

## IMPACT OF FLUORIDE ON PUBLIC HEALTH: AN OVERVIEW

\*B. R. Pandey<sup>1,2</sup>, Geetika Rawat<sup>3,4</sup>

<sup>1</sup>Faculty of Science & Technology, Sai Nath University, Ranchi, Jharkhand, India, <sup>2</sup>Sky Institute, Lucknow, Uttar Pradesh, India, <sup>3</sup>Research Scholar, SaiNath University, Ranchi, Jharkhand India &

<sup>4</sup>Department of Chemistry, Drona College of Management & Technical Education, Dehradun, Uttarakhand, India

**\*Address for Correspondence:** Dr. B. R. Pandey, Dean, Faculty of Science & Technology, Sai Nath University, Ranchi, Jharkhand, India & Director (Research), Sky Institute, Lucknow, Uttar Pradesh, India  
**Email ID :** drbrpandey@gmail.com

### ABSTRACT

Fluoride is the most electronegative and reactive of all elements and items, in nature, are rarely found in its elemental state. Fluorine combines directly at ordinary or elevated temperatures with all elements other than oxygen and nitrogen and therefore reacts vigorously with most organic compounds. Rocks, soil, water, air, plants and animals all contain fluoride in widely varying concentrations. Because of this variation, the sources and their relative importance for human beings also vary. Fluoride is abundant in the environment and the main source of fluoride to humans is drinking water. It has been proved to be beneficial in recommended doses, and at the same time its toxicity at higher levels has also been well established. Fluoride gets accumulated in hard tissues of the body and has been known to play an important role in mineralization of bone and teeth. At high levels, it has been known to cause dental and skeletal fluorosis. There are suggested effects of very high levels of fluoride on various body organs and genetic material. Fluoride enters the body by ingestion and inhalation and in extreme cases of acute exposure, through the skin. Not all the fluoride that is ingested or inhaled is absorbed, and a portion is excreted by various means. The use of fluorine or fluoride containing materials in industry leads not only to an increase in occupational exposure but also, in some cases, to increased general population exposure. More significant than the preceding sources are deposits of high fluoride rocks that in some areas cause a large increase in the fluoride content of water or food. There are many parts of the country where this exposure to fluoride is sufficiently high to cause endemic fluorosis. In some areas of the world e.g. India, Kenya and South Africa, levels of fluoride can be much higher than 25 ppm as per WHO report. Fluoride has beneficial and toxic effects on human beings. With exposure to optimal levels of fluoride in drinking water, there is a clearly demonstrated cariostatic effect. The extent of caries reduction by various methods is influenced by the initial caries prevalence and the standard of health care in the community. Fluoride has been used in the treatment of osteoporosis, though beneficial effects have been reported, the dose-response relationships and efficacy need further clarification. The most important toxic effect of fluoride on human beings is skeletal fluorosis which is endemic in areas with soils, and water containing high fluoride concentrations. In non-endemic areas, skeletal fluorosis has occurred as a result of industrial exposure. This condition, whether of endemic or industrial origin, is normally reversible by reducing fluoride intake. In endemic fluorosis areas, developing teeth exhibit changes ranging from superficial enamel mottling to severe hypoplasia of the enamel and dentine. Patients with kidney dysfunction may be particularly susceptible to fluoride toxicity. Fluoride has been found to be an endocrine disruptor that can affect the bones, brain, thyroid gland, pineal gland and even blood sugar levels. There have been several human and animal studies linking fluoride to brain damage including lower IQ in children and studies have shown that fluoride toxicity can lead to a wide variety of health problems, including thyroid disease, arthritis, dementia, bone fractures, bone cancer, osteosarcoma, genetic damage and cell death, increased tumour and cancer rate, disrupted immune system, damaged sperm and increased infertility etc. The present review focuses on the effects of fluoride on human beings.

**Keywords:** Fluoride; Caries Reduction; Dental Fluorosis; Skeletal Fluorosis; Neurotoxicity; Genotoxicity; Nephrotoxicity; Cancer

## INTRODUCTION

The term fluorine and fluoride are used interchangeably in the literature as generic terms. Fluorine is a corrosive pale yellow gas. Fluoride is the most electronegative and reactive of all elements and items, in nature, are rarely found in its elemental state. It is highly reactive, participating in reactions with virtually all organic and inorganic substances. Fluorine is usually found in soil, air, food and water as fluorides. It accounts for about 0.3 g/kg of the Earth's crust and exists in the form of fluorides in a number of minerals, of which fluorspar, cryolite and fluorapatite are the most common. In industrial settings, fluorine and its compounds are used in producing uranium, plastics, ceramics, pesticides, and pharmaceuticals. Fluorochlorohydrocarbons are used in refrigeration and aerosol propellant applications.<sup>[1]</sup> Fluoride is the simplest anion of fluorine. Its salts and minerals are important chemical reagents and industrial chemicals, mainly used in the production of hydrogen fluoride for fluorocarbons. Hydrogen fluoride is a colorless, pungent liquid or gas with a boiling point of 19.5 °C. It is highly soluble in water, in which it forms hydrofluoric acid. Sodium fluoride is a colorless to white solid that is moderately soluble in water. Fluorosilicic acid, which is also known as hexafluorosilicic acid, is a colorless solid that is highly soluble in water.<sup>[2,3,4]</sup> Inorganic fluorine compounds are used in industry for a wide range of purposes. They are used in aluminium production and as a flux in the steel and glass fiber industries. They can also be released to the environment during the production of phosphate fertilizers (which contain an average of 3.8% fluorine), bricks, tiles and ceramics. Fluorosilicic acid, sodium hexafluorosilicate and sodium fluoride are used in municipal water fluoridation schemes<sup>[2,4]</sup>. Although sodium fluoride is soluble in water<sup>[2]</sup>, aluminium, calcium and magnesium fluorides are only sparingly so<sup>[5]</sup>. Fluoride is determined by means of an ion-selective electrode, which makes it possible to measure the total amount of free and complex-bound fluoride dissolved in water. The method can be used for water containing at least 20 µg/litre.<sup>[3]</sup> For rainwater in which fluoride was present at a concentration of 10 µg/litre, a detection limit of 1 µg/litre was reported.<sup>[6]</sup> A method using a fluoride-selective electrode and an ion analyzer to determine fluoride at levels of 0.05–0.4 mg/litre has been described.<sup>[7]</sup>

Fluoride-containing compounds, such as sodium fluoride or sodium monofluorophosphate are used in topical and systemic fluoride therapy for preventing tooth decay. They are used for water fluoridation and in many products associated with hygiene. Originally, sodium fluoride was used to fluoridate water, hexafluorosilicic acid ( $\text{H}_2\text{SiF}_6$ ) and its salt sodium hexafluorosilicate ( $\text{Na}_2\text{SiF}_6$ ) are more commonly used additives, especially in the United States. Fluoridation of community drinking water to prevent dental caries is considered as one of the ten most important public health achievements of the 20th century.<sup>[8]</sup> Concurrent with the decline in dental caries has been an increase in the prevalence of dental fluorosis, a side effect of fluoride exposure. Dental fluorosis remains highly prevalent worldwide. As recently as 2005, 23% of persons in the United States aged 6 to 39 years had very mild or greater enamel fluorosis.<sup>[9]</sup> Although safe and even healthy at low concentrations, sustained consumption of large amounts of soluble fluoride salts have been found dangerous. The lethal dose of sodium fluoride (NaF) for most adult humans is estimated at 5 to 10 g (which is equivalent to 32 to 64 mg/kg elemental fluoride/kg body weight).<sup>[10,11,4]</sup> Ingestion of fluoride can produce gastrointestinal discomfort at doses at least 15 to 20 times lower (0.2–0.3 mg/kg or 100 to 150 mg for a 50 kg person) than lethal doses.<sup>[12]</sup> Although helpful for dental health in low dosage, chronic exposure to fluoride in large amounts interferes with bone formation. In this way, the most widespread examples of fluoride poisoning arise from consumption of ground water that is abnormally fluoride-rich.<sup>[13]</sup> For optimal dental health, the World Health Organization recommended a level of fluoride from 0.5 to 1.0 mg/L (milligrams per litre), depending on climate.<sup>[14]</sup> Adverse effects become possible at fluoride levels far above this recommended dosage. The United States Health and Human Services Department recommended a maximum of 0.7 milligrams of fluoride per liter of water – the lower limit of the current recommended range of 0.7 to 1.2 milligrams.<sup>[15]</sup> In India an estimated 60 million people have been poisoned by well water contaminated by excessive fluoride, which is dissolved from the granite rocks. The effects are particularly evident in the bone deformations of children. Similar or larger problems are anticipated in other countries

problems are anticipated in other countries including China, Uzbekistan, and Ethiopia.<sup>[13]</sup> Historically, most cases of acute fluoride toxicity have followed accidental ingestion of sodium fluoride based insecticides or rodenticides.<sup>[16]</sup> Currently, in advanced countries, most cases of fluoride exposure are due to the ingestion of dental fluoride products.<sup>[17]</sup> Other sources include glass-etching or chrome-cleaning agents like ammonium bifluoride or hydrofluoric acid,<sup>[18,19]</sup> industrial exposure to fluxes used to promote the flow of a molten metal on a solid surface, volcanic ejecta (for example, in cattle grazing after an 1845–1846 eruption of Hekla and the 1783–1784 flood basalt eruption of Laki), and metal cleaners. Malfunction of water fluoridation equipment has happened several times, including a notable incident in Alaska<sup>[12]</sup> Twenty percent of modern pharmaceuticals contain fluorine<sup>[20]</sup>. These organofluorine compounds are not sources of fluoride poisoning. The carbon-fluorine bond is too strong to release fluoride.

A number of potential and established adverse effects including cognitive impairment, hypothyroidism, dental and skeletal fluorosis, enzyme and electrolyte derangement and cancer have been reported.<sup>[21]</sup> Children may experience gastrointestinal distress upon ingesting excessive amounts of flavoured toothpaste. Between 1990 and 1994, over 628 people, mostly children, were treated after ingesting too much fluoride-containing toothpaste. While the outcomes were generally not serious, gastrointestinal symptoms appear to be the most common problem reported.<sup>[22]</sup> Several studies have shown that high levels of fluoride exposure may lead to adverse effects on brain<sup>[23]</sup>, bones leading to skeletal fluorosis<sup>[24-26]</sup>, kidney,<sup>[27-32]</sup> teeth leading to dental fluorosis<sup>[24]</sup>, thyroid<sup>[25,33]</sup> and also produce adverse effects on aquatic organisms.<sup>[34,35]</sup> The present review focuses on adverse effects of fluoride on human health.

## CHEMOBIOKINETICS AND METABOLISM

It has been reported that a large proportion of the ingested and inhaled fluorides is rapidly absorbed through the gastrointestinal tract and through the lungs, respectively. Absorbed fluoride is carried by the blood and is excreted via the renal system or taken up the calcified tissues. Most of the fluoride

bound in the skeleton and teeth has a biological half-life of several years. The concentration of fluoride in the calcified tissues is a function of exposure and age. No significant accumulation occurs in the soft tissues. Renal excretion appears to be based on glomerular filtration followed by a variable tubular reabsorption, which is higher at low pH and low urinary flow rates. Fluoride passes through the placenta and occurs in low concentrations in saliva, sweat and milk.<sup>[36-38]</sup> It has been documented that the ingestion of optimally fluoridated water does not have an adverse effect on bone.<sup>[39-43]</sup> The kidneys play the major role in the removal of fluoride from the body. Normally kidneys are very efficient and excrete fluoride very rapidly. In case of persons with severely impaired renal function, who may not be on renal dialysis, decreased fluoride removal may occur. No cases of dental fluorosis or symptomatic skeletal fluorosis have been reported among persons with impaired renal function; however, the overall health significance of reduced fluoride removal is uncertain and continued follow-up is recommended especially for children with impaired renal function.<sup>[44]</sup> Urine tests have been used to ascertain rates of excretion in order to set upper limits in exposure to fluoride compounds and associated detrimental health effects. Ingested fluoride initially acts locally on the intestinal mucosa, where it forms hydrofluoric acid in the stomach. Scientific studies have shown that untoward effects of fluoride may be due to the formation of aluminium fluoride complexes.<sup>[25,45,33,46,47]</sup>

## EFFECTS ON HUMAN BEING

### Beneficial Effects

With exposure of optimal levels of fluoride in the drinking water (0.7-1.5 mg/liter, depending on climatic conditions), there is a clearly demonstrated cariostatic effect. The extent of caries reduction by various methods is influenced by the initial caries prevalence and the standard of health care in the community. Fluoridation of community drinking water to prevent dental caries is considered as one of the ten most important public health achievements of the 20th century.<sup>[48]</sup> Fluoride has been used in the treatment of osteoporosis, though beneficial effects have been reported, the dose-dependent relationships and efficacy need further clarification.

### **Toxic Effects**

Scientific studies have shown that prolonged use of fluoride at recommended levels does not produce any harmful physiological effects in the human. Harmful effects in humans can occur with the ingestion of fluoride beyond the safe limits. These toxic effects may be either acute or chronic.

### **Acute toxicity**

Acute toxicity occurs due to single ingestion of a large amount of fluoride. Ingestion of an acute fatal dose of fluoride is very rare. The amount of fluoride considered lethal when taken orally is 35-70 mg F per kg body weight. This is equivalent to 5-10 g sodium fluoride for a 70-kg adult and 1-2 g sodium fluoride for a 15-kg child.<sup>[49]</sup> Symptoms of acute toxicity occur rapidly. Some of the symptoms of acute toxicity are diffuse abdominal pain, diarrhea, vomiting, excess salivation, and thirst. Rapid measures should be taken to reduce fluoride absorption.<sup>[36]</sup>

As is evident from earlier studies, most cases of acute fluoride toxicity have followed accidental ingestion of sodium fluoride based insecticides or rodenticides.<sup>[16]</sup> Currently, in advanced countries, most cases of fluoride exposure are due to the ingestion of dental fluoride products.<sup>[17]</sup> Other sources include glass-etching or chrome-cleaning agents like ammonium bifluoride or hydrofluoric acid,<sup>[18,19]</sup> industrial exposure to fluxes used to promote the flow of a molten metal on a solid surface, volcanic ejecta and metal cleaners. Malfunction of water fluoridation equipment has happened several times, including a notable incident in Alaska.<sup>[12]</sup>

### **Chronic toxicity**

Long-term ingestion of smaller amounts of fluoride in drinking water may cause chronic toxicity. Excessive fluoride more than 8 ppm in drinking water daily for many years can lead to skeletal fluorosis. Severe cases are normally found only in warm climates where drinking water contains very high levels of fluoride. Due to chronic toxicity, bone density slowly increases; the joints stiffen and becomes painful. At higher levels of ingestion from 2 to 8 mg daily, skeletal fluorosis may arise. Whereas dental fluorosis is easily recognized, the skeletal involvement is not clinically obvious until the advanced stage and early cases may be

misdiagnosed as rheumatoid or osteoarthritis. In India an estimated 60 million people have been poisoned by well water contaminated by excessive fluoride, which is dissolved from the granite rocks. The effects are particularly evident in the bone deformations of children. Similar or larger problems are anticipated in other countries including China, Uzbekistan, and Ethiopia.<sup>[13]</sup>

Fluoride has been found to be an endocrine disruptor that can affect the bones, brain, thyroid gland, pineal gland and even blood sugar levels. There have been several human and animal studies linking fluoride to brain damage including lower IQ in children and studies have shown that fluoride toxicity can lead to a wide variety of health problems, including thyroid disease, arthritis, dementia, bone fractures, bone cancer, osteosarcoma, genetic damage and cell death, increased tumour and cancer rate, disrupted immune system, damaged sperm and increased infertility etc.

### **Skeletal Fluorosis**

The most important toxic effect of fluoride on human being is skeletal fluorosis, which is endemic in areas with soils and water containing high fluoride concentrations. The sources of fluoride that contribute to the total human intake vary geographically between endemic fluorosis areas, but the symptoms are generally similar. They range from skeletal histological changes, through increases in bone density, bone morphometric changes and exostoses to crippling skeletal fluorosis. This condition is usually restricted to tropical and subtropical areas and is frequently complicated by factors such as calcium deficiency or malnutrition. In non-endemic areas, skeletal fluorosis has occurred as a result of industrial exposure. This condition, whether of endemic or industrial origin, is normally reversible by reducing fluoride intake. High systemic fluoride exposures can lead to skeletal fluorosis, a condition hallmarked by osteosclerosis, ligament calcifications, and often accompanying osteoporosis, osteomalacia, or osteopenia.<sup>[50,51]</sup> Skeletal fluorosis can be complicated by malnutrition.<sup>[52]</sup> Early symptoms of skeletal fluorosis include sporadic and stiffness of joints, headache, stomach-ache muscle weakness. The next stage is osteosclerosis (hardening and calcifying of the bones) and finally

the spine, major joints, muscles and nervous system are damaged. Bone changes observed in human skeletal fluorosis are structural and functional, with a combination of osteosclerosis, osteomalacia, osteoporosis and exostosis formation and secondary hyperpara-thyroidism in a proportion of patients. At very high fluoride concentrations, stages 2 and 3 of skeletal fluorosis are likely to occur. The clinical signs of these stages are chronic joint pain, dose related calcification of ligaments, osteosclerosis, possible osteoporosis of long bones and in severe cases, muscle wasting and neurological defects. Because some of the clinical symptoms mimic arthritis, the first two clinical phases of skeletal fluorosis could be easily misdiagnosed. Established clinical complications of skeletal fluorosis include arthritis, radiculomyelopathy, quadriplegia and pathological bone fractures<sup>[53,54]</sup>.

### Dental Fluorosis

Concurrent with the decline in dental caries has been an increase in the prevalence of dental fluorosis, a side-effect of fluoride exposure. Dental fluorosis, which is characterized by discoloured, blackened, mottled or chalky white teeth, is a clear indication of over exposure to fluoride during childhood when teeth were developing. Discolouration is always horizontally aligned on the enamel surface, discolouration shall be away from the gums, and the discolouration shall occur in teeth in pairs. These effects are not apparent if the teeth were already fully grown prior to the fluoride exposure, therefore, the fact that an adult may show no signs of dental fluorosis does not necessarily mean that his or her fluoride intake is within the safe limits. Dental fluorosis remains highly prevalent worldwide. As recently as 2005, 23% of persons in the United States aged 6 to 39 years had very mild or greater enamel fluorosis.<sup>[9]</sup> Fluorosis is a toxic manifestation of chronic (low-dose, long-term) fluoride intake. To prevent fluorosis from occurring in the most prominent and/or most susceptible teeth, the most critical time to avoid fluoride exposure is the first three to six years of a child's life. Effect of fluoride on enamel formation can follow several pathogenic pathways including effect on ameloblasts, effect on nucleation and crystal growth in all stages of enamel formation and effect on calcium

homeostasis generally with dental fluorosis as an indirect result.<sup>[55]</sup>

### Neurotoxicity

According to studies of National Research Council (NRC 2006) adverse effects of high fluoride concentrations in drinking water may be of concern and ingestion of high fluoride concentrations may damage the brain as fluorides have the ability to interfere with the functions of brain.<sup>[56]</sup> The results of experimental studies conducted on animal models have shown that fluoride accumulates in the brain and alters mental behaviour including effects on learning and memory in a manner consistent with a neurotoxic agent.<sup>[57, 58]</sup> Besides these studies, there have been number of animal experiments showing that fluoride can damage the brain and impact learning and behaviour. The results of an experimental study conducted on animal model where the rat hippocampal neurons were incubated with various concentrations (20 mg/L, 40 mg/L, and 80 mg/L) of sodium fluoride *in vitro* have shown that fluoride neurotoxicity may target hippocampal neurons.<sup>[59]</sup> Although acute fluoride poisoning may be neurotoxic to adults, most of the epidemiological information available on associations with children's neurodevelopment is from China, where fluoride generally occurs in drinking water as a natural contaminant, and the concentration depends on local geological conditions. In many rural communities in China, populations with high exposure to fluoride in local drinking-water sources may reside in close proximity to populations without high exposure.<sup>[56]</sup> Fluoride readily crosses the placenta.<sup>[60]</sup> Fluoride exposure to the developing brain, which is much more susceptible to injury caused by toxicants than is the mature brain, may possibly lead to permanent damage.<sup>[61]</sup>

Research findings have demonstrated that rats generally require five times more fluoride to reach the same plasma levels in humans.<sup>[62]</sup> further, one animal experiment found effects at remarkably low doses.<sup>[63]</sup> In this study, rats fed for one year with 1 ppm fluoride in their water (the same level used in fluoridation programs), using either sodium fluoride or aluminium fluoride, had morphological changes to their kidneys and brains, an increased uptake of aluminum in the brain, and the formation of beta-amyloid deposits which are associated with

Alzheimer's disease. Other animal studies have found effects on the brain at water fluoride levels as low as 5 ppm.<sup>[64]</sup> The impact of fluoride on intelligence has been reported by a number of studies. Fluoride exposure may lower IQ as evidenced by several studies from China, Iran, India and Mexico. These studies have reported an association between fluoride exposure and reduced IQ. One of these studies, Lin et al. 1991 indicates that even just moderate levels of fluoride exposure (e.g., 0.9 ppm in the water) can exacerbate the neurological defects of iodine deficiency.<sup>[65]</sup> The results of other studies have shown IQ reductions at different levels of fluoride exposure ranging from 0.3ppm to 4.12 ppm.<sup>[66-80,87,88]</sup>

A study was conducted to assess the relationship between exposure to different drinking water fluoride levels and children's intelligence in Madhya Pradesh state, India, which indicates that exposure to fluoride, is associated with reduced intelligence in children. The study has shown a significant inverse relationship between intelligence and the water fluoride level, and intelligence and the urinary fluoride level and clearly reflects that children exposed to fluoride are at risk for impaired development of intelligence.<sup>[78]</sup>

In addition to above studies, other studies also confirm that fluoride has negative impacts on intelligence.<sup>[79-85]</sup> In a meta-analysis Tang *et al.* concluded that children who lived in an endemic fluoride area had five times higher odds of developing low IQ than those who lived in a non-fluoride area.<sup>[85]</sup> Research studies have demonstrated that fluoride can penetrate the blood brain barrier.<sup>[80-83,85,86]</sup> Also, it can pass through the placenta to the fetus,<sup>[80-83,85,87]</sup> and with subsequent continuous exposure to fluoride during childhood, it may have adverse effects on the developing brain, thereby causing decreased intelligence in children.<sup>[80-83,85]</sup>

It has been observed that fluoride may cause a reduction in the cholinesterase activity in the brain leading to altered utilization of acetylcholine thereby affecting the transmission of nerve impulses in the brain tissue.<sup>[88-90]</sup> NaF has been found to alter the levels of dopamine, serotonin, 5-hydroxyindoleacetic acid, homo-vanille acid, norepinephrine, and epinephrine, in the hippocampus and neocortex regions of the rat brain.<sup>[91]</sup> Yu *et al.* demonstrated changes in neurotransmitters and their receptors in the fetal

brain from the endemic fluorosis area.<sup>[81]</sup> Thyroid hormones play an important role in the development of the brain. In a study, Susheela *et al.* found that elevated fluoride uptake may cause iodine deficiency in fluorotic individuals, even when they reside in non-iodine deficient areas.<sup>[92]</sup> Choi *et al.* have reviewed different studies related to effect of fluoride on children's neurodevelopment and concluded that children in high-fluoride areas had significantly lower IQ scores than those who lived in low-fluoride areas and emphasized that fluoride's impact on the developing brain should be a "high research priority."<sup>[93]</sup>

Research studies on human subjects have revealed an association between fluoride exposure and impaired visual-spatial organization.<sup>[94-96]</sup> Further, an association between prenatal fluoride exposure and 'fetal brain damage' has also been investigated.<sup>[97-100]</sup>

### Genotoxicity

The genotoxic effects of fluoride have been observed in several experimental studies using mice as animal model.<sup>[101]</sup> However these experimental studies could not provide evidence of effect of fluoride on chromosomes in bone marrow or sperm cells even at fluoride levels 100 times higher than that in fluoridated water.<sup>[102-108]</sup>

The results of another study to investigate genotoxic effects of fluoride in hamster bone marrow cells and cultured hamster ovarian cells have shown that fluoride may not cause chromosomal damage thereby indicating that fluoride may not be a genetic hazard.<sup>[109]</sup> Other research studies have also shown that fluoride has not caused genetic mutations in the most widely used bacterial mutagenesis assay (the Ames test) over a wide range of fluoride levels.<sup>[110-112]</sup> In addition to these studies, there is no sufficient scientific basis to confirm that ingestion of fluoride at levels found in community water fluoridation (0.7-1.2 ppm) would have adverse effects on human reproduction.<sup>[101]</sup> In a recent study, the effects of fluoride on formation of teeth and bones and the influence of genetics have been described and it has been argued that the interaction of an individual's genetic background has offered new insight into fluoride's physiological effects. Fluoride has been shown to induce osteoclastogenesis in mice. Fluorides appear to mediate their

actions through the MAPK (Mitogen-activated protein kinase) signaling pathway and can lead to changes in gene expression, cell stress, and cell death. Different strains of inbred mice demonstrate differential physiological responses to ingested fluoride. Genetic studies in mice are capable of identifying and characterizing fluoride-responsive genetic variations.<sup>[113-121]</sup>

## OTHERS

It has been observed that fluoride accumulates in the human pineal gland to very high level.<sup>[122]</sup> Research studies on animal model have also revealed that fluoride reduces melatonin production leading to an earlier onset of puberty.<sup>[123]</sup> In one of the fluoridation trials in the U.S.,<sup>[124]</sup> it has been found that on average young girls in the fluoridated community reached menstruation 5 months earlier than girls in the non-fluoridated community. Fluoride has been found to affect thyroid function. In one of the studies, the results have shown a lowering of thyroid function, among otherwise healthy people, at 2.3 ppm fluoride in water.<sup>[125]</sup> Other studies have also revealed that fluoride exposure at higher levels has been associated with thyroid changes.<sup>[126-130]</sup> Fluoride toxicity at high levels has been associated with impaired kidney function and even urolithiasis.<sup>[131,132]</sup> Fluoride may cause cancer. Several population – based studies have been reported indicating the potential link between water fluoride levels and cancer. These studies have shown that chronic fluoride ingestion is a possible cause of uterine cancer and bladder cancer and there may be a link with osteosarcoma.<sup>[133-136]</sup> Research studies have shown that fluoride is an enzyme disruptor. There are 66 enzymes which are affected by fluoride ingestion, including P450 oxidases, as well the enzyme which facilitates the formation of flexible enamel.<sup>[137-144]</sup> Further, scientific studies have proved the association of chronic fluoride ingestion with hyperkalaemia and consequent ventricular fibrillation.<sup>[145]</sup>

High fluoride levels may cause reproductive problems as evidenced from the animal studies which showed that fluoride administered to male animals of different species at high doses produced disorders on male reproductive systems by damaging sperm and increasing the rate of infertility species.<sup>[146-156]</sup> further, several

epidemiological studies from USA, China and India have shown that exposure of males and females to high levels of fluoride caused different reproductive disorders viz. Increased rates of infertility among couples, reduced level of circulating testosterone in males, disruption of reproductive hormones in men etc.<sup>[69,157,159-168]</sup>

## DISCUSSION

Fluoride, the ionic form of fluorine, is a natural component of the biosphere and 13th most abundant element in the crust of the earth. It is, therefore, found in a wide range of concentrations in virtually all inanimate and living things. Fluorides are naturally present in the soil, rocks, and water throughout the world, with higher concentrations in areas where there have been recent/past pyroclastic activities or geologic uplift. Fluorides are also widely used in many industrial processes. The major sources of systemic fluoride exposure are the diet (food and water) and fluoride-containing dental products.

Fluoride is known to have a protective effect against tooth decay by preventing demineralization of tooth enamel during attack by acid-producing plaque bacteria. In infants and young children with pre-erupted teeth, ingested fluoride is incorporated into the developing enamel, making the teeth more resistant to decay. Drinking fluoridated water or brushing teeth with fluoride toothpaste raises the concentration of fluoride in saliva and plaque fluid, which reduces the rate of enamel demineralization during the caries process and promotes the remineralization of early caries lesions. When ingested in water, fluoride is absorbed and secreted back into saliva, where it can again act to inhibit enamel demineralization. A constant, low-level of fluoride in the mouth has been shown to combat the effects of plaque bacteria, which are fuelled by dietary sugars. Drinking fluoridated water accomplishes this through both topical and systemic actions.

Based on extensive research, the U.S. Public Health Service (USPHS, 1986) established the optimum concentration for fluoride in the water in the United States in the range of 0.7-1.2 ppm. This range effectively reduces tooth decay while minimizing the occurrence of dental fluorosis. The optimum level is dependent on the annual average of the maximum daily air temperature in the c

geographic area.<sup>[169]</sup> Public health authorities worldwide agree that community water fluoridation (CWF) is the most effective public health measure to reduce the burden of dental caries, reducing both its prevalence within a population and its severity in individuals who are affected. With a history dating back to the 1940s in the US, CWF is now practiced in over 30 countries around the world, providing over 370 million people with optimally fluoridated water. Epidemiological evidence of its efficacy and safety has been accumulating for over six decades. The fluoride concentrations recommended for CWF have been set based on data from both animal toxicology studies and human epidemiological studies to provide a daily oral exposure that confers maximum

Benefit without appreciable risk of adverse effects. Naturally occurring concentrations of fluoride in water in some parts of the world (e.g. parts of China, Africa, and India) are much higher than those found in fluoridated water, and in some of these regions high fluoride intakes are known to cause problems in teeth and bones (dental and skeletal fluorosis).

With exposure of optimal levels of fluoride in the drinking water (0.7-1.5 mg/litre, depending on climatic conditions), there is a clearly demonstrated cariostatic effect. The extent of caries reduction by various methods is influenced by the initial caries prevalence and the standard of health care in the community. Fluoridation of community drinking water to prevent dental caries is considered as one of the ten most important public health achievements of the 20th century.<sup>[48]</sup>

Fluoride has been used in the treatment of osteoporosis, though beneficial effects have been reported, the dose-dependent relationships and efficacy need further clarification.

The effectiveness of fluoride is defended on two grounds: (1) Fluoride inhibits enzymes that breed acid producing oral bacteria whose acids eat away tooth enamel. Contrary to it, some scientists now believe that the harmful effect of high level of fluoride on other useful enzymes outweighs the beneficial effect on caries prevention (2) Fluoride ions bind with calcium ions, strengthening tooth enamel as it forms in children. But some researchers now believe that excessive fluoride intake leads to loss of calcium from the tooth

matrix, aggravating cavity formation throughout life rather than remedying it and so causing dental fluorosis. Severe, chronic and cumulative oral exposure can cause the incurable crippling of skeletal fluorosis.

Excessive systemic exposure to fluorides can lead to disturbances of bone homeostasis (skeletal fluorosis) and enamel development (dental/enamel fluorosis). The severity of dental fluorosis is also dependent upon fluoride dose and the timing and duration of fluoride exposure. Fluoride's actions on bone cells predominate as anabolic effects both *in vitro* and *in vivo*. More recently, fluoride has been shown to induce osteoclastogenesis in mice. Fluorides appear to mediate their actions through the MAPK signaling pathway and can lead to changes in gene expression, cell stress, and cell death.

The latest information shows that fluorosis is endemic in at least 25 countries across the globe. As far as Indian scenario is concerned, fluoride level in ground water varies substantially in different regions. High concentrations of fluoride (>1.5 mg/l) have been reported in the states of Haryana, Delhi, Rajasthan, Karnataka, Uttar Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Kerala, Jammu and Kashmir, Punjab, Orissa, Himachal Pradesh, and Bihar.

<sup>[170]</sup> In India an estimated 60 million people have been poisoned by well water contaminated by excessive fluoride, which is dissolved from the granite rocks. The effects are particularly evident in the bone deformations of children. Similar or larger problems are anticipated in other countries including China, Uzbekistan, and Ethiopia.<sup>[13]</sup> Fluoride has a potential to cause major adverse human health problems, while having only a modest dental caries prevention effect. A number of potential and established adverse effects including cognitive impairment, hypothyroidism, dental and skeletal fluorosis, enzyme and electrolyte derangement and cancer have been reported.<sup>[21]</sup> Children may experience gastrointestinal distress upon ingesting excessive amounts of flavoured toothpaste. Between 1990 and 1994, over 628 people, mostly children, were treated after ingesting too much fluoride-containing toothpaste. While the outcomes were generally not serious, gastrointestinal symptoms appear to be the most common problem reported.<sup>[22]</sup>

Several studies have shown that high levels of fluoride exposure may lead to adverse effects on brain<sup>[23]</sup>, bones leading to skeletal fluorosis<sup>[24-26]</sup>, kidney,<sup>[27-32]</sup> teeth leading to dental fluorosis<sup>[24]</sup>, thyroid<sup>[25,33]</sup>. Prolonged or high exposure to fluoride has been found to be an endocrine disruptor that can affect the bones, brain, thyroid gland, pineal gland and even blood sugar levels. There have been several human and animal studies linking fluoride at higher doses to brain damage including lower IQ in children and studies have shown that fluoride toxicity can lead to a wide variety of health problems, including thyroid disease, arthritis, dementia, bone fractures, osteosarcoma (bone cancer), genetic damage and cell death, increased tumour and cancer rate, disrupted immune system, damaged sperm and increased infertility etc.

Fluoride is not considered to be essential for human growth and development but it is considered to be beneficial in the prevention of dental caries (tooth decay). As a result, intentional fluoridation of drinking water and the development of fluoride containing oral care products (toothpastes and mouth rinses), foods (fluoridated salt) and supplements (fluoride tablets) have been employed since the early 20th century in several parts of the world as a public health protective measure against tooth decay. Additional exposure to fluoride comes from naturally occurring water (tap and mineral) beverages, food, and to a lesser extent from other environmental sources. Individual and population exposures to fluoride vary considerably and depend on the high variability in the levels of fluoride found in tap (be it natural or the result of intentional fluoridation of drinking water) and mineral waters and on individual dietary and oral hygiene habits and practices. The emerging picture from all risk assessments conducted on fluoride is that there 'exists a narrow margin between the recommended intakes for the prevention of dental carries and the upper limits of exposure'. Invariably, all assessments to date call for continued monitoring of the exposure of humans to fluoride from all sources and an evaluation of new scientific developments on its hazard profile. Millions of children around the world are exposed to high concentration of fluoride in water and are therefore, potentially at risk. For the benefit of the

upcoming generations, urgent attention needs to be focused on this substantial public health problem. Further, efforts are warranted to reduce unethical discharge of fluoride compounds into the environment. Measures should be taken to use fluoride to our advantage in achieving optimal health. Multidisciplinary scientific research at molecular level on animal models and also at-risk human populations who are susceptible to the unwanted or potentially adverse effects of fluoride action using latest biotechnological approach is need of the day in order to understand molecular basis of fluoride action and to develop preventive measures to safe guard the public health.

## ACKNOWLEDGEMENTS

The authors are thankful to Shri Sanjay Dixit, Scientist, Sky Institute, Lucknow, Uttar Pradesh, India for extending his help in preparing the manuscript.

## REFERENCES

1. United States Environmental Protection Agency, "Fluoride," Report to Congress section 112 (n) (16), Clean Air Act, Washington, DC, USA, 2000.
2. IARC (1982) Some aromatic amines, anthraquinones and nitroso compounds, and inorganic fluorides used in drinking-water and dental preparations. Lyon, International Agency for Research on Cancer, pp. 237–303 (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 27).
3. Slooff W et al., eds. (1988) Basisdocument fluoriden. Bilthoven, Netherlands, National Institute of Public Health and Environmental Protection (Report No. 758474005).
4. IPCS (2002). *Environmental health criteria 227 (Fluoride)*. Geneva: *International Programme on Chemical Safety, World Health Organization*. p. 100. ISBN 92-4-157227-2
5. US EPA (1985a) Drinking water criteria document on fluoride. Washington, DC, US Environmental Protection Agency, Office of Drinking Water (TR-823-5).
6. Barnard WR, Nordstrom DK (1982) Fluoride in precipitation. i. Methodology with fluoride-selective electrode. Atmospheric

- Environment, 16:99.
7. Liu JW et al. (1987) Measurement of low level fluoride in water and water-based products using a fluoride electrode and an ion analyzer with automatic calibration program. *Journal of Micronutrient Analysis*, 3:295–305.
8. Achievements in Public Health, 1900-1999: Fluoridation of Drinking Water to Prevent Dental Caries, 1999.
9. Beltran-Aguilar ED, Barker LK, Canto MT, Dye BA, Gooch BF, Griffin SO, et al. (2005). Surveillance for dental caries, dental sealants, tooth retention, edentulism, and enamel fluorosis—United States, 1988-1994 and 1999-2002. *MMWR Surveill Summ* 54:1-43.
10. Gosselin, RE; Smith RP; Hodge HC (1984). *Clinical toxicology of commercial products*. Baltimore (MD): Williams & Wilkins. pp. III–185–93. ISBN 0-683-03632-7.
11. Baselt, RC (2008). *Disposition of toxic drugs and chemicals in man*. Foster City (CA): Biomedical Publications. pp. 636–40. ISBN 978-0-9626523-7-0.
12. Bradford D. Gessner; Michael Beller; John P. Middaugh; Gary M. Whitford (13 January 1994). "Acute fluoride poisoning from a public water system". *New England Journal of Medicine* 330 (2): 95–99. doi:10.1056/NEJM199401133300203. PMID 8259189
13. Pearce, Fred (2006). *When the Rivers Run Dry: Journeys Into the Heart of the World's Water Crisis*. Toronto: Key Porter. ISBN 978-1-55263-741-8
14. WHO Expert Committee on Oral Health Status and Fluoride Use. Fluorides and oral health 1994.
15. <http://www.reuters.com/article/2011/01/08/us-usa-fluoride-idUSTRE7064CM20110108>
16. Nochimson G. (2008). Toxicity, Fluoride. *e Medicine*. Retrieved 2008-12-28 .
17. Augenstein WL, Spoerke DG, Kulig KW, et al. (November 1991). "Fluoride ingestion in children: a review of 87 cases". *Pediatrics* 88 (5): 907–12. PMID 1945630 .
18. Wu ML, Deng JF, Fan JS (November 2010). "Survival after hypocalcemia, hypomagnesemia, hypokalemia and cardiac arrest following mild hydrofluoric acid burn." *Clinical Toxicology (Philadelphia, Pa.)* 48 (9): 953–5. doi:10.3109/15563650.2010.533676. PMID 21171855.
19. Klasaer AE, Scalzo AJ, Blume C, Johnson P, Thompson MW (December 1996). "Marked hypocalcemia and ventricular fibrillation in two pediatric patients exposed to a fluoride-containing wheel cleaner." *Annals of Emergency Medicine* 28 (6): 713–8. doi:10.1016/S0196-0644(96)70097-5. PMID 8953969.
20. Emsley 2011, p. 178.
21. National Research Council (NRC), Fluoride in Drinking Water : A Scientific Review of EPA's Standards, National Academies Press, Washington, DC, USA, 2006.
22. Jay D. Shulman; Linda M. Wells (1997). "Acute Fluoride Toxicity from Ingesting Home-use Dental Products in Children, Birth to 6 Years of Age." *Journal of Public Health Dentistry* 57 (3): 150–158. doi:10.1111/j.1752-7325.1997.tb02966.x. PMID 9383753
23. Choi AL, Sun G, Zhang Y, Grandjean P (2012). "Developmental fluoride neurotoxicity: a systematic review and meta-analysis". *Environ. Health Perspect. (Systematic review & Meta-analysis)* 120 (10): 1362–8. doi:10.1289/ehp.1104912. PMC 3491930. PMID 22820538
24. McDonagh, Marian S.; Whiting, Penny F; Wilson, Paul M.; et al. (7 October 2000). "Systematic review of water fluoridation". *BMJ* 321 (7265): 855–859. doi:10.1136/bmj.321.7265.855. PMC 27492. PMID 11021861.
25. National Research Council (2006). Fluoride in Drinking Water: A Scientific Review of EPA's Standards. Washington, DC: National Academies Press. ISBN 0-309-10128-X. Lay summary (PDF) – NRC (September 24, 2008)
26. Gupta R, Kumar AN, Bandhu S, Gupta S (2007). "Skeletal fluorosis mimicking seronegative arthritis." *Scand. J. Rheumatol.* 36 (2): 154–5. doi:10.1080/03009740600759845. PMID 17476625
27. Cousins MJ, Skowronski G, Plummer JL. Anaesthesia and the kidney. *Anaesth Intensive Care*. 1983 Nov;11(4):292-320.
28. Baden JM, Rice SA, Mazze RI. Deuterated methoxyflurane anesthesia and renal function

- in Fischer 344 rats. *Anesthesiology*. 1982 Mar;56(3):203-6.
29. Mazze RI. Methoxyflurane nephropathy. *Environ Health Perspect*. 1976 Jun;15:111-9.
30. Cousins MJ, Greenstein LR, Hitt BA, Mazze RI. Metabolism and renal effect of enflurane in men. *Anesthesiology* 1976; 44:44-53.
31. VanDyke R. Biotransformation of volatile anesthetics with special emphasis on the role of metabolism in the toxicity of anesthetics. *Can Anaesth Soc J* 1973; 20:21-33.
32. White AE, Stevens WC, Eger EI II, Mazze RI, Hitt BA. Enflurane and methoxyflurane metabolism at anesthetic and subanesthetic concentrations. *Anesth Analg* 1979;58:221-4.
33. Strunecká A, Strunecký O, Patocka J (2002). "Fluoride plus aluminum: useful tools in laboratory investigations, but messengers of false information" (PDF) *Physiol Res* 51(6): 557–64. PMID 12511178
34. Camargo, Julio A. (January 2003). *Fluoride toxicity to aquatic organisms: a review* "Chemosphere 50 (3): 251–264. doi:10.1016/S0045-6535(02)00498-8.
35. Joseph A. Cotruvo. "Desalination Guidelines Development for Drinking Water: Background" (PDF). Retrieved January 26, 2015 .
36. Whitford GM. The metabolism and toxicity of fluoride. 2nd rev. ed. Monographs in oral science. Vol. 16. Basel, Switzerland: Karger; 1996.
37. Whitford GM. The physiological and toxicological characteristics of fluoride. *J Dent Res* 1990;69:539-49.
38. Whitford GM. Intake and metabolism of fluoride. *Adv Dent Res* 1994; 8:5-14.
39. Cauley JA, Murphy PA, Riley TJ, Buhari AM. Effects of fluoridated drinking water on bone mass and fractures: The study of osteoporotic fractures. *J Bone Min Res* 1995;10:1076-86.
40. Gordon SL, Corbin SB. Summary of workshop on drinking water fluoridation influence on hip fracture on bone health. (National Institutes of Health, 10 April, 1991) *Osteoporos Int* 1992;2:109-17.
41. Jacobsen SJ, O.Fallon WM, Melton LJ 3rd. Hip fracture incidence before and after the fluoridation of the public water supply, Rochester, Minnesota. *Am J Public Health* 1993;83:743-5.
42. Karagas MR, Baron JA, Barrett JA, Jacobsen SJ. Patterns of fracture among the United States elderly: Geographic and fluoride effects. *Ann Epidemiol* 1996;6:209-16.
43. Suarez-Almazor ME, Flowerdew G, Saunders LD, Soskolne CL, Russell AS. The fluoridation of drinking water and hip fracture hospitalization rates in two Canadian communities. *Am J Public Health* 1993;83:689-93.
44. US Department of Health and Human Services, Public Health Service. Review of fluoride: Benefits and risks. Washington, DC; Report of the Ad Hoc Subcommittee on Fluoride; 1991.
45. Baez, J.; Baez, Martha X.; Marthaler, Thomas M. (2000). "Urinary fluoride excretion by children 46 years old in a south Texas community". *Revista Panamericana de Salud Pública/Pan American Journal of Public Health* 7 (4): 242–248. doi:10.1590/s1020-49892000000400005.
46. Li L (2003). *The biochemistry and physiology of metallic fluoride: action, mechanism, and implications*. "Crit. Rev. Oral Biol. Med. 14 (2): 100–14. doi:10.1177/154411130301400204. PMID 12764073.
47. Chiba J, Kusumoto M, Shirai S, Ikawa K, Sakamoto S (March 2002). *The influence of fluoride ingestion on urinary aluminum excretion in humans*. "Tohoku J. Exp. Med. 196 (3): 139–. 49. doi:10.1620/tjem.196.139. PMID 12002270.
48. Centers for Disease Control and Prevention. 1999. Achievements in public health, 1990–1999: fluoridation of drinking water to prevent dental caries. *MMWR* 48(41):933–940.
49. Mellberg JR, Ripa LW. Fluoride metabolism. *Fluorides in Preventive Dentistry-Theory and clinical Applications*. Quintessence Publishing Co Limited; 1983. p. 81-102.
50. Christie DP (1980). The spectrum of radiographic bone changes in children with fluorosis. *Radiology* 136:85-90.
51. Wang W, Kong L, Zhao H, Dong R, Li J, Jia Z, et al. (2007). Thoracic ossification of ligamentum flavum caused by skeletal fluorosis. *Eur Spine J* 16:1119-1128.

52. Teotia M, Teotia SP (2008). Nutritional bone disease in Indian population. *Indian J Med Res* 127:219-228.
53. R. T. Hainmanot, "Neurological complications of endemic skeletal fluorosis, with special emphasis on radiculo-myelopathy," *Paraplegia*, vol. 28, no. 4, pp. 244-251, 1990.
54. J. C. Gerster, S. A. Charhon, P. Jaeger et al., "Bilateral fractures of femoral neck in patients with moderate renal failure receiving fluoride for spinal osteoporosis," *British Medical Journal*, vol. 287, no. 6394, pp. 723-725, 1983
55. Fejerskov O, Thylstrup A, Larsen MJ. Rational use of fluorides in caries prevention. A concept based on possible cariostatic mechanisms. *Acta Odontol Scan* 1981;39:241-9.
56. NRC (National Research Council). 2006. Fluoride in Drinking Water: A Scientific Review of EPA's Standards. Washington, DC:National Academies Press.
57. Chioca LR, Raupp IM, Da Cunha C, Losso EM, Andreatini R. 2008. Subchronic fluoride intake induces impairment in habituation and active avoidance tasks in rats. *Eur J Pharmacol* 579:196-201.
58. Mullenix PJ, Denbesten PK, Schunior A, Kernan WJ. 1995. Neurotoxicity of sodium fluoride in rats. *Neurotoxicol Teratol* 17:169-177.
59. Zhang M, Wang A, Xia T, He P. 2008. Effects of fluoride on DNA damage, S-phase cell-cycle arrest and the expression of NF- $\kappa$ B in primary cultured rat hippocampal neurons. *Toxicol Lett* 179:1-5.
60. Agency for Toxic Substances and Disease Registry. 2003. Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine (Update). Available: <http://www.atsdr.cdc.gov/toxprofiles/tp11.pdf>
61. Grandjean P, Landrigan P. 2006. Developmental neurotoxicity of industrial chemicals. *Lancet* 368(9553):2167-2178.
62. Sawan RMM et al. (2010) Fluoride Increases Lead Concentrations in Whole Blood and in Calcified Tissues from Lead-Exposed Rats. *Toxicology*. 271 1-2: 21-26.
63. Varner JA et al. (1998). Chronic Administration of Aluminum-Fluoride or Sodium-Fluoride to Rats in Drinking Water: Alterations in Neuronal and Cerebrovascular Integrity. *Brain Research*. 78 (1-2): 284-98.
64. Liu H, et al. (1988). Analysis of the effect of fluoride on male infertility in regions with reported high level of fluoride (endemic fluorosis). *Journal of the Medical Institute of Suzhou* 8(4):297-99.
65. Lin Fa-Fu; et al (1991). The relationship of a low-iodine and high-fluoride environment to subclinical cretinism in Xinjiang. *Endemic Disease Bulletin* 6(2):62-67 (republished in *Iodine Deficiency Disorder Newsletter* Vol. 7(3):24-25).
66. Xiang Q, et al. (2003a). Effect of fluoride in drinking water on children's intelligence. *Fluoride*. 36: 84-94.
67. Xiang Q. (2003b). Blood lead of children in Wamiao-Xinhuai intelligence study. *Fluoride*. 36: 138.
68. Ding Y et al. (2010). The relationships between low levels of urine fluoride on children's intelligence, dental fluorosis in endemic fluorosis areas in Hulunbuir, Inner Mongolia, China. *Journal of Hazardous Materials*. doi:10.1016/j.jhazmat.2010.12.097.
69. Xu Y, et al. (1994). The effect of fluorine on the level of intelligence in children. *Endemic Diseases Bulletin* 9(2):83-84.
70. Yao Y, et al. (1997). Comparative assessment of the physical and mental development of children in endemic fluorosis area with water improvement and without water improvement. *Literature and Information on Preventive Medicine* 3(1):42-43.
71. Yao Y, et al. (1996). Analysis on TSH and intelligence level of children with dental Fluorosis in a high fluoride area. *Literature and Information on Preventive Medicine* 2(1):26-27.
72. An J, et al. (1992). The effects of high fluoride on the level of intelligence of primary and secondary students. *Chinese Journal of Control of Endemic Diseases* 7(2):93-94.
73. Poureslami HR, et al. (2011). Intelligence quotient of 7 to 9 year-old children from an

- area with high fluoride in drinking water. *Journal of Dentistry and Oral Hygiene* 3(4):61-64.
74. Eswar P, et al. (2011). Intelligent quotients of 12-14 year old school children in a high and low fluoride village in India. *Fluoride* 44:168-72.75. Seraj B, et al. (2006). [Effect of high fluoride concentration in drinking water on children's intelligence]. [Study in Persian] *Journal of Dental Medicine* 19(2):80-86.
  76. Hong F, et al. (2001). Research on the effects of fluoride on child intellectual development under different environments. *Chinese Primary Health Care* 15(3):56-57 (republished in *Fluoride* 2008; 41(2):156-60).
  77. Wang X, et al. (2001). Effects of high iodine and high fluorine on children's intelligence and thyroid function. *Chinese Journal of Endemiology* 20(4):288-90.
  78. Yang Y, et al. (1994). The effects of high levels of fluoride and iodine on intellectual ability and the metabolism of fluoride and iodine. *Chinese Journal of Epidemiology* 15(4):296-98 (republished in *Fluoride* 2008; 41:336-339).
  79. Lu Y, et al (2000). Effect of high-fluoride water on intelligence of children. *Fluoride* 33:74-78.
  80. Sudhanshu Saxena, Anjali Sahay, and Pankaj Goel. Effect of fluoride exposure on the intelligence of school children in Madhya Pradesh, India. *J Neurosci Rural Pract.* 2012 May-Aug; 3(2): 144-149 .
  81. Rocha-Amador D, Navarro ME, Carrizales L, Morales R, Calderon J. Decreased intelligence in children and exposure to fluoride and arsenic in drinking water. *Cad Saude Publica.* 2007;23(Suppl 4):S579-87.[PubMed]
  82. Zaho LB, Liang GH, Zhang DN, Wu XR. Effect of a high fluoride water supply on children's intelligence. *Fluoride.* 1996;29:190-2.
  83. Trivedi MH, Verma RJ, Chinoy NJ, Patel RS, Sathawara NG. Effect of high fluoride water on intelligence of school children in India. *Fluoride.* 2007;40:178-83.
  84. Wang SX, Wang ZH, Cheng XT, Li J, Sang ZP, Zhang XD, et al. Arsenic and fluoride exposure in drinking water: Children's IQ and growth in Shanyin county, Shanxi Province, China. *Environ Health Perspect.* 2007;115:643-7. [PMC free article] [PubMed]
  85. Tang QQ, Du J, Ma HH, Jiang SJ, Zhou XJ. Fluoride and Children's Intelligence: A Meta-analysis. *Biol Trace Elem Res.* 2008; 126:115-20. [PubMed]
  86. Spittle B. Psychopharmacology of fluoride: A review. *Int Clin Psychopharmacol.* 1994;9:79-82.[PubMed]
  87. Opydo-Szymaczek J, Borysewicz-Lewicka M. Transplacental passage of fluoride in pregnant Polish women assessed on the basis of fluoride concentrations in maternal and cord blood plasma. *Fluoride.* 2007;40:46-50.
  88. Guan ZZ, Wang YN, Xiao KQ, Dai DY, Chen YH, Liu JL, et al. Influence of chronic fluorosis on membrane lipids in rat brain. *Neurotoxicol Teratol.* 1998;20:537-42. [PubMed]
  89. Vani ML, Reddy KP. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. *Fluoride.* 2000;33:17-26.
  90. Basha PM, Madhusudhan N. Pre and post natal exposure of fluoride induced oxidative macromolecular alterations in developing central nervous system of rat and amelioration by antioxidants. *Neurochem Res.* 2010; 35:1017-28. [PubMed]
  91. Chirumari K, Reddy PK. Dose-dependent effects of fluoride on neurochemical milieu in the hippocampus and neocortex of rat brain. *Fluoride.* 2007;40:101-10.
  92. Susheela AK, Bhatnagar M, Vig K, Mondal NK. Excess fluoride ingestion and thyroid hormone derangements in children living in Delhi, India. *Fluoride.* 2005;38:151-61.
  93. Anna L. Choi, Guifan Sun, Ying Zhang, and Philippe Grandjean . Developmental Fluoride Neurotoxicity: A Systematic Review and Meta-Analysis. *Environ Health Perspect* 120:1362-1368 (2012). <http://dx.doi.org/10.1289/ehp.1104912> [Online 20 July 2012]
  94. Calderon J et al. (2000). Influence of fluoride exposure on reaction time and visuospatial organization in children. *Epidemiology* 11(4): S153.
  95. Li J, Yao L, Shao QL, Wu CY. 2004. Effects of

- high fluoride level on neonatal neurobehavioural development. Chinese Journal of Endemiology 23:464-465 (republished in Fluoride 41:165-70).
96. Rocha-Amador D et al. (2009). Use of the Rey-Osterrieth Complex Figure Test for neurotoxicity evaluation of mixtures in children. Neurotoxicology 30(6):1149-54.
  97. Han H, Cheng Z, Liu W. 1989. Effects of fluorine on the human fetus. Chinese Journal of Control of Endemic Diseases 4:136-138 (republished in Fluoride 41:321-6).
  98. Du L. 1992. The effect of fluorine on the developing human brain. Chinese Journal of Pathology 21(4):218-20 (republished in Fluoride 41:327-30).
  99. Dong Z, et al. (1993). Determination of the contents of amino-acid and monoamine neurotransmitters in fetal brains from a fluorosis-endemic area. Journal of Guiyang Medical College 18(4):241-45.
  100. Yu Y et al. (1996) Neurotransmitter and receptor changes in the brains of fetuses from areas of endemic fluorosis. Chinese J Endemiology 15: 257-259 (republished in Fluoride 41(2):134-8).
  101. National Research Council. Health effects of ingested fluoride. Report of the Subcommittee on Health Effects of Ingested Fluoride. Washington, DC: National Academy Press; 1993.
  102. Dunipace AJ, Zhang W, Noblitt TW, Li Y, Stookey GK. Genotoxic evaluation of chronic fluoride exposure: Micronucleus and sperm morphology studies. J Dent Res 1989;68:1525-8.
  103. Kram D, Schneider EL, Singer L, Martin GR. The effects of high and low fluoride diets on the frequencies of sister chromatid exchanges. Mutat Res 1978;57:51-5.
  104. Li Y, Dunipace AJ, Stookey GK. Effects of fluoride on the mouse sperm morphology test. J Dent Res 1987;66:1509-11.
  105. Li Y, Dunipace AJ, Stookey GK. Lack of genotoxic effects of fluoride in the mouse bone-marrow micronucleus test. J Dent Res 1987;66:1687-90.
  106. Li YM, Heerema NA, Dunipace AJ, Stookey GK. Genotoxic effects of fluoride evaluated by sister-chromatid exchange. Mutat Res 1987;192:191-201.
  107. Li YM, Zhang W, Noblitt TW, Dunipace AJ, Stookey GK. Genotoxic evaluation of chronic fluoride exposure: Sister-chromatid exchange study. Mutat Res 1989;227:159-65.
  108. Zeiger E, Gulati DK, Kaur P, Mohamed AH, Revazova J, Deaton TG. Cytogenetic studies of sodium fluoride in mice. Mutagenesis 1994;9:467-71.
  109. Martin GR, Brown KS, Singer L, Ophaug R, Jacobson-Kram D. Cytogenic and mutagenic assays on fluoride. In: Fluorides, effects on vegetation, animals and humans. Schupe JL, Peterson HB, Leone NC, editors. Salt Lake City: Paragon Press; 1983. p. 271-80.
  110. Li Y, Dunipace AJ, Stookey GK. Absence of mutagenic and antimutagenic activities of fluoride in Ames salmonella assays. Mutat Res 1987;120:229-36.
  111. Martin GR, Brown KS, Matheson DW, Lebowitz H, Singer L, Ophaug R. Lack of cytogenetic effects in mice or mutations in salmonella receiving sodium fluoride. Mutat Res 1979;66:159-67.
  112. Tong CC, McQueen CA, Brat SV, Williams GM. The lack of genotoxicity of sodium fluoride in a battery of cellular tests. Cell Biol Toxicol 1988;4:173-86.
  113. Weston CR, Davis RJ (2007). The JNK signal transduction pathway. Curr Opin Cell Biol 19:142-149.
  114. Wagner EF, Nebreda AR (2009). Signal integration by JNK and p38 MAPK pathways in cancer development. Nat Rev Cancer 9:537-549.
  115. Thrane EV, Refsnes M, Thoresen GH, Lag M, Schwarze PE (2001). Fluoride-induced apoptosis in epithelial lung cells involves activation of MAP kinases p38 and possibly JNK. Toxicol Sci 61:83-91.
  116. Zhang M, Wang A, He W, He P, Xu B, Xia T, et al. (2007). Effects of fluoride on the expression of NCAM, oxidative stress, and apoptosis in primary cultured hippocampal neurons. Toxicology 236: 208-216.
  117. Karube H, Nishitai G, Inageda K, Kurosu H, Matsuoka M (2009). NaF activates MAPKs and induces apoptosis in odontoblast-like cells. J Dent Res 88:461-465.

118. Thomas AB, Hashimoto H, Baylink DJ, Lau KH (1996). Fluoride at mitogenic concentrations increases the steady state phosphotyrosyl phosphorylation level of cellular proteins in human bone cells. *J Clin Endocrinol Metab* 81:2570-2578.
119. Wu LW, Yoon HK, Baylink DJ, Graves LM, Lau KH (1997). Fluoride at mitogenic doses induces a sustained activation of p44mapk, but not p42mapk, in human TE85 osteosarcoma cells. *J Clin Endocrinol Metab* 82:1126-1135.
120. Lau KH, Baylink DJ (1998). Molecular mechanism of action of fluoride on bone cells. *J Bone Miner Res* 13:1660-1667.
121. E.T. Everett . Fluoride's Effects on the Formation of Teeth and Bones, and the Influence of Genetics . *J Dent Res* 90(5):552-560, 2011
122. Luke J. (2001). Fluoride deposition in the aged human pineal gland. *Caries Research* 35: 125-128.
123. Luke J. (1997). The Effect of Fluoride on the Physiology of the Pineal Gland. Ph.D. Thesis. University of Surrey, Guildford
124. Schnitzler CM, et al. (1990). Bone fragility of the peripheral skeleton during fluoride therapy for osteoporosis. *Clinical Orthopaedics* .(261): 268-75.
125. Bachinskii PP, et al. (1985) Action of the body fluorine of healthy persons and thyroidopathy patients on the function of hypophyseal-thyroid the system. *Probl Endokrinol (Mosk)* 31: 25-9.
126. U.S. National Research Council (2006).
127. Department of Health & Human Services. (U.S. DHHS) (1991). Review of Fluoride: Benefits and Risks. Report of the Ad Hoc Committee on Fluoride, Committee to Coordinate Environmental Health and Related Programs. Department of Health and Human Services, USA.
128. Galletti P, Joyet G. (1958). Effect of fluorine on thyroidal iodine metabolism in hyperthyroidism. *Journal of Clinical Endocrinology* 18: 1102-1110.
129. Stecher P, et al. (1960). The Merck Index of Chemicals and Drugs. Merck & Co., Inc, Rathway NJ. p. 952
130. Waldbott GL, et al. (1978). Fluoridation: The Great Dilemma. Coronado Press, Inc., Lawrence, Kansas.
131. Johnson WJ, et al. (1979). Fluoridation and bone disease in renal patients. In: Johansen E, Taves DR, Olsen TO, Eds. Continuing Evaluation of the Use of Fluorides. AAAS Selected Symposium. Westview Press, Boulder, Colorado. pp. 275-293.
132. Vineet Dhar, Maheep Bhatnagar . Physiology and toxicity of fluoride. *Indian J Dent Res*, 20(3), 2009. 350- 354
133. European Commission, "Critical review of any new evidence on the hazard profile, health effects, and human exposure to fluoride and the fluoridating agents of drinkingwater," Scientific Committee on Health and Environmental Risks (SCHER), 2011.
134. P. Grandjean and J. H. Olsen, "Extended follow-up of cancer incidence in fluoride-exposed workers," *Journal of the National Cancer Institute*, vol. 96, no. 10, pp. 802–803, 2004.
135. E. Tohyama, "Relationship between fluoride concentration in drinking water and mortality rate from uterine cancer in Okinawa prefecture, Japan," *Journal of Epidemiology*, vol. 6, no. 4, pp. 184–191, 1996.
136. E. B. Bassin, D. Wypij, R. B. Davis, and M. A. Mittleman, "Agespecific fluoride exposure in drinking water and osteosarcoma (United States)," *Cancer Causes and Control*, vol. 17, no. 4, pp. 421–428, 2006.
137. R. Hamilton, "Biochemical effects of fluoride on oral bacteria," *Journal of Dental Research*, vol. 69, pp. 660–667, 1990.
138. Y. Iwami, S. Hata, C. F. Schachtele, and T. Yamada, "Simultaneous monitoring of intracellular pH and proton excretion during glycolysis by *Streptococcus mutans* and *Streptococcus sanguis*: effect of low pH and fluoride," *Oral Microbiology and Immunology*, vol. 10, no. 6, pp. 355–359, 1995.
139. M. Czajka, "Systemic effects of fluoridation," *Journal of Orthomolecular Medicine*, vol. 27, pp. 123–130, 2012.
140. G. F. Judd, *Good Teeth: Birth to Death—Prescription for Perfect Teeth*, Research Publications Co., Glendale, Calif, USA, 1997.

- 141.O. Barbier, L. Arreola-Mendoza, and L.M.DelRazo, "Molecular mechanisms of fluoride toxicity," *Chemico-Biological Interactions*, vol. 188, no. 2, pp. 319–333, 2010.
- 142.R. Yolken, P. Konecny, and P.McCarthy, "Acute fluoride poisoning," *Pediatrics*, vol. 58, no. 1, pp. 90–93, 1976.
- 143.M. Okazaki, "Mg<sup>2+</sup>-F<sup>-</sup> interaction during hydroxyapatite formation," *Magnesium*, vol. 6, no. 6, pp. 296–301, 1987.
- 144.M. Teotia, S. P. S. Teotia, and K. P. Singh, "Endemic chronic fluoride toxicity and dietary calcium deficiency interaction syndromes of metabolic bone disease and deformities in India : year 2000," *Indian Journal of Pediatrics*, vol. 65, no. 3, pp. 371–381, 1998.
- 145.M. E. McIvor, C. E. Cummings, M. M. Mower et al., "Sudden cardiac death from acute fluoride intoxication: the role of potassium," *Annals of Emergency Medicine*, vol. 16, no. 7, pp. 777–781, 1987.
- 146.Kour K, Singh J. (1980). Histological finding of mice testes following fluoride ingestion. *Fluoride*. 13: 160-162.
- 147.Chinoy NJ, Sequeira E. (1989). Effects of fluoride on the histoarchitecture of reproductive organs of the male mouse. *Reproductive Toxicology*. 3: 261-7.
- 148.Chinoy NJ, et al. (1991). Microdose vasal injection of sodium fluoride in the rat. *Reproductive Toxicology*. 5: 505-12.
- 149.Susheela AK, Kumar A. (1991). A study of the effect of high concentrations of fluoride on the reproductive organs of malerabbits, using light and scanning electron microscopy. *Journal of Reproductive Fertility*. 92: 353-60.
- 150.Chinoy NJ, Narayana MV (1994). In vitro fluoride toxicity in human spermatozoa. *Reproductive Toxicology*. 8:155-9.
- 151.Kumar A, Susheela AK. (1994). Ultrastructural studies of spermiogenesis in rabbit exposed to chronic fluoride toxicity. *International Journal of Fertility and Menopausal Studies*. 39:164-71.
- 152.Narayana MV, et al. (1994). Reversible effects of sodium fluoride ingestion on spermatozoa of the rat. *International Journal of Fertility and Menopausal Studies*. 39: 337-46.
- 153.Narayana MV, Chinoy NJ. (1994). Effect of fluoride on rat testicular steroidogenesis. *Fluoride*. 27: 7-12.
- 154.Elbetieha A, et al. (2000). Fertility effects of sodium fluoride in male mice. *Fluoride*. 33: 128-134.
- 155.Ghosh D, et al. (2002). Testicular toxicity in sodium fluoride treated rats: association with oxidative stress. *Reproductive Toxicology*. 16: 385.
- 156.Zakrzewska H, et al. (2002). In vitro influence of sodium fluoride on ram semen quality and enzyme activities. *Fluoride*. 35: 153-160.
- 157.Freni SC. (1994). Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. *Journal of Toxicology and Environmental Health*. 42: 109-121.
- 158.Neelam, K, et al. (1987). Incidence of prevalence of infertility among married male members of endemic fluorosis district of Andhra Pradesh. In: *Abstract Proc Conf Int Soc for Fluoride Res*. Nyon, Switzerland.
- 159.Hao P, et al. (2010). Effect of fluoride on human hypothalamus-hypophysis-testis axis hormones. *Journal of Hygiene Research* 39(1):53-55.
- 160.Chen YC, et al. (1997). Nutrition survey in dental fluorosis-afflicted areas. *Fluoride*. 30(2):77-80.
- 161.Susheela AK and Jethanandani P (1996). Circulating testosterone levels in Skeletal Fluorosis patients. *Clinical Toxicology*. 34 (2): 1-7.
- 162.Barot VV. (1998). Occurrence of endemic fluorosis in human population of North Gujarat, India: human health risk. *Bulletin of Environmental Contamination and Toxicology*. 61: 303-10.
- 163.Ortiz-Perez D, et al. (2003). Fluoride-induced disruption of reproductive hormones in men. *Environmental Research* 93:20-30.
- 164.Sprando RL, et al. (1998). Testing the potential of sodium fluoride to affect spermatogenesis: a morphometric study. *Food and Chemical Toxicology*. 36: 1117-24.
- 165.Sprando RL, et al. (1997). Testing the potential of sodium fluoride to affect spermatogenesis in the rat. *Food and Chemical Toxicology*. 35: 881-90.

166. Sprando RL, et al. (1996). Effect of intratesticular injection of sodium fluoride on spermatogenesis. Food and Chemical Toxicology. 34: 377-84.
167. NRC (2006) . National Research Council of the National Academies, Fluoride in Drinking Water: A Scientific Review of EPA's Standards. Washington, DC: National Academies Press.
168. US Department of Health and Human Services, Centers for Disease Control, Dental Disease Prevention Activity. Water fluoridation: A manual for engineers and technicians. Atlanta; 1986.
169. Susheela AK. Fluorosis: Indian scenario. A Treatise on Fluorosis. Fluorosis Research and Rural Development Foundation; 2001. p. 13-5.