using radioactive fluoride. The symptoms in teeth generally observed were chalkiness, mottling and hypoplasia. In addition, the affected tooth is subject to an abrupt wear and erosion of enamel away from the dentine (Chinoy, 1995). Udall and Kellers (1952) in their field studies have found poor reproduction, diarrhoea and overgrowth of the hoofs due to high consumption of fluoride in cattle.

There is some information available on the role of fluoride on aquatic animals, especially fishes. Experiments undertaken by Neuhold and Singler (1960) in fish exposed to poisonous amounts of NaF causes weight loss and periods of violent movement. Studies from our laboratory revealed alterations in the activities of some enzymes of muscle and liver of *Channa punctatus* exposed to NaF. Biochemical changes in the liver, kidney and brain were also observed (Chinoy et al., 1994a).

**EFFECTS OF FLUORIDE ON GENERAL BODY METABOLISM**

The toxicity of fluoride is aggravated mainly through it adverse effect on general body and tissue metabolism. Therefore the role of fluoride on general body metabolism is presented here.

**FLUORIDE AND PROTEIN METABOLISM**

Fluoride is known to reduce protein synthesis (Hongslo and Holland, 1979), which is mainly due to impairment of the polypeptide chain initiation (Hoerz and McCarty, 1971). Holland (1979) reported that fluoride inhibits growth of cells *in vitro*,

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due to inhibition of protein and DNA synthesis which are the main targets for the cytotoxic action of fluoride. Protein synthesis can be depressed by fluoride at different levels including nucleus, ribosomes, mitochondria and hyaloplasm (Uslu, 1985). The reduced levels of protein has further been found to be due to weak incorporation of amino acids into proteins in rabbit dental pulp in vitro (Helgeland, 1976). The protein levels in stomach, duodenum and ileum of fluoride treated rabbits was declined (Shashi et al., 1987). A reduction in protein concentrations have also been observed in the reproductive tissues of mice, rats, and guinea pigs intoxicated with NaF in different doses (Chinoy and Sequeira, 1989a; Chinoy et al., 1991a; 1992a; 1993a; 1994b,c; 1995; 1997a,b; Patel et al., 1994).

Polyacrylamide gel electrophoresis of proteins of testis and cauda epididymis of NaF treated rats revealed disappearance of some proteins, induction of some new proteins and some were found to be resistant to NaF action (Chinoy et al., 1995; 1997a).

**FLUORIDE AND CARBOHYDRATE METABOLISM**

Fluoride has been traditionally known as an inhibitor of glycolysis, and to induce dramatic changes in carbohydrate metabolism mainly in the processes of utilization or storage of carbohydrates. In rabbits treated with fluoride a decline in glycogen concentration in spleen, lens, liver and skeletal muscle occurred (Shashi et al., 1988). On the contrary, glycogen accumulation occurred in fluoride treated fishes (Shaikh and Hiradhar, 1985; Chinoy et al., 1994a) and in liver, muscle, vas deferens and uterus of rats and mice (Chinoy and Sequeira, 1989a; Chinoy et al., 1991a; 1992a; 1993a; 1994b; 1995).
Suttie et al. (1974) demonstrated inhibition of enolase mediated glycolysis by cell culture studies where NaF was added to the medium. A decrease in the isocitrate dehydrogenase and accumulation of citrate (which is known to be a negative effector for phosphofructokinase) was reported by Dousset et al. (1987) in guinea pigs treated with HF. Inhibition of this enzyme could be another factor which leads to a decrease in glycolysis. Shearer and Suttie (1970) studied the changes in the concentrations of liver glycolytic and tricarboxylic acid cycle intermediates of rats fed fluoride (450 to 600 ppm for 3 days) and found an elevation of liver citrate concentrations. Shearer et al. (1971) also reported that fluoride affects the carbohydrate metabolism mainly through inhibition of the glycolytic pathway rather than the tricarboxylic acid pathway in rats.

In guinea pigs exposed to hydrogen fluoride (HF), a significant enhancement in plasma cholesterol and glucose-6-phosphate dehydrogenase activity occurred. The increased cholesterol biosynthesis was correlated with the acceleration of pentose phosphate pathway due to the enhanced production of NADPH by HF (Dousset et al., 1987).

FLUORIDE AND LIPID METABOLISM

Interaction of fluoride and lipid metabolism assumes considerable significance since fluoride is involved in arteriosclerosis. Saralakumari et al. (1988) reported that in rats supplemented with 100 ppm of fluoride resulted in marked reduction in plasma free fatty acids. The liver and serum lipid fractions were also affected. However, the treatment brought about a noticeable increase in total lipids, triglycerides and phospholipids in the serum which points to the formation of a fatty liver. However,
according to Leipzig et al. (1967), excess fluoride intake decreased the triglycerides but failed to influence serum cholesterol. Unaltered levels of cholesterol in reproductive tissues and serum of rodents treated with NaF at a dosage of 10 mg/kg body weight for 30 days were reported by Chinoy and Sequeira (1989a) and Chinoy et al. (1993a). Normal levels of serum cholesterol were also found in endemic human populations of North Gujarat (Chinoy et al., 1994d). However, a long term (70 days) treatment with fluoride in rodents resulted in increase in cholesterol in testis and a decrease in the activities of 3β and 17β HSD and circulating testosterone levels (Narayana and Chinoy, 1994a). In the liver of rabbits treated with NaF, triglycerides were decreased with a concomitant inhibition of lipase activity (Singh et al., 1985), while, Townsend and Singer (1977) obtained an increase in triglyceride level in serum in guinea pigs. Hence, it is likely that fluoride may interfere with lipid metabolism and possibly affect the energy turnover.

**FLUORIDE AND SOFT TISSUE INTERRELATIONSHIPS : GENOTOXIC EFFECTS OF FLUORIDE**

Conflicting reports are available in the literature regarding the genotoxic effects of fluoride. Information available is very limited on this aspect and the results that have been published are inconclusive (Smith, 1985). The literature review suggests three different observations: (1) Fluoride has no genotoxic effects. Thompson et al. (1985) found no fluoride induced increase in the frequencies of chromosomal aberrations or Sister Chromatid Exchanges (SCEs) in human lymphocyte cultures. Sodium fluoride even at maximum tolerance dosage did not cause chromosome damage detectable with micronucleus assay (Li et al., 1987) in mouse bone marrow.
Moreover, Martin et al. (1979) showed that life time consumption of 50 ppm fluoride did not cause detectable chromosome damage in bone marrow or testis cells of mice. Leonard et al. (1977) in their *in vitro* studies also observed no increase in the chromosome aberrations in the leucocytes of cattle with signs of chronic fluoride poisoning, when compared to control animals. (2) The second observation is that fluoride is a mutagenic agent and causes DNA and chromosome damage even at a dose of 0.45 ppm (Mohamed and Chandler, 1982) in mice. Jachimczak and Skotarczak (1978) have reported that sodium fluoride induced chromosome aberrations in cultured human leucocytes. Similarly, NaF caused chromosome aberrations in cultured ovarian oocyte of mice, ewes and cows (Jagiello and Lin, 1974). Recent studies carried out by Sheth et al. (1994) revealed an increase in the frequency of SCE’s in endemic human population of North Gujarat as compared to control. (3) Fluoride has synergetic effects with certain known mutagens (Vogel, 1973).

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 and efforts in this direction are underway at present in our laboratory.

**TERATOGENIC EFFECTS OF FLUORIDE**

Embryo and fetal toxicity from high doses of fluoride have been reported in experimental animals. High doses of fluoride (3 to 12 mg/kg body weight/day) have been found to cause abortions, necrosis of placentae and affect fetal growth in rats (Devoto et al., 1972). Studies carried out by Glenn et al. (1982) suggest that fluoride
may also exert effects on human fetal growth. Babies, whose mothers had received fluoride tablets during pregnancy were somewhat heavier and slightly longer at birth, and prematurity was much less frequent as compared to control. Doses of 0.5 to 2 mg fluoride were found to be lethal to chick embryos (Spira, 1956). However, NaF at a dose of 3 mg/kg body weight/day failed to produce still births in mice (Fleming and Greenfield, 1954).

The incidence of Down’s Syndrome with increasing concentrations of fluoride has also been reported in human population residing in endemic areas in Sweden (Berglund et al., 1980). Berry (1962), however did not find the difference in the occurrence of Down’s Syndrome in populations between areas with low (<0.2 mg/litre) and high (0.8-2.6 mg/litre) fluoride levels.

**FLUORIDE AND ENDOCRINE FUNCTIONS**

Extensive investigations carried out during the past one decade showed that fluoride toxicity is not confined to the bone and dental tissues alone, but involvement of more than one endocrine organ is evident in adults as well as children affected with skeletal fluorosis. Sivakumar (1977) observed normal levels of growth hormone in fluorotic subjects without genu valgum. However, in subjects with genu valgum the mean values were significantly elevated. There appears to be sufficient evidence that high concentrations of fluoride inhibit the action of the trophic hormones of the pituitary (Jentzer, 1954). Further studies carried out by Das and Susheela (1991) in rabbits treated with fluoride and fluorotic patients revealed low levels of plasma cortisol suggesting adrenal hypofunction. This hypofunction may be due to reduction in ascorbic acid contents as well as a depletion of steroid dehydrogenase activity in the
adrenal gland of rabbits (Rao and Susheela, 1979).

**FLUORIDE AND THYROID GLAND FUNCTION**

Contradictory reports are available regarding fluoride and thyroid gland metabolism. Siddiqui (1955) reported an increased incidence of goitre cases with an enhanced fluoride content of the environment and a decrease with an increase in iodine content of water. Epidemiological investigation on workers exposed to inorganic fluoride chemical factory revealed their hypothyroid condition (Zhang et al., 1992). Experimental studies in rabbits have suggested that high fluoride intake could cause hypertrophy of para-follicular cells of the thyroid (Poonam Khan, 1985). An increase in lipid component and significant reduction in the levels of RNA and DNA of the thyroid gland have also been reported in chronic fluoride intoxication in rabbits (Shashi, 1988, 1993). Chongwan and Daijei (1988) demonstrated swelling of mitochondria with disintegrated cristae in follicular epithelial cells of thyroid gland of rabbits after fluoride intoxication. In addition to morphological changes reported by many workers, functional alterations in thyroid gland metabolism were found in experimental animals subjected to excess feeding of fluoride. Our recent studies in human population affected by fluorosis revealed alterations in serum thyroid hormones, T₃, T₄ and TSH (Chinoy and Narayana, 1992; Michael et al., 1996). On the contrary, studies carried out by Teotia and Teotia (1991) did not find any correlation between chronic fluoride ingestion and thyroid gland function.

**FLUORIDE AND PARATHYROID FUNCTIONS**

The parathyroid gland plays an essential role in endemic fluorosis since its
function is to mainly regulate calcium metabolism. Fluoride is known to stimulate parathyroid and thereby enhance circulating parathormone levels. Faccini and Care (1965) reported elevated levels of serum immunoreactive parathyroid hormone in young sheep reared on water containing 100 ppm fluoride. The enhanced PTH levels would be implicated in the process of bone resorption often observed in some bones of animals in which experimental fluorosis was induced. Teotia and Teotia (1973) also reported an increase in parathyroid hormone (PTH) levels manifesting secondary hyperparathyroidism in patients with skeletal fluorosis and in children living in endemic areas. Teotia et al. (1978) opined that the observed changes in man such as osteosclerosis, hypermineralization, osteoclastic resorption of trabeculae and other effects are the attributes of interaction between the changes that occur in the PTH - thyrocalcitonin axis. Community based studies strongly suggest that calcium status is modified by PTH, which turn is responsible for bone changes observed in fluorosis.

**FLUORIDE AND REPRODUCTION**

The studies on the role of fluoride on reproductive status has received inadequate attention. Therefore, there is paucity of data and the existing data is controversial. The interrelationship of fluoride and reproductive functions were first reported by Messer et al. (1973), who found that fluoride plays an important role in reproduction and its deficiency is a cumulative factor for fertility impairment in female mice. They further demonstrated that mice with low fertility improved their reproductive capacity, litter production and breeding performance when maintained on high fluoride diet (Messer et al., 1974). However, these results were contradicted and opposed by Tao and Suttie (1976) who claimed that fluoride was not involved in
maintenance of reproduction. Testicular cells of adult rats given oral NaF upto 84 mg/kg body weight did not show DNA strand breaks (Skare et al., 1986). On the contrary, in mice fed on 125, 250 and 500 ppm fluoride, spermatogenesis was reported to be impaired (Ridha, 1978). Degenerative changes, such as atrophy and necrosis of seminiferous tubules, lack of differentiation and maturation of spermatocytes have been shown in the testis of F- treated mice (Kour and Singh, 1980). These results were supported by the detailed studies carried out by Chinoy and Sequeira (1989b), who reported that fluoride ingestion to mice at a dose of 10 mg/kg body weight for 30 days caused a decrease in the diameter of seminiferous tubules and their epithelium due to denudation and vacuolisation of cells rendering them devoid of sperm. Further studies of Chinoy et al. (1991b) revealed that a single microdose vasal injection of NaF to rats also exhibited similar changes in testicular histoarchitecture affecting the spermatogenic process. The electron microscopic studies in rabbits revealed changes in the structural integrity of testis by fluoride, affecting spermatogenic elements (Susheela and Kumar, 1991). Narayana and Chinoy (1994a) have reported that testicular steroidogenesis, Leydig cell functions and serum testosterone levels were altered after fluoride treatment in rats.

The circulating androgen levels were decreased during fluoride intoxication and hence the target organs whose structural and functional integrity is dependent on androgens, were adversely affected. In cauda epididymis, after fluoride treatment, confluence of tubules occurred resulting in larger tubules, decrease in epithelial cell height with denudation of cells in the lumen, which was devoid of sperm. These structural changes contributed towards alterations in cauda epididymal metabolism and function (Chinoy and Sequeira, 1989a,b; Chinoy et al., 1991c; 1992a).
Recent studies in our laboratory have revealed inhibition of sperm acrosomal enzymes, namely, hyaluronidase and acrosin (Narayana and Chinoy, 1994b). Schoff and Lardy (1987) reported that fluoride is a strong inhibitor of glycolysis and respiration process in spermatozoa. The sperm of NaF treated rabbits when stained with silver nitrate (specific for acrosomal integrity) exhibited head to head agglutination, deflagellation and loss of acrosome (Chinoy et al., 1991c). These alterations in sperm structure and metabolism are the result of the hostile internal milieu of epididymis affecting sperm maturation which ultimately led to a decline in sperm count, motility and their fertilizability and subsequently to a significant reduction in fertility after NaF treatment (Chinoy, 1991a; Chinoy and Sequeira, 1992). Human spermatozoa lost their motility in vitro in the presence at 250 nM NaF within 20 minutes incubation (Chinoy and Narayana, 1994).

The histoarchitecture of vas deferens of fluoride treated mice indicated nuclear pycnosis in the epithelial region, clumping of stereocilia, increase in thickness of lamina propria and muscle coat as well as absence of sperm in the lumen (Chinoy and Sequeira, 1989b). In rabbits treated with NaF (10 mg/kg body weight) for 18 to 29 months revealed loss of stereocilia on the epithelial cell lining the lumen of the vas deferens with abundant mucus droplets (Susheela and Kumar, 1991). Alterations in histology of other sex accessory organs were also observed (Chinoy and Sequeira, 1989b; Chinoy et al., 1991b).

Epidemiological study of gynaecological problems in female workers in a superphosphate manufacturing plant offers a direct evidence of fluoride effects in human pregnancies. The female workers were found to suffer more menstrual irregularities, vaginal and uterine inflammation, more frequent toxicosis during
pregnancy with hypotension and threatened abortions, a higher percentage of untimely discharge of the amniotic fluid and weakness of birth activity (Kuznetsova, 1969a,b). In male patients with fluorosis, Tokar and Savechenko (1977) found reduced amount of testosterone and enhanced concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH).

Fluoride in concentrations over 60 ppm in the feed of dairy cows interfered with breeding efficiency by increased services per conception (Hobbs et al., 1954). A dose of 150 to 300 mg fluoride per kg body weight over 0-72 hour interval blocked gonadotropin stimulation of the rabbit ovary (Guercio and Cazzola, 1941). Similarly, fluoride at a dose of 10mg/kg in a 21 day period blocked follicular development in guinea pigs (Sanfilippo, 1946). Studies on histopathological changes in rabbit ovary during experimental fluorosis revealed atrophy of follicles along with oocyte disintegration and marked necrosis of cells (Shashi, 1990).

The above results clearly indicate some controversy on the role of fluoride on soft tissue functions, especially reproductive organs in female mice.

REVERSAL OF FLUORIDE EFFECTS

Fluoride is a potent health hazard and affects virtually every phase of body metabolism. In view of the millions of people afflicted 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, are cheap and have promising results in mitigation of fluoride induced health hazards in endemic populations.
FLUORIDE AND MINERALS

The extent of fluoride toxicity in a cell or tissue is influenced by many minerals, due to its strong affinity to form complexes with minerals and elements. Some divalent ions like Ca²⁺, Mg²⁺ phosphorus (P) etc., form strong complexes and thus reduce fluoride action.

Extensive studies on calcium kinetics showed that the cumulative retention of radioactive calcium was enhanced in animals fed with fluoride, higher retention of fluoride was observed in those receiving low calcium diets (Krishnamachari, 1978). Urinary calcium excretion, calcium deposition into bone and mass of exchangeable calcium was not affected by fluoride. However, calcium absorption was inhibited and its removal rate was elevated in bones of fluoride treated calves (Ramberg et al., 1970).

Administration of 30, 50, 150 or 450 ppm additional fluoride to pigs increased the bone fluoride. High dietary calcium-phosphorus, however, lowered bone fluoride concentrations in swine (Forsyth et al., 1972a). The increase in fluoride level was still higher, when they were maintained on low calcium phosphorus diet (Forsyth et al., 1972b). Therefore Ca²⁺ phosphorus interaction with fluoride has been reported to be highly effective in lowering fluoride action.

FLUORIDE AND VITAMINS

Wadhwani (1952) induced fluorosis in monkeys on normal and scorbutic diets and noted higher mortality in scorbutic animals. He observed that stiffness of limbs and restricted movements disappeared and death postponed when each animal was given 20 mg vitamin C. Studies carried out by Chinoy and Co-workers (Chinoy et al.,
1991c, 1993a; 1994b,c; 1995;1997a,b; Narayana and Chinoy, 1994b) in rodent models further revealed the therapeutic role of ascorbic acid or calcium as they brought about marked recovery in fluoride induced toxicity. However, recovery was most pronounced in animals given both ascorbic acid and calcium due to their synergistic/additive effect. According to Muhler and Hine (1959), elevated levels of vitamin A, C and D, mitigated the symptoms of fluorosis. Marks (1975) found that vitamin E reduced reproduction abnormalities in rats. Vitamin E has a powerful anti-oxidant effect within the animal body particularly for lipids. In rats, the main symptoms of vitamin E deficiency are degeneration of testis, regression in the ovary, changes in ovulation and abnormalities of gestation.

Vitamin D acts as a regulator of calcium and phosphate metabolisms. It is used in treating conditions such as reduced renal functions, calcium malabsorption and osteoporosis. The rodent data (Chinoy and Sharma, 1997) revealed that ingestion of Vitamin E and/or Vitamin D to fluorotic mice manifested a significant recovery in all NaF induced effects in both vital and reproductive organs.

**FLUORIDE AND AMINOACIDS**

Suttie et al. (1974) reported that addition of various amino acids to the growth media in concentrations is excess to those present in the media enhanced the growth of fluoride treated L-Cells wherein glycine and glutamine were found to be the most potent. Another report (Chinoy and Mehta, 1997) has revealed that supplementation of aminoacids (Glycine and Glutamine) alone and in combination was beneficial in mitigation of fluoride induced effects in male mice.

On basis of these studies further reports by Chinoy and Mehta (1997b) have
revealed that a protein supplemented diet would be beneficial, while, a protein deficient diet aggravated fluoride toxicity in male mice. The basis for the protective effect is not yet clear, but may involve slight changes in intracellular fluoride concentrations, or some as yet undetermined metabolic alterations.

Therefore, to bridge these gaps, the present work has been undertaken. The work incorporated highlights the interrelationship of fluoride and reproductive functions in female mice. The work embodied in the thesis also emphasizes the role of some therapeutic agents in overcoming fluoride toxicity.