INTRODUCTION

Pollution is an undesirable change in the physical, chemical or biological characteristic of our environment. An increase in human population combined with industrialization leads to an unavoidable problem of environmental pollution. The problems resulting from organisms exposed to many new industrial chemicals and hazardous byproducts are now only beginning to receive their just share of attention by government and private research agencies. Only recently has the public become cognizant of these real and potential dangers to man and his environment. While this is true in the case of affluent western societies, in the developing countries the environmental awareness is gaining acceptance at a snail's pace.

Fluoride: Its Importance as a Pollutant

Of the several pollutants, which pose serious health problems, perhaps fluoride needs special mention as it is known to have beneficial effects in reducing dental caries, in appearance of tooth by way of early eruption time and in alignment in the dental arches (Marthaler, 1987). It is also known to reduce frequency and severity of periodontal disease and is even suggested as a therapeutic drug for the treatment of osteoporosis (Cohen *et al.*, 1969). On the other hand, excessive fluoride ingestion during early ages, results in mottled enamel and accumulation of fluoride in the bones over a period of time, ultimately leads to crippling bone deformities known as `skeletal fluorosis'. The deleterious effects overrules its beneficial effects, due to the fact that all around the world people suffer from the excessive fluoride effects and is one of the major cause of concern in India.

Occurrence of endemic fluorosis with varying intensity has been reported by several workers in different states of India (Figure 1). The pioneer investigations were made by Shortt and his associates in 1937. Day (1940) reported dental fluorosis from several districts of the erstwhile Punjab. Khan and Wig (1945) reported advanced cases of endemic skeletal fluorosis from the same areas. More systematic studies have been carried out by Singh, Jolly and their cowork-

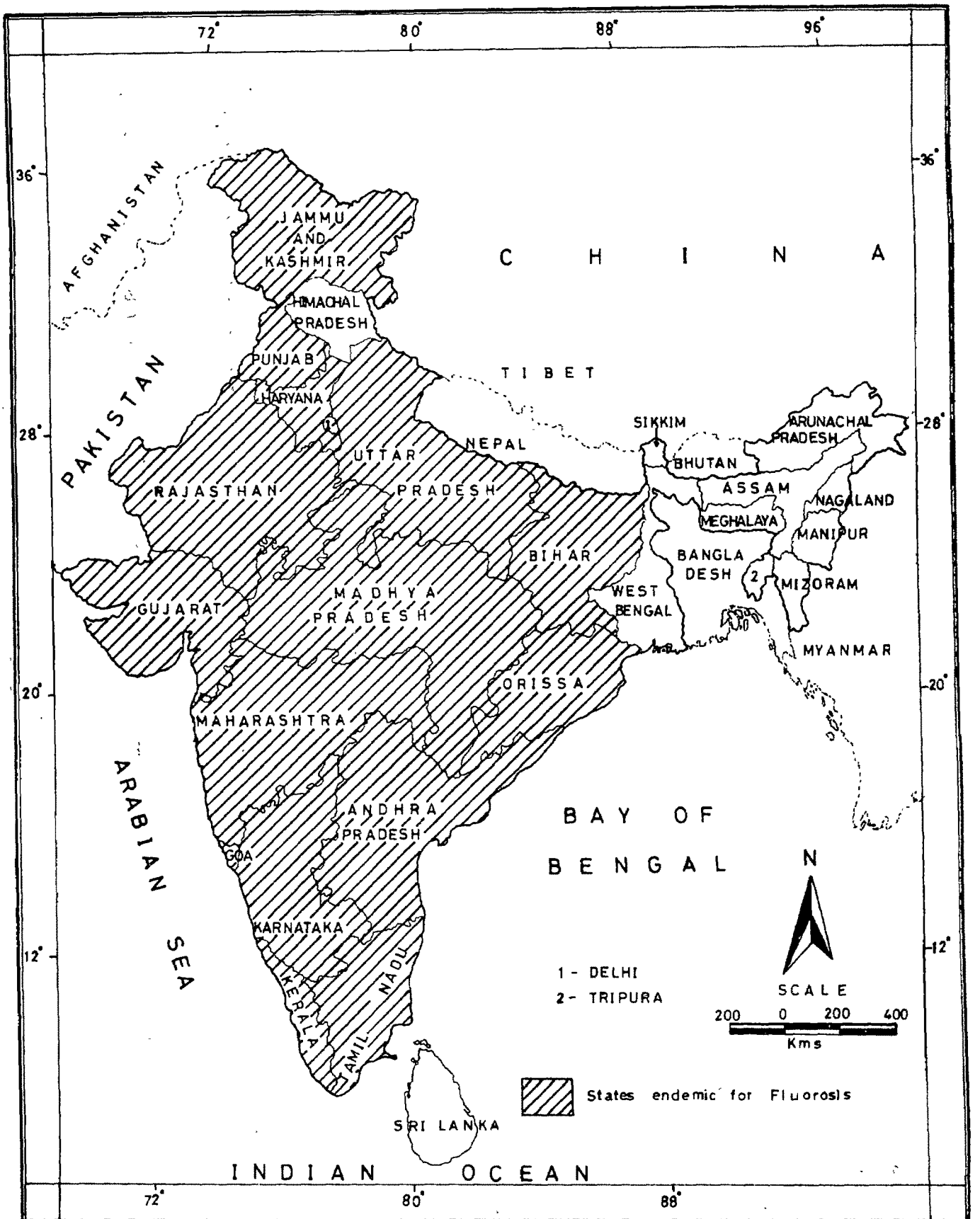

Fig. 1. Map of Indian Subcontinent showing states endemic for Fluorosis

[Source: Susheela A.K.(1991) "Prevention and Control of Fluorosis" National Technology Mission on Drinking Water]

ers (Singh *et al.*, 1962; Jolly *et al* , 1969). Singh *et al.* (1962), estimated that the belt of high fluoride level in drinking water covered atleast one fourth of Punjab and exposed roughly five million people to the toxic potentialities of fluoride.

Thergaonkar and Bhargava (1974), and Tamboli *et al* (1980), reported dental and skeletal fluorosis from Rajasthan. Vast areas in the southern part of India, covering the states of Andhra Pradesh (Pandit *et al.*, 1940; Raghavachari and Venkataramanan, 1942; Siddiqui, 1955; Reddy *et al.*, 1969), Tamil Nadu (Pandit *et al.*, 1940) and Karnataka (Krishnamachari and Sivakumar, 1976), were detected to contain appreciable amount of fluoride in natural water, with a large segment of population in the endemic villages suffering from dental and skeletal fluorosis. These investigators observed a positive correlation of the rate of prevalence and degree of the disease, with the level of fluoride in drinking water.

Krishnamachari and Krishnaswamy (1973) reported from certain parts of India, which were already described as fluorosis affected areas, a new fluoride toxicity syndrome known as `genu valgum'. This form of fluorosis was prevalent chiefly among the adolescents between 10 to 25 years of age and manifested within a shorter period of exposure to fluoride. The interaction of fluoride ions with some other nutritional factors has been suggested to be the possible cause of this skeletal pathology (Krishnamachari and Krishnaswamy, 1973; 1974). Genu valgum has also been reported from Tamil Nadu and Karnataka (Krishnamachari and Sivakumar, 1976). In certain parts of India, industrial fluorosis has also been reported (Vashi *et al.*, 1981).

Source and Cycle of Fluoride in Nature

Fluorine, due to its high electronegativity is widely distributed in a combined form in a number of naturally occuring minerals, including fluorspar (CaF_2); cryolite ($AlF_3.3NaF$), topaz, tourmaline, the micas, etc., in which it is found in combination with silicates; but

particularly in association with phosphorus as fluoroapatite ($Ca_5F.(PO_4)_3$). The fluorine content of phosphate rock is often as high as 3-4%. In certain areas, particularly where rock phosphate is found, such as North Africa (Morocco), parts of North and South America, China and Iceland, the fluorine content of the soil may reach 0.15% and of water, especially from underground sources, 15 ppm. The effects of fluorides to the health of man stem largely from dissolved fluoride present in many supplies of drinking water. However, suspended particulate fluorides may also have some health importance.

The occurrence of fluoride in the water is dependent on a number of factors: **a**. availability and solubility of parent fluoride containing minerals in contact with water, **b**. the porosity of rocks and soils through which the water flows, **c**. the rate of flow, **d**. the pH as well as temperature of water and **e**. the concentration of certain ions *viz*., calcium and hardness of water (Bell and Ludwig, 1970). Hence, the subsoil waters, because of its greater opportunity to interact with fluoride bearing minerals, contain higher fluoride than surface waters. Since fluoride in water is derived from the earth, a fairly good correlation between soil and water contents of fluoride was observed in different parts of the world (Daver, 1945; Williamson, 1953; Cholak, 1959).

Natural sources of atmospheric fluorides are volcanoes (Noguchi *et al*., 1963) and dust storms in areas where soils are rich in fluoride (Williamson, 1953). Gaseous wastes from a number of industries also contaminate the atmosphere with fluoride. Of these, the following industries form the major source: **1**. aluminium production by electrolytic processes, where the source of fluorine is the cryolite used as a flux in the small furnaces; **2**. brickwork, where the source is usually the local clay, although coal is sometimes a contributory factor; **3**. in glass, enamel and certain colour works, where fluorine compounds are used as fluxes; **4**. calcination of ironstone, where the source is mainly the fluorine-rich ore itself; **5**. steel and metal works, when the method of production involves the use of large amounts

of fluorspar in the flux; **6.** potteries, where the sources are clay and other materials (Allcroft, 1954). All these sources may act to increase the fluoride level of rain or precipitation. The steam discharged from fumaroles of active volcanoes may also contain considerable quantities of fluoride, so that the fluoride levels of rain in areas of volcanic activity may also be substantially increased (Zies, 1929; Noguchi *et al.*, 1963).

Almost in all the foods the presence of fluoride ranges from traces to hundreds of ppm. Natural concentrations in the foliage of most plants range from 2 to 10 mg F^-/kg. In USA, fluoride concentration in 107 alfalfa samples from areas assumed to be free of industrial pollution were found to range from 0.8 to 36.5 mg/kg (Marier and Rose, 1971). Those parts of the plants (vegetables and fruits) that are consumed by man normally have fluoride contents of the order of 0.1 to 0.4 mg/kg. However, high levels of fluoride have been found in cereals. Singer and Ophaugh (1979), found fluoride concentrations of 2.0 to 2.1 mg/kg in barley and rice processed in unfluoridated water as compared to 4.3 and 6.4 mg/kg respectively, if processed in fluoridated water. Certain plants such as taro, yams and cassava, which constitute the staple diet in many tropical areas particularly in South America and in the Pacific, have been found to contain relatively high fluoride levels. Tea leaves are also reported to contain high levels of fluoride (Singer *et al.*, 1967).

McClure (1949), in his report on `Fluorine in foods' emphasized that the solid foods have been considered to contribute only slightly to the total daily fluoride intake, at least in Western-style diets. From McClure's analysis it would appear that most foods are low in fluoride, having a content of 0.1 to 1.0 mg F^-/kg of dry weight and would contribute a maximum of 0.27 mg F^-/day. More recent studies, however, have shown that there are great individual variations in the daily intake of fluorides from solid food (Leverett, 1982).

Considering the numerous sources described earlier, chances of fluoride entering the food

chain is more and rightly therefore. Marier (1972) has warned that the fluoride cycle in our ecosystem is no less dangerous than other pollutants which are given more emphasis by environmental biologists. Moreover, the journey of fluoride in the food chain involves transfer of sodium fluoroacetate, which is much more toxic than fluoride itself (Groth,1975).

Absorption of Fluoride

Uptake of fluoride from the gut is passive in nature and no active transport mechanism is involved in the process. Stookey *et al.* (1964b), from their experiments with rats established that: **1.** the rate of fluoride transfer from the intestinal lumen is proportional to the surface area of intestine, **2.** metabolic inhibitors like sodium cyanide, sodium iodoacetate or 2,4-dinitrophenol did not alter the rate of fluoride diffusion and **3.** the temperature variations from 20°C to 37°C did not influence the rate of fluoride diffusion from the intestine. The site of absorption is stomach as well as intestine, but more in the latter (Perkinson *et al.*, 1955; Carlson *et al.*, 1960b; Foster and Rush, 1961).

Fluoride absorption takes place in two distinct phases, first rapid phase followed by a slower phase. In first hour, 72-75% of the ingested fluoride is absorbed (Wallace-Durbin, 1954; Zipkin and Likins, 1957). After first one and half hour 85% of the fluoride was reported to be absorbed (Zipkin and Likins, 1957). In next 24 hours only a small amount was absorbed (Ericsson, 1958; Stookey *et al.*, 1963; 1964a). The slow phase may be due to disappearance of gradient absorption to a near equilibrium point with fluoride entering and leaving the gut almost at an equal rate. Hein *et al.* (1956), showed that when fluoride was given intravenously it was secreted to intestine. The plasma level of fluoride, therefore, plays an important role in determining the net transfer across the gut. Fluoride absorption decreases when there is elevated level of fluoride in blood and increases when the concentration is low (Lawrenz *et al.*, 1940; Stookey *et al.*, 1963; Savchuck and Armstrong, 1951;

Zipkin and McClure, 1952).

The absorption of fluorides depends upon the degree of solubility, the degree of ionization and the ability to form complexes with other substances in the gut. The ingested fluoride compounds, depending upon their chemical nature, may dissociate to free fluoride ions prior to their absorption or may be absorbed as such (Zipkin and Likins, 1957; Ericsson *et al.*, 1961). There are ample evidences which suggest that the absorption of fluoride is faster when it was taken with water than when it was taken with food (Lawrenz and Mitchell, 1941; Weddle and Muhler, 1954). The reduced rate of absorption arises possibly due to its lower solubility and interference of food constituents (Lawrenz *et al.*, 1939). High fat content of the food is known to promote fluoride absorption (Miller and Phillips, 1955; Buttner and Muhler, 1958;Bixilar and Muhler, 1960). It may be due to delayed gastric emptying time by the presence of fat.

Presence of high concentration of inorganic ions, such as calcium, magnesium and aluminium most effectively reduce fluoride absorption by forming complex forms with fluoride (Weddle and Muhler, 1954; Wagner, 1959; Spencer *et al.*, 1980), and controversial reports regarding effects of molybdenum on the absorption of fluoride have appeared (Stookey *et al.*, 1962; 1964a; Ericsson, 1966).

Inhaled fluorides, in the form of vapour, gas or dust with particular size are almost completely absorbed from the respiratory system (Machle and Largent, 1943; Collings *et al.*, 1951; Largent, 1961).

Distribution of Fluoride in the Body

The maximum amount of fluoride in the body is found in skeletal tissues. However, small but significant quantity is also found in soft tissues and body fluids.

In hard tissues

Fluoride has a marked affinity to hard tissues and a good proportion of the ingested fluoride is being incorporated and retained by the hard tissues. The level of fluoride in the hard tissues is dependent upon a number of variables: **a**. the amount of fluoride ingested; **b**. chemical forms of fluoride in the diet; **c**. the duration of ingestion; **d**. age of the animal and **e**. vascularity and metabolic activity of the tissues (Moller, 1982). The amount of fluoride absorbed by the skeleton is usually more closely related to the amount present in the drinking water than to that contained in the diet. Zipkin *et al.* (1958), showed a linear relationship between the fluoride level of the water supply and the average fluoride concentration of human bone. When the concentration of fluoride in water is between 1 to 20 ppm, about half of the ingested fluoride appears to be retained by the skeleton. Most of the remainder is rapidly excreted via urine and about 5 to 10% excreted via the faeces (Largent, 1961).

Bones can accumulate fluoride throughout the life (Smith *et al.*, 1935), though the rate of uptake is faster in young animals (Zipkin and McClure, 1952; Suttie and Phillips, 1959). During the active phase of bone growth, when large proportions of crystallites are still forming, fluoride is incorporated directly inside the crystal lattice (Bauer *et al.*, 1955). The older bones acquire fluoride by surface exchange. Uptake of fluoride by the surface exchange is also greater in young animals due to the hydration and larger surface area of the crystals.

The dental tissues take up fluoride more rapidly during their periods of formation and calcification (Wallace-Durbin, 1954; Weidmann, 1962). Even after the dental growth is terminated, the dental tissues may acquire considerable quantity of fluoride, during which the mineral is incorporated in incompletely calcified teeth (Jenkins, 1955; Weidmann, 1962).

Hard tissue references — dates back to 1982!!

In soft tissues

Compared to skeletal tissues, soft tissues accumulate less quantity of fluoride. Chronic administration of fluoride is known to elevate the level of fluoride in liver, kidney, muscle, lung, heart, spleen and brain (Harvey, 1952; Venkateswarlu and Narayana Rao, 1957; Shupe *et al.*, 1963). However, the aorta and placenta may accumulate a substantial amount of fluoride (Gardner *et al.*, 1952; Ericsson and Ullberg, 1958; Smith *et al.*, 1960; Greever *et al.*, 1971) due to the trapping of fluoride in the zones of ectopic calcification (Call *et al.*, 1965; Smith *et al.*, 1960). Kidney also has been reported to contain more amount of fluoride than the other soft tissues (Shupe *et al.*, 1963). Wagner (1962) reported that fluoride levels in soft tissues increased with age. This increase of fluoride concentration was not dependent on the total intake of fluoride by the animals as it grew but dependent on the reduced uptake of fluoride by calcified tissues (Ericsson and Ullberg, 1958; Smith *et al.*, 1960) and thereby tending to push the plasma level of fluoride high. This in turn leads to more deposition in soft tissues (Wagner, 1962). Engel *et al.*(1961), reported that the anion binding property of intracellular colloidal proteins may be the reason for the increase in fluoride in the soft tissues, whereas some other investigators reported mineral status of soft tissue cells in the animal may be the reason for higher concentration of fluoride (Foster *et al.*, 1960; Griffith *et al.*, 1963).

The literature regarding the capacity of fluoride to cross various membrane barriers of cells is scanty or not clear. The available reports indicate that fluoride probably is transferred only partially across the salivary (Carlson *et al.*, 1960b; Gedalia *et al.*, 1963), mammary (Perkinson *et al.*, 1955; Suttie *et al.*, 1957) and placental barriers (Knouff *et al.*, 1936; Ericsson and Ullberg, 1958; Brzezinski *et al.*, 1961). However, studies on the kinetics of fluoride penetration clearly indicate that blood-brain barrier fails to exclude fluoride from nervous tissue (Geeraerts *et al.*, 1986).

In blood

In normal blood, about 3/4 of the total fluoride is in the plasma (Carlson *et al.*, 1960a). The total fluoride in the blood is of the order of 0.1 to 0.2 ppm. Only 10% of the total fluoride in the plasma remains in ionic form, while the rest of the fluoride is associated with albumin and is called as organic fluoride. A third fraction of components of organically bound fluoride has also been identified. This fraction represents 20% of total fluoride of the blood (Papez *et al.*, 1980). Similar to the well known chloride shift, migration of fluoride takes place from plasma to erythrocyte when the blood pH is lowered and in opposite direction when the pH is raised (Carlson *et al.*, 1960a).

The fluoride level in the blood is maintained within the narrow limits, indicating an efficient homoeostatic regulation of fluoride in the body (Smith *et al.*, 1950; Singer and Armstrong, 1960; 1964). Effective regulation of plasma fluoride level in the humans throughout the day has been demonstrated in three normal individuals consuming a normal diet and fluoridated water (Singer and Armstrong, 1960). Patients with metabolic bone diseases treated with large doses of sodium fluoride (50-100 mg/day) for 10 to 34 weeks, showed that there is only a transitory rise in plasma fluoride content during the early weeks of treatment and later comes to within a pretreatment value range (Armstrong *et al.*, 1964). When Smith *et al.* (1950) studied two groups of populations supplied with 23 fold difference in fluoride content, only a 3 fold difference in blood levels of fluoride was exhibited.

Singh and Jolly (1961) reported blood levels of fluoride in patients suffering from skeletal fluorosis in India at about 1.5 ppm with a range of 0.5 to 6.1 ppm. Srikantia and Siddiqui (1965) reported 2.9 ppm of fluoride in a study of blood fluoride levels in 31 male adults between 18 and 40 years of age, who had consumed well water with fluoride contents of between 6.8 and 8.2 ppm and who exhibited various degrees of skeletal fluorosis. Shupe

et al.(1963), demonstrated the ability of cattle to regulate the fluoride level of the blood.

Excretion of Fluoride

Fluoride is excreted mainly through urine and partly through faeces. Very small amount of fluoride is also lost through milk (Perkinson *et al.*, 1955), saliva (Carlson *et al.*, 1960b), nails as well as hair (Elsair *et al.*, 1982) and tears (Hodge *et al.*, 1970).

Many of the reports showed that 20-30% of the ingested fluoride appear in the urine within first 3 to 4 hours of ingestion (Zipkin *et al.*, 1957; Carlson *et al.*, 1960a). The concentration of urinary fluoride is fairly proportional to the amount of fluoride taken (Gedalia, 1958), so it has been recognized as the most reliable parameter for assessing the exposure to fluoride (Hodge *et al.*. 1970).

There are conflicting reports of urinary fluoride excretion (Machle *et al.*, 1942; Largent,1961). However, the urinary fluoride excretion of patients on large therapeutic doses of fluoride (60 mg F^-/day) was reasonably consistent from period to period (Rich *et al.*, 1964).

Variations in urinary fluoride excretion can be found from individual to individual. Even in the same individual there will be variations from hour to hour and day to day. So, only studies over a considerable period will give consistency in results. Buttner *et al.* (1961), showed day to day variations between two individuals, both in concentration and content. Infants and children excrete less fluoride in their urine than the adults when exposed to the same fluoride concentration (Zipkin *et al.*, 1956; Gedalia, 1958). This may be because of greater absorption of fluoride into the tissues at the formative ages.

The available data indicate that fluoride excretion tends to be lowered when there is kidney disease or injury (Yudkin *et al.*, 1954; Largent, 1961). Linsman and McMurry (1943) and

Maes *et al.* (1960) described in their studies on autopsy and biopsy samples of bones from the patients who suffered chronic interstitial nephritis, that the samples showed higher amounts of fluoride compared to normal samples indicating that, if renal function is impaired to the extent that fluoride excretion is slowed and reduced, absorbed fluoride will tend to remain elevated longer in the blood, thereby offering an opportunity for more fluoride to deposit in various tissues.

The fluoride excretion tends to decrease during pregnancy. Urinary fluoride level declines significantly at the second half of pregnancy and returns to the normal level after delivery (Gedalia *et al.*, 1959). The decline in urinary fluoride level may be due to rapid uptake of the fluoride by the growing foetus (Hanhijarvi, 1981).

The insoluble fluoride compounds as well as those rendered insoluble by dietary factors are excreted through faeces.

The question of amount of fluoride lost through sweat is debatable but it is likely that, with higher humidity and higher climatic temperature, a relatively high proportion of fluoride can be lost through sweat.

Fluoride Toxicity

The acute toxicity of fluoride in man is not common and only occurs by accidental intake with food or by absorption of a massive dose through lungs due to inhalation of fluoride containing vapours. The acute lethal dose of fluoride for man is probably about 5 g in the range of 2-10 g (Goodman and Gillman, 1975).

Roholm recorded 112 cases of acute toxicity in humans (see review by Roholm, 1937) and Greenwood (1940) added 18 more to the list. The largest incidence of acute toxicity involving 263 cases of fluoride poisoning was reported from Oregon State Hospital, USA (Lidbeck *et*

al., 1943). The major symptoms of acute poisoning are vomiting, pain in abdomen, diarrhoea, convulsions, spasms, general weakness as well as muscle weakness and collapse. But in some of the cases nausea, unconsciousness, impaired swallowing, high temperature, perspiration, thirst and difficulty in speech have been reported (Roholm, 1937). According to Goodman and Gillman (1975), respiratory paralysis or cardiac failure is the usual cause of death. Robinowitch (1945), reported a death due to the altered calcium metabolism after taking large amount of sodium fluoride.

Acute poisoning is most commonly seen in pigs and is nearly always due to accidental ingestion of too much sodium fluoride, which is used as an anthelminthic against round worms (*Ascaris*) and stomach worms (*Hyostrongylus*) in the pig. This compound can act as a violent poison, concentrations as low as 4-5% being extremely toxic and fatal to pigs. Poisoning following the use of insect powder is uncommon, but has been described in the dog (Holmes, 1946). The dusting powder responsible, contained 40% sodium fluoride. Egyed and Brisk (1967) have reported cases of poisoning in farm stock due to sodium fluorosilicate used as a pesticide. The lethal dose is 100 g for the horse and 200 g for the bovine. Padberg (1972) has noted poisoning in cattle due to potassium ammonium bifluoride used as a wood preservative. The chief signs were diarrhoea and fall in milk yield. Volcanic erruptions can cause acute fluoride poisoning in sheep, as the ash may contain up to 2000 ppm of fluoride (Georgsson and Petursson, 1972).

The clinical signs of acute poisoning in animals are those of corrosive poisoning: gastroenteritis, vomiting, abdominal pain, diarrhoea, muscular weakness and, in severe cases, collapse and death. There have been reports of fatal haemorrhage in pigs castrated after dosing with sodium fluoride (Bain, 1953; Hardenbergh, 1957). Fluoride is known to delay the clotting time and is often used as an anticoagulant. In sheep poisoned with fluorosilicate, Egyed and Rosner, (1968), have recorded salivation, inappetence, dullness, dyspnoea,

recumbency, clonic convulsions and increase in heart rate. Post mortem findings in pigs dead from fluoride poisoning consists of haemorrhagic gastroenteritis and marked congestion of the liver and kidneys.

The toxicity of inorganic fluorine compounds varies and to some extent depends upon their solubility in water above certain minimum levels of intake. Sodium fluoride and sodium fluorosilicate are the most toxic and calcium fluoride is the least toxic of the compounds studied experimentally.

It has been suggested that horses are more tolerant to fluoride than cattle and sheep, but Allcroft (1954), suggests that it is probable that the difference in feeding and management between horses and ruminants may account for this opinion. In general the descending order of susceptibility of farm animals appears to be: calves, pigs, horses and poultry (Allcroft, 1954). The level of fluoride as rock phosphate which is on the borderline of toxicity for cattle, sheep and pig is about 100 ppm of the total dry ration. For chicks the level is of the order of 350 ppm and for laying hens 530 ppm (Clarke *et al.*, 1981).

Effects on teeth

Teeth are extremely sensitive to dietary fluoride level and the dental changes induced by fluoride is one of the early, easily detectable and sensitive index for fluorosis in man and animals. Excessive amounts of fluoride incorporation in the enamel during the course of development of teeth produces a condition well described clinically as dental mottling.

Dean (1933; 1934), observed a qualitative variation in the distribution of mottled enamel among persons using a common water supply containing fluoride and a quantitative diversity in the incidence among children from different endemic areas. The microscopic structure of mottled teeth was first described by McKay and Black in 1916. They reported varying de-

grees of discolouration of the enamel surface in ground sections of mottled teeth. Later on so many reports have been appeared regarding the microscopic structure of mottled teeth by using different techniques (Ainsworth, 1933; Newbrun, 1957; Awazawa, 1962).

Several studies have been made regarding the effects of fluoride on teeth by using various animals as models. Smith *et al.*, (1931), concluded from their experiments with rats that irrespective of the mode of administration, sodium fluoride influences the developing tooth structure. Dean *et al.* (1934), demonstrated minute striations on the incisor teeth of rats and the severity of these alterations are proportional to the concentration of fluoride in the drinking water. These striations were followed by the formation of irregular brown patches and finally the enamel became white and brittle.

Weber and Yaeger (1964) from their microradiographic studies on the effect of toxic doses of fluoride on the teeth of new born rats, reported a disorganization of ameloblastic layer and disturbance in the pattern of enamel mineralization. Bowes and Murray (1936) reported a higher protein content in fluorised enamel than in non-mottled enamel. This was confirmed by Bhussry (1959), who reported a higher nitrogen content in mottled enamel than in normal enamel. Many investigators argued that there is no appreciable difference in the calcium, phosphorus, magnesium and carbonate content of mottled and non-mottled teeth (Bowes and Murray, 1936; Armstrong and Brekhus, 1937; Ockerse, 1943).

Effects on skeletal system

Shortt *et al.* (1937a, b), first reported from their studies in India, about the chronic effects of fluoride on skeletal system. Later on many reports have come through from various parts of the world about endemic fluorosis, notably Sri Lanka (Clark, 1942), China (Lyth, 1946), South Africa (Ockerse, 1942), Japan (Hamamoto *et al.*, 1954), USA (Leone *et al.*, 1954; Zipkin *et al.*, 1958) and Europe (Odenthal and Wieneke, 1959). The initial symptom noted is

the pain and stiffness of spine. In later stages vertebral column become rigid and the patients develop `pocker back' appearance (Shortt *et al.*, 1937a, b; Lyth, 1946; Krishnamachari and Krishnaswamy, 1973). Accompanying spinal deformity there is stiffness in various joints which ultimately leads to crippling fluorosis with fixed flexion deformities of hip and knees and reduced mobility of thoracic cages resulting in the fixation of chest in the position of inspiration (Singh *et al.*, 1962a, 1963; Jolly, 1981).

The maximum changes are detected in the spine with calcification of various ligaments resulting in marked osteophytes. The vertebral bodies are larger than normal and show marked lipping. The vertebrae show altered proportions and measurements in all the planes but the striking abnormality is the gross reduction of the anteroposterior diameter of the spinal canal. The vertebrae are also fused at many places, a fact which explains the marked limitation of movements. The intervertebral foramina are narrowed and rendered irregular, a finding which explains the presence of radicular manifestations.

The ribs are large with rough surfaces and osteophytes projecting along the attachments of muscles, membranes and ligaments. The other bones, including those of the limbs, the sternum and mandible have many prominent osteophytes at the attachments of ligaments, membranes, tendons and muscular insertions, thus making the various markings and ridges thick and prominent. The interosseous membrane between the tibia and fibula and between the radius and ulna are calcified in variable degree (Singh *et al.*, 1962a).

In addition, a survey of the endemic areas of Andhra Pradesh has revealed the prevalence of `genu valgum', a new form of skeletal fluorosis. The most striking radiological feature of it, includes severe osteoporosis at the lower end of femur and upper ends of tibia and fibula, rarefaction of metacarpal bones along with the generalized osteosclerosis at upper limb bones and axial skeleton (Krishnamachari and Krishnaswamy, 1973). In animals the major ra-

diological symptoms of skeletal fluorosis are increased superperiosteal hyperosteosis, endosteal porosity, narrowing of marrow cavity and all possible combination of these radiological manifestations (Shupe *et al.*, 1963).

There are many reports about the effects of fluoride on the chemical composition of bone. Zipkin *et al.* (1960), recorded that even though fluoride concentration increases in bones after fluoride exposure, there are no changes in calcium and phosphorus. With increasing exposure time/dose they found that there was slight increase in magnesium and decrease in carbonate. The citrate content decreased markedly with increased fluoride. Wollinsky *et al.* (1972) reported from their experiments on rats that, there was a decrease in lipid and citrate content and a decreased glucose utilization and lactate formation. Femurs of fluoride treated rats exhibited a decrease in mechanical strength as manifested by a decrease in ultimate stress to breaking as well as decrease in limit and modulus of elasticity. Hac (1972), reported that rapid, high deposition of fluoride in young rats altered the concentration of total hexosamines and the hexosamines corresponding to the acid glycosaminoglycans precipitable by acetylpyridinium chloride, in the cancellous growing ends of the bones. Prince and Navia (1983), demonstrated that specific alterations of bone glycosaminoglycans result from fluorosis independently of changes in body weight and age.

Effect on soft tissues

Chronic fluoride poisoning is known to cause a variety of pathological manifestations in soft tissues in both animals and man. The fluoride ions have great affinity for calcium (Neuman *et al.*, 1950; Call *et al.*, 1965) and high calcium content in the skeletal muscle makes it more susceptible to the toxic effects of fluoride (Hogen *et al.*, 1965). Fluoride also enhances the permeability of sarcolemma, and certain phosphokinase levels are raised in the blood stream due to the high permeability of the sarcolemma (Kaul and Susheela, 1974). In the rabbit, elec-

tron microscopic studies revealed that all components of muscle fibres including the actin and myosin are affected by fluoride (Kaul *et al.*, 1974). Kaul and Susheela (1974), reported changes in size as well as shape of muscle fibres and deterioration of muscle fibre after feeding 50 mg F^-/kg b.w. to rabbits for 45 days. In patients with skeletal fluorosis Kapila *et al.* (1983), noticed marked degenerative changes in muscle fibres. The sarcoplasm of muscle fibres showed focal areas of necrosis with loss of striation. More or less similar changes in muscles have been observed in fluoride poisoned rabbits (Shashi, 1989). The process of atrophy found in most of the fibres may be attributed to the inhibition of protein synthesis by fluoride (Ravel *et al.*, 1966; Goodchaux and Atwood, 1976). A recent study by Shashi *et al.* (1992) revealed that chronic administration of 20 and 50 mg F^-/kg b.w. significantly reduced protein and amino acids in the muscles of rabbit.

Liver is the major target organ which handles many toxic elements that gain entry into an animal. Chronic fluoride administration is known to affect the structure and function of hepatic tissue. Shaikh (1985) observed necrosis and fatty infiltration in the liver of fish exposed to 80 ppm fluoride for 72 h. Similar changes have also been observed in the liver of regenerating lizards which receive subacute fluoride for 35 days (Suresh, 1989). However, the biochemical response to sublethal dose of fluoride in the liver varies from animal to animal and with the mode of administration (Pillai, 1983; Shaikh and Hiradhar, 1985; Suresh and Hiradhar, 1990b).

It has been known that kidney is the most affected organ in acute fluoride toxicosis (Ogilvie, 1953; Yum *et al.*, 1976; Kessabi *et al.*, 1981). The site of injury in the kidney is the proximal convoluted tubular epithelium. However, the collecting tubules and glomeruli have also been affected but to a lesser extent by fluoride (Takagi and Shiraki, 1982). Lim *et al.* (1975) noticed shrunken glomeruli, focal haemorrhage, swelling and necrosis of the proximal and distal tubules in acute toxicity with stannous fluoride and sodium fluoride. In chronic

fluoride poisoning, the histological features are dilation of Henle's loops in the juxtacortical area of the medulla, soon followed by the flattening of the epithelium in the convoluted tubules in the cortex and the distention of the tubules (Pindborg, 1956, 1957). Electronmicroscopically, Kosek *et al.* (1972), observed an impairment of the mitochondria in the proxiaml tubules of rats given sodium fluoride. Numura(1973), noted an increased vacuolization, swelling of microvilli and rarefaction of the cytoplasmic matrix in the proximal tubular cells given sodium fluoride chronically. Muehlberger (1930) reported albuminuria in rats treated with sodium fluoride.

In acute fluoride toxicity, the most striking changes in humans are also found in the gastrointestinal mucosa (Waldbott, 1963). The pathology includes acute haemorrhagic gastroenteritis, patches of hyperemia, oedema and haemorrhage (Roholm, 1936). Stomach and intestinal disorders are common in acute fluoride toxicity (Czerwinski and Lankosz, 1977). Waldbott (1978) noticed gastrointestinal problems in 45% of fluorosis patients in Sicily. Oral administration of sodium fluoride at a dose of 10 mg/kg b.w. for 24 months cause significant cytomorphologic abnormalities of the duodenal mucosa in rabbits (Susheela and Das, 1988). According to them excess fluoride doses bring about severe acid lesions in the intestinal mucosa. Which may be the possible cause for the gastrointestinal complaints of human subjects afflicted with chronic fluoride toxicity and fluorosis.

The manifestations of the initial phase of fluorosis points to injury of the central nervous system and the spinal cord. In humans, the neurological complications in advanced fluorosis in the form of partial and complete paralysis of arms and legs, headache, vertigo, spasticity in the extremities, visual disturbances and impaired mental acuity have been reported (Waldbott *et al.*, 1978). Cerebral damage has been noticed in fish exposed to fluoridated water (Shaikh, 1985). Metabolic alterations have also been observed in the brain of fluoride poisoned subjects (Shashi, 1992).

A positive correlation between fluoride toxicity and infertility has been known since 1925 (Schulz and Lamb, 1925) and it is now well established that fluoride affects the structure and function of the male reproductive organs. Messer *et al.* (1973), reported impaired reproduction in mice fed 100 and 200 ppm fluoride as sodium fluoride in drinking water. A lack of maturation, differentiation of spermatocytes and loss of spermatogenesis in mice given fluoridated water have also been reported (Kaur and Singh, 1980). Tokar and Savchenko (1977) recorded reduced amounts of testosterone and increased concentration of follicle stimulating hormone in patients with fluorosis. Ridha *et al.* (1978) observed impaired spermatogenesis in mice given 125-500 ppm fluoride in feed. Microscopic studies of testis structure in chicken by Medhi *et al.* (1983) revealed that 600 ppm fluoride in feed impaired the initiation of spermatozoa. Recently through their light and scanning electronmicroscopic study, Susheela and Kumar (1991) argued that oral administration of fluoride (10 mg NaF/kg b.w.) to rabbits for 29 months induces infertility by preventing spermatogenesis.

There have been some studies on the effect of fluoride on endocrine system. Rao and Susheela (1979) observed adrenal dysfunction in rabbits treated with 50 mg NaF/kg b.w. for 200 days. Li *et al.* (1990) reported adrenal hypofunction in rats after long term administration of fluoride. More recently Das and Susheela (1991b) recorded hypocortisolemia in fluorosis patients as well as in fluoride treated animals. Hyperparathyroidism and increased levels of calcitonin have also been recorded in patients with skeletal fluorosis (Teotia *et al.*, 1978; Srivastava *et al.*, 1989).

It is apparent from the aforementioned review that although there are plenty of informations available on the multiferious effects of fluoride on animal structure and function, there is an absolute dearth of information regarding its effect on growth and development of an organism. In recent years analysis of pollutant effects on growth, development and reproduction

have been gaining significance in toxicity studies. Hence it was thought desirable to study the effect of fluoride on a developing organism. An organism like chick would serve as an ideal model for such a study, because being a bird with higher metabolic activity, the manifestations of the effects of the toxicant would be more pronounced in chick. Moreover, fluoride in the form of sodium fluoride, cryolite and sodium fluorosilicate are used as pesticide against ectoparasites in poultry industry (Clarke *et al.*, 1981). Fluoride supplementation through feed is even recommended for increasing the bone strength in coop-reared chickens (Merkley, 1976). Therefore, there are possibilities that the chicks get affected with fluoride toxicity. To assess the extent of damage caused by fluoride to growing chicks, the following investigations were carried out:

1. The acute toxicity of fluoride to postnatal chicks.
2. The effect of fluoride on body growth and development of digestive organs of chicks.
3. Effect of experimental fluoride poisoning on the cellular proliferative and synthetic activities of the post-hatched developing chicks.
4. Fluoride induced alterations in the haemogram of growing chicks.
5. Alterations in the energy metabolism of developing chicks subjected to chronic fluoride poisoning.
6. Fluoride induced changes in glucose transport and uptake mechanism of growing chicks.