Effect of inorganic fluoride on living organisms of different phylogenetic level

Article *in* Zhurnal evoliutsionnoĭ biokhimii i fiziologii · October 2011

DOI: 10.1134/S002209301105001X · Source: PubMed

2 authors:

Natalia Agalakova
Russian Academy of Sciences, Saint-Petersburg
53 PUBLICATIONS **853** CITATIONS
SEE PROFILE

Gennadii P Gusev
78 PUBLICATIONS **435** CITATIONS
SEE PROFILE

ISSN 0022-0930, Journal of Evolutionary Biochemistry and Physiology, 2011, Vol. 47, No. 5, pp. 393—406. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © N. I. Agalakova, G. P. Gusev, 2011, published in Zhurnal Evolyutsionnoi Biokhimii i Fiziologii, 2011, Vol. 47, No. 5, pp. 337—347.

REVIEWS

Effect of Inorganic Fluoride on Living Organisms of Different Phylogenetic Level

N. I. Agalakova and G. P. Gusev

Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia E-mail: agalak@iephb.ru

Received July 19, 2010

Abstract—This review summarizes literature data on pathways of inorganic fluoride intake to the plant, animal and human organisms, its metabolism, distribution and accumulation in the organism, the fluoride forms in biological tissues, toxic effects of fluoride on physiological and reproductive functions of living organisms of different phylogenetic groups, as well as clinical symptoms of insufficient and excessive intake of fluoride in human organism.

DOI: 10.1134/S002209301105001X

Key words: fluoride accumulation, membrane transport, metabolism, toxicity, fluorosis.

INTRODUCTION

Effect of inorganic fluoride on living organisms attracts a great interest of researchers. First, for the long time fluoride was considered one of the microelements essential for the human normal physiological development and life activity. According to recommendation of the World Health Organization, fluoride concentration in drinking water preventive for dental and gingival diseases should be 0.7–1.2 mg/l; therefore, starting from the early 1940s, many countries introduced supplementation of the drinking water, milk, salt, food additives, medications, dental products with fluoride compounds [1, 2]. The medical society considers water fluoridation one of the most considerable public health achievements of the XX century along with the food pasteurization, water purification, and immunization against infectious diseases. Besides, the fluoride ability to stimulate the bone tissue re-calcification underlies application of its preparations for osteoporosis treatment, although the results of such treatment are contradictory due to multiple side effects and dosage instability [3].

However, recently such wide use of fluoride, especially fluoridation of the drinking water, raises the intensive scientific and political debates and numerous protests of human population [1]. Indeed, no clinical disorders due to fluoride deficiency were described, and the reliable proofs for fluoride necessity for the human life activity or evidence for its biochemical role in organism are absent. To some degree this is explained by that the wide distribution of fluoride in environment makes it difficult the "pure" experiment, whereas in the case of human it is impossible for ethic reasons; therefore, it is extremely difficult to prove that fluoride is a vitally essential element for living organisms (like, for instance, Cu, Co, Zn). On the contrary, the toxic fluoride effects on human health are well known. In the regions with high content of fluoride in environment, its chronic excessive intake to the human and animal organisms leads to development of fluorosis, an endemic disease characterized by pathological alterations in

the structure of tooth and bone tissues [4–6]. In the developed countries the risk of toxic fluoride effect on the human health increases due to uncontrolled consumption of fluoridated water and fluoride-containing food supplements and dental products [1, 3]. Moreover, due to high reactive capacity of fluoride the level of its toxicity for living organisms is even higher than that, for example, of lead, while the threshold between safe and toxic doses was not determined.

Second, inorganic fluoride compounds (HF, NaF, CaF_2, sodium hexafluorosilicate Na_2SiF_6, hexafluorosilicic acid H_2SiF_6, etc.) are widely used in industry and agriculture; as a result, the anthropogenic contamination of environment with fluoride-containing compounds can increase fluoride concentrations in the biogeocenoses up to toxic levels. The coal and fuel burning results in the 10–20-fold increase of fluoride content in the air. In rivers and coastal sea waters the fluoride concentration rises 10–100 times due to waist of contaminated water from factories producing metals (especially aluminum), glass, bricks, phosphate fertilizers, etc. The waist of fluoridated drinking water also increases 5 times the fluoride concentration in the rivers-recipients. Phosphate fertilizers, insecticides, and pesticides are the main sources of fluoride contamination of soil and agricultural products. Monitoring of conditions of the water and terrestrial ecosystems has shown that the soluble fluoride compounds are accumulated in plants and animals and induce development of various pathologies, as well as cause the negative effect on human health [6–11].

The goal of the present work was to summarize the currently accumulated data on pathways of intake, distribution, and accumulation of inorganic fluoride in living organisms, its metabolism, physiological role, and toxic effects on humans and animals.

INTAKE, ACCUMULATION, AND METABOLISM OF FLUORIDE IN ORGANISM

Intake of fluoride into plant and animal organisms. Fluoride enters plants predominantly from air through stomata on the leave surface, epidermis, and cuticle, and accumulate it in cell walls [10]. The fluoride accumulation by roots is insignificant, although it is substantially elevated after addition of phosphate fertilizers [6]. The animal organism consumes fluoride from water and to the lesser degree from the plant food and accumulates it in the calcified structures—exoskeletons of invertebrates and bone and tooth tissues of vertebrates [9, 11].

Intake of fluoride into the human organism. Consumption of fluoride by the human organism occurs from multiple sources and depends on the diet peculiarities [7, 8, 12–15]. The content of fluoride in the majority of fruits, vegetables, and meat is insignificant (0.1–5 mg/kg). The exceptions are sea fish (6–27 mg/kg) and some kinds of tea (up to 200 mg/kg). Therefore, the main source of fluoride intake for human organism is drinking water. Adult human consumes, on average, 0.5–1.0 mg F per day with food products and 2.0–3.0 mg with water containing 1 mg F/l. On the whole, the daily fluoride consumption by the human organism varies from less than 0.01 mg for infants to 2.1 mg for children, and 2.5–4.0 mg for adults. However, the consumption of up to 27 mg per day is not rare for the humans living in the regions with high fluoride content in environment. The fluoride concentration increases considerably (up to 100 times) in the agricultural products cultured in vicinity of industrial fluoride sources, in the regions of intense use of phosphate fertilizers or irrigation with water with fluoride concentration of more than 10 mg/l, as well as in the process of food cooking in water with high fluoride concentration [8, 15]. The entrance of fluoride into the human organism can increase due to consumption of bottle water from underground sources, fluoridated milk, salt, food supplements [1, 2, 5].

Another source of significant fluoride entrance into the human organism is the stomatological products. The fluoride compounds at a concentration of 500–1500 mg/l are present in the toothpaste, mouth rinses, chewable tablets or drops for caries prevention. The professional dental gel contains 5000–24000 mg F/l, the preparations for osteoporosis treatment—50–100 mg F/l [1, 2, 3].

The risk of excessive fluoride entrance to the organism through respiration or skin contact exists for the workers of industrial factories (aluminum, phosphate ore, etc.) [16, 17]. High fluoride

concentrations in the air, causing negative effects on the human health, are observed in the regions where coal is used for heating [7].

Absorption. The experiments with laboratory animals and observations on humans have shown that the main pathway of fluoride intake into the organism is its absorption from gastrointestinal tract [12]. The fluoride ions from soluble compounds (NaF, HF, Na_2PO_3F) are absorbed practically completely. He presence of food and some two- and three-valent cations (Al, Mg, Ca), forming insoluble compounds with fluoride, considerably delay or decrease the fluoride absorption. Thus, if fluoride is consumed as tablets on empty stomach, its bioavailability is almost 100% and absorption reaches the peak after 30 min; if the same dose is consumed with a glass of milk, its bioavailability decreases to 70%, while with the calcium-enriched food—to 60% [18, 19].

Fluoride not absorbed in stomach is rapidly absorbed in small intestine [12]. A small amount of fluoride (to 7%) can be absorbed from mouth [20]. Fluoride in gas form is partially or completely absorbed from the respiratory pathways, while insoluble fluoride particles are deposited in bronchioles and nasal cavities and can also be absorbed with time [21]

Mechanisms of fluoride transport across cell membranes. The transmembrane migration of fluoride ions in the epithelial cells of mouth, stomach, and urinary bladder, as well as fluoride reabsorption by the renal tubule cells are pH-dependent processes and occur by the way of non-ion diffusion in the form of non-dissociated HF acid [12]. The experiments with artificial lipid bilayers have shown that HF by its permeability coefficient is close to that of water, which is higher, on average, million times than that for fluoride ions [22]. However, the fluoride absorption by cells of the rat small intestine is insensitive to luminal pH, which suggests the existence of a membrane carrier responsive for cotransport of F^- and H^+ or exchange of F^- and OH^- [23]. The studies on human erythrocytes showed that fluoride ions could be transported via anion exchanger [24].

Blood. After entering an organism, approximately 80–90% of fluoride is absorbed for one hour from the gastrointestinal tract to blood [12]. The fluoride concentration in the blood plasma reaches maximum for 20–60 min, then it rapidly declines by usually reaching the basic level for 3–6 h. The mean fluoride concentration in the blood plasma of healthy adults when measured after starvation correlates directly with its content in drinking water [25]. Thus, fluoride concentration in the blood plasma of human consuming water with 1 mg F/l (52.6 μM) varies from 0.5 to 1.5 μM/l. Deviations from this ratio depend on differences in the rates of fluoride clearance by kidneys, relative accumulation by bone tissue, and age; it can be lower in children and young people and higher in elderly people [26]. The fluoride concentration in the cord blood is ~75% of that in the mother blood, and the fluoride is rapidly accumulated in fetus calcified tissues and teeth [27].

Distribution in organs and tissues. Fluoride is rapidly spread from plasma into intracellular and extracellular fluids, tissues, and organs [12]. Experiments with radioactive fluoride have shown that its intracellular concentration depends on pH gradient and is by 10–50% lower than in blood plasma, but changes simultaneously and proportionally to that in plasma [38]. The content of fluoride in specialized fluids (cerebrospinal fluid, saliva, milk) is by 50% lower than in plasma, but is proportional to that in plasma. The fluoride concentration equilibrium between plasma and well-vascularized organs (heart, lungs, and liver) is achieved faster than between plasma and skeletal muscles, skin, and other organs.

Excretion of fluoride from an organism by kidneys. On average, approximately 50% of fluoride consumed by an organism every day is effectively excreted with urine through kidneys [12]. In healthy individuals the renal fluoride clearance (complete clearance of the blood from given substance) is significantly higher (35–45 ml/min) than that of chlorine, iodide, and bromide (1–2 ml/min) [29]. The organism fluoride clearance depends on the functional kidneys mass, the glomerular filtration rate, and urine pH; for example, in patients with abnormal renal function and the decreased level of glomerular filtration, as well as in the aged people, when the number of normally functioning nephrons decreases, the plasma fluoride content rises.

Accumulation of fluoride in bone tissue. Approximately 50% of fluoride absorbed daily by healthy humans of the middle age are reversibly incorpo-

rated into calcified tissues for 24 h, with the uptake of fluoride by bone tissue even exceeding that of calcium [12, 13]. As a result, about 99% of fluoride accumulated by an organism during life are accumulated in bones, teeth enamel and dentine, the rest of fluoride is distributed between highly vascularized soft tissues and blood. The fluoride distribution reflects the individual long-term fluoride consumption and is oppositely proportional to the age and stage of skeleton formation. In children, up to 75% of the daily consumed fluoride are accumulated in skeletal tissue; in adults this value decreases to 60%, whereas in elderly people this process shifts toward excretion.

FORMS OF FLUORIDE IN BIOLOGICAL TISSUES

The work [30] was the first to describe two fluoride fractions in the blood plasma—the "exchangeable" fluoride that easily exchanges with radioactive fluoride ^{18}F and is easily available for diffusion in the form of HF and the "non-exchangeable" fluoride that is not available for exchange or diffusion and whose content can be measured only after sample burning. Further chromatographic study of the human blood plasma with radioactive fluoride (^{18}F) showed the absence of the organic fluoride (covalently, by C–F bond, connected with proteins) in the blood plasma [31]. Thus, the animal tissues contain only various forms of the inorganic fluoride: (1) ionic fluoride in the form of free ions, which can be measured by ion-selective electrodes, (2) non-ionic fluoride in the forms of complexes such as: (a) HF at low pH values; (b) fluoride bound with metal ions (Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, etc.); (c) fluoride absorbed or complexed on the mineral-organic sediments, for example, in saliva; d) inorganic fluoride incorporated into apatite elements of bones and teeth. All these inorganic fluoride fractions can be transformed into ionic inorganic fluoride and their content can be measured [32].

Organic fluoride compounds in the living organisms are quite rare, and the biosynthetic pathways of transformation of the inorganic into the organic fluoride currently are unclear. The fluoride ions are weak nucleophiles in the water due to high capability for hydration, and they are not oxidized, like other halogens, by haloperoxidases. This makes difficult the biosynthesis of organic fluoride compounds by the way of the C–F bond formation. Besides, it is not always possible to reliably determine whether the given organism synthesizes fluoride-containing compounds or accumulates them from environment. Several plant species from the arid Australia and Africa regions (Gastrolobium spp., Oxylobium spp., Dichapetalum spp., *Acacia georginae*, *Palicourea marcgravii*) have the ability to convert the soluble fluoride extracted from soil into the organic monofluoroacetic acid, an extremely toxic substance representing danger for herbivorous animals [10]. The seeds of the African plant *Dichapetalum toxicarious* contain a series of fluoroorganic metabolites such as ω-fluoro-9.10-dihydroxystearic, monofluoroleinoic, monofluoropalmitoic, monofluoromiristoil acids [33]. Two bacterial species *Streptomyces cattleya* and *Streptomyces calvus* are able to accumulate fluoroorganic metabolites and to synthesize fluoroacetate and 4-fluorothreonine from inorganic fluoride [34].

The first example of enzymatic formation of covalent C–F bond was obtained with the mutant enzymes of glycosyltransferase able to produce α-fluoroglycosides as temporary intermediate products of sugar activation by dinitrophenol acting as substrates for glycosylation [35]. However, only in bacteria *S. cattleya* [34] there was identified the only natural enzyme able to convert inorganic fluoride into the organic one by the way of biological C–F bond formation. This enzyme—fluorinase (5'-fluoro-5'-deoxyadenosine synthase, EC 2.5.1.63)—catalyzes reaction of binding of fluoride ion and S-adenosyl-L-methionine on the way of biosynthetic formation of fluoroacetate and 4-fluoromethionine. In [34], several intermediate metabolites with C–F bonds were identified *in vitro*; however, it is not clear whether they might be formed and accumulated in the organism. At present in tissues of other phylogenetic animal groups the fluoroorganic metabolites have not been found.

TOXIC EFFECTS OF FLUORIDE ON LIVING ORGANISMS

On the whole, the fluoride toxicity rises with

increase in fluoride concentration in the air or water, in time of exposure, temperature elevation (and, respectively, metabolism intensification) and a decreases in parallel with an increase of water hardness due to formation of complexes of fluoride with calcium ions and a rise of the organism species-specific size [9–11].

Microorganisms. The ability of fluoride to suppress the life activity of oral flora provided a wide application of fluoride-containing dental products for prevention and treatment of oral and gingival diseases [36]. Fluoride ions inhibit activity of glycolytic enzymes thus decreasing ATP synthesis and suppressing all energy-consuming processes (for example, gluconeogenesis) in cells. Under natural conditions, the deposit of atmospheric fluoride in the soil (5300 mg/kg) is accompanied by a decrease in the soil bacterial biomass and activity of their enzyme systems [37].

Algae and cyanobacteria. Fluoride is able either to suppress or to intensify growth of populations of algae and cyanobacteria depending on its concentration, time of exposure, and alga species [9, 38, 39]. For example, growth of population of the cryptomonade *Rhodomonas lens* in experiments was stimulated by 20–30% in the presence of 25–100 mg/l fluoride, whereas the growth of the dinoflagellate *Amphidinium carteri* and gaptophite algae *Pavlova lutheri* was suppressed by 25–30% at a fluoride concentration of 100 mg/l. Some algae are tolerant even to as high fluoride concentrations as up to 200 mg/l in water. The toxic fluoride effects include inhibition of respiratory activity, photosynthesis, and nitrogen fixation, membrane damage with subsequent release of pigments and electrolytes.

Lichens. In the vicinity of an aluminum factory, lichens accumulated 400–600 μg F/g of dry weight (as compared to less than 10 μg/g in uncontaminated regions) [40]. The symptoms of toxic fluoride action include chlorosis, necrosis, weakening and loss of thalli binding to rocks and tree bark. The most sensitive to fluoride are fruticose lichens, whose survival decreased to 1% for 4 years of observations. The majority of foliose lichen species also lost up to 88% of biomass, whereas crustose lichens were the most resistant to fluoride action and gradually occupied areas of dying lichens of other species.

Higher plants. In areas of anthropogenic contamination, accumulation of fluoride by plants directly correlates with emission of fluoride-containing compounds to air and produces toxic effect on plants, especially on young, growing tissues of foliose plants and needles of coniferous plants. The features of fluoride toxicity for plants are expressed in the slowing of growth, development of chlorosis, necrotic spots, which reduces the effective photosynthetic leaf area, in a decrease in the content of photosynthetic pigments and activity of intracellular enzymes—peroxidase, superoxide dismutase, ATPase, acid phosphatase [10, 41].

The experiments with a few species of leafy and coniferous plants have shown that the fluoride toxicity depends on plant species. The lower HF concentration inducing 65% necrosis of the leaf area varied from 0.35 (gladioli *Gladiolus grandiflorus*) to 21.3 μg/m^3 (the medicago *Medicago sativa*) [42]. The lower HF concentrations inducing needle necrosis in the 2-year old black spruce (*Picea mariana*) and the 3-year old white spruce (*P. glauca*) were 4.4 μg/m^3 and 13.2 μ/m^3, respectively [43].

Fluoride accumulation by the plants also affects their reproductive functions—a reduction of the number of flowers (conifers) and a decrease in the size of seeds. For example, in the region of industrial pollution with the fluoride on the most contaminated site (11.4 μg F/m^3 of air) the production of seeds on the fir (*Abies balsamea*), black spruce (*P. mariana*) and larix (*Larix laricina*) decreased by 76.4%, 87.4% and 100%, respectively [44]. Gradually, the toxic effects of fluoride on these tree species led to their replacement by more tolerant species—birch and alder.

Invertebrates. The extremely high natural fluoride concentrations were found in some species of Antarctic crustacean [45], for example, exoskeleton of euphaside *Euphausia crystallorophias* contained 5477 μg F/g, whereas copepods *Calanus propinquus* and *Euchaeta Antarctica* contained as low as 1.44 and 0.87 μg/g, respectively. Up to 99% of fluoride is concentrated in exoskeleton, especially intensively during the periods of formation of a new exoskeleton after moulting, which some authors consider as a protective mechanisms against fluoride intoxication or the way to make exoskeleton more rigid due to formation of

fluoroapatite [9, 45]. Thus, the total fluoride concentration in the crab *Portunus depurator*, shrimps *Crangon vulgaris* and *Leander serratus*, living in the water contaminated by the aluminum plant emission (3.4 mg) was 17–18 μg/g of dry weight, whereas the fluoride level in muscles did not exceed 3.3 μg/g [46].

The toxicity of fluoride to various invertebrate species is quite different. In acute (48–96 h) experiment the most sensitive to the toxic fluoride action were amphipods *Hyalella azteca* (LC_{50} 14.6 mg/l). The lethality was lower in the mayfly *Hexagenia limbata* (LC_{50} 32.3 mg/l), fly *Chironomus tentans* (LC_{50} 124.1 mg/l), and daphnia *Daphnia magna* (LC_{50} 282.8 mg/l) [48]. A similar pattern was observed in the chronic 10–28-day long experiment for the delay of animal growth. One more biological parameter described for invertebrates and found to be sensitive to the fluoride action is fertility of females. In the 90-day long experiment, two amphipod species, *Grandidierella lutosa* and *G. lignorum*, kept in the water with 6.9 mg F/l, produced less eggs by 30% as compared with control animals [48].

The terrestrial invertebrates inhabiting polluted soils in the vicinity of industrial fluoride sources also accumulate fluoride. The highest fluoride amounts (on average, 110 μg/l) were found in the organisms of scavengers, such as myriapods and woodlouses [49]. Representatives of predator spiders (Arachnida: Araneae), opiliones (Arachnida: Opiliones), earth worms (Oligochaeta), snails (Gastropoda: Pulmonata), bugs (Insecta: Coleoptera), and peripods (Arthropoda: Chilopoda) contained 393, 258, 190, 148, 50, and 48 μg F/g, respectively. Since the terrestrial invertebrates are prey for birds and small mammals, they can be one of the fluoride sources for vertebrate organisms.

Fish. Fluoride accumulation in the fish organism occurs in the calcified structures, being more intensive at the early stages of growth [11]. Thus, the 90-day long experimental exposure of youngsters of sturgeon *Acipenser baerii* with 4–62.5 mg F/l in water was accompanied by a considerable fluoride accumulation in the bone tissues (to 3200 mg/kg of dry weight), cartilage (1400 mg/kg), gills (389 mg/kg), and skin (100 mg/kg), whereas in liver, intestine, pylorus, and muscles no increase in fluoride concentration was observed [50]. The mugil (*Mugil auratus*, M. *cephalus*, and *M. labrosus*) inhabiting areas of waist of fluoride-enriched waters (2–3 mg/l) contained 4–5 times more fluoride in bones and muscles (320 and 14.6 mg/kg of wet weight, respectively) than the fish inhabiting waters distant from the fluoride sources (73 and 3.8 mg/kg, respectively) [51].

The toxicological experiments have shown that rainbow trout is the most sensitive species to fluoride. Thus, LC_{50} for the rainbow trout *Oncorhynchus mykiss* in the 480-h long experiment was 2.7–4.7 mg F/l as compared with 75–91 mg/l for carp *Cyprinus carpio* [52]. The symptoms of the first phase of acute poisoning of fish with fluoride are manifested as apathetic behavior and partial lethargy accompanied by anorexia, hypoexcitability, decelerated respiration, skin darkening, an increase of secretion of mucous membranes, at the second phase of poisoning the incoherent helpless movements with the loss of balance are observed and the death as a consequence of partial or complete muscle paralysis [11]. The chronic toxic fluoride action on *Danio rerio* is manifested as development of tooth fluorosis: changes in the structure of enamel-like layer, and an increase in the content of organic material [53].

Observations on behavior of two salmon species (*Oncorhynchus tshawytscha* and *O. kisutch*) migrating to the river in vicinity of aluminum plant revealed high lethality and delay of migration at the water fluoride concentration of 0.3–0.5 mg/l [54]. However, it is to be noted that the natural fish populations are adapted to specific fluoride concentrations in environment. Thus, the healthy reproducing populations of trout inhabit rivers of the Yellowstone Park and Nevada lakes, in which the water fluoride concentration reaches 13–14 mg/l [11].

High fluoride concentrations in water affect reproduction of fish. The hatching of fertilized eggs of fresh-water fish *Catla catla* was delayed by 1 and 2 h after an increase in the water fluoride concentration from 3.6 to 7.3–17 mg/l [55]. The toxic fluoride effect is manifested in the chaotic movement of embryos inside the eggs, which often results in the premature break of vitellin membrane, death of embryo, and immediate yolk coagulation. The survived embryos often have a deformed skeleton [11].

Amphibians. The toad *Bufo melanostictus* inhabiting the region of fluoride contamination accumulated up to 2736 mg F/kg in bones as compared to 241 mg/kg in the control region, with a decrease in the hemoglobin content, the number of erythrocytes, and hematocrit, whereas the erythrocyte volume increased [56]. Under experimental conditions, the survival of adult *R. pipiens* individuals decreased after an increase in the fluoride concentration to 50–300 mg/l, at 250 and 300 mg/l all animals died for 30 days [57]. The tadpoles of *Rana temporaria* delayed metamorphosis, while activity of their thyroid hormones decreased [58].

Birds. Under experimental conditions, the hens consuming up to 1300 mg F/l with the diet accumulated fluoride mainly in bones (up to 2600 mg/l) and to the lesser degree in kidneys (31.8 mg/l), liver (19.2 mg/l), and muscles (6.7 mg/l) [59]. The maintenance of hens with excess of fluoride in the water (10 and 14 mg F/l) was accompanied by a decrease in the food consumption and a decrease of bird weight with a rise in weight of skeleton [60]. The tolerance limits for egg-laying hens were found to be 4453 mg F/day for 74 weeks, for chickens—1.2 g NaF/kg of food or water, for embryos—400 μg NaF [60, 61]. The lethal doses for embryos and chickens were 800 μg and 2.4 g NaF/l of water, respectively.

The consumption of excessive fluoride amount leads to disturbance of bird reproduction—a decrease of the eggs laying capacity, a reduction of the weight and size of eggs, deterioration of shell quality [59]. The eggs of owl *Otus asio* after prolonged consumption of 40 and 200 mg F/kg had considerably lower volume, weight, and length. The weight and body length of the bird nestlings kept on diet with 200 mg F/kg were at hatching by 10% lower as compared to the nestlings of birds on control diet [62].

Mammals. The small mammals inhabiting anthropogenic fluoride sources in the heavily contaminated soils accumulate the most considerable fluoride amounts in bones. Thus, the fluoride content in bones of field voles (*Microtus agrestis*) and mice (*Apodemus sylvaticus*) inhabiting near an aluminum production plant was 910–11 000 μg/g and 1800–17 200 μg/g, respectively, as compared to 23–540 μg/g in bones of voles from relatively uncontaminated regions [63], with the fluoride accumulation accompanied by serious tooth damage in these animals. The experimental housing of field vole (*M. agrestis*), bank vole (*Clethrionomys glareolus*), and wood mouse (*A. sylvaticus*) on the water with 40 or 80 mg F/l for 84 days resulted in the premature animal death, whereas teeth of survived animals had significant morphological alterations and damages [64]. The maintenance of female voles *M. agrestis* on diet with high fluoride concentration led to a reduction in the number of offsprings, a decrease in the size and weight of offsprings, a rise of newborn lethality; in males of *C. glareolus*, high fluoride doses led to a damage of testicular tissue—epithelium hemorrhage, necrosis and apoptosis of epithelium [65, 66].

The consumption of fluoride in carnivores usually is insignificant, as fluoride is accumulated predominantly in bones of their preys. Nevertheless, the red foxes (*Vulpes vulpes*) inhabiting holes of fluoride-contaminated soils accumulated in bones from 283 to 1650 μg F/g [67]. The chronic consumption of considerable fluoride amounts with diet by foxes at fur farms resulted in the fall of milk production and subsequent lethality of pups [68]. The survival of adult minks (*Mustela vison*) decreased only at a relatively high fluoride concentration in diet—350 mg/kg [69]; however, only 14% of the pups survived the 3-week age, the survived animals and their offsprings having bone microfractures and radiographic alterations.

Large mammals are often used for monitoring the environment pollution with fluoride from industrial sources. Thus, the horns of deer *Capreolus capreolus* inhabiting regions of aluminum production accumulated from 2070 to 12 000 mg F/kg of ash (in control 262–277 mg/kg of ash) [70]. 75% of the studied deer had tooth fluorosis manifested as defects of dentine and enamel mineralization (hypomineralization and hypermineralization), black tooth discoloration, jaw fractures [71]. The reduced fluoride level in the diet inducing fluorosis-like effects was obtained in the experiments on the white-tail deer (*Odocoileus virginianus*), in which the tooth damage was observed at 35 mg F/kg; the higher fluoride concentrations also induced hyperostosis of long bones [72].

It is interesting that marine vertebrates whose ration includes krill, for instance, Antarctic seals (*Leptochynotus weddelli* and *Lobodon carcinopha-*

gus) and whales (fin whale *Balaenoptera physalus*) accumulate considerable fluoride concentrations (to 6400 and 18 600 mg/kg, respectively) in bones without any signs of toxicity, while its content in soft tissues is comparable to that for terrestrial animals, which seems to be an example of adaptation of animals to high fluoride concentrations [73, 74].

In some world regions the problem of chronic excessive fluoride consumption by agriculture animals is extremely important due to serious loss in the animal farming. The symptoms of chronic fluoride intoxication include weight loss, ligament rigidity, tooth and skeletal fluorosis, as well as the decreased milk production, reduced reproductive capacity of the animals. Based on clinical symptoms of tooth fluorosis, the tolerance level was found to be 30–40 mg F/kg of food for the milk cows and 150 mg F/kg for sheep; for water these values were 2.5–4.0 mg F/l for cows and 12–15 mg F/l for sheep [6].

EFFECT OF FLUORIDE ON HUMAN HEALTH

Acute toxicity. The amount of pure fluoride considered lethal for human after oral administration is 35–70 mg/kg of body weight, which is equivalent to 5–10 g NaF for the human weighing 70 kg or 1–2 g NaF for the child weighing 15 kg [13, 21]. The well-soluble fluoride compounds (NaF) are more toxic than the insoluble or poorly soluble ones (CaF_2). Symptoms of acute fluoride intoxication develop rapidly and are characterized by diffuse abdominal pain, diarrhea, nausea and vomiting, increased thirst and saliva production, weakness, and convulsions. Respiratory effects include hemorrhage, pulmonary edema, tracheal bronchitis, shortness of breath, damage of gastric mucosa accompanied by hemorrhage, loss of epithelium, formation of ulcers. Hypocalcemia and hypercalcemia lead to the cardiac arrest, hypocalcemia and inhibition of cell enzymes affect CNS [21]. Besides, cases of severe damage of respiratory organs as well as disturbances in heart functioning and death of workers after accidental skin contact with hydrofluorotic acid were observed at industrial workplaces [17].

Tolerance limits. The optimal fluoride amount preventing tooth diseases, but not producing side effects on human health were found to be from 0.01–1 mg/day for the children to 3–4 mg/day for adults. The upper tolerance limit of human organism to fluoride is from 0.7–1.3 mg/day for children to 6 mg/day for adults [13].

Fluoride deficiency. The consequence of hypofluorosis for organism, often observed at the water fluoride content of less than 0.5 mg/l is development of tooth caries. The primary cause of this disease is the life activity of bacterial flora on the tooth surface [75]. The metabolic activity of bacteria induces pH fluctuations to result in demineralization of the hard tooth tissues, an increase in porosity of tooth enamel and dentine, disturbances in connection between organic and inorganic parts of enamel and dentine, heterogeneous tooth erosion, formation of cavities and cracks, gradual development of pulpit and periodontal disease. The fluoride ions prevent solubility of tooth tissues by incorporation into the mineral enamel structures, replacement of hydroxyl groups (formation of fluoroapatite), and a decrease in carbonate content, thus improving their stability [76]. Moreover, fluoride suppresses activity of cariogenic bacterial flora in the mouth, thereby it decreases the mouth acidity [36].

Recently, there appeared the point of view that development of caries is not a result of fluoride deficiency, but depends on several causes including peculiarities of diet, hygienic habits, genetic factors. Therefore, the necessity of wide fluoride application including water fluoridation raises certain doubt [1]. Moreover, the review of clinical investigations showed that application of fluoride-containing supplements did not produce any favorable effects on the milk teeth, although the preventive importance of fluoride for the health of constant teeth so far does not cast doubts.

Excess of fluoride. As early as in the first half of the XX century the clinical observations performed in the arid and volcanic regions with high fluoride content in environment (India, countries of the African continent, south of the USA, Central Europe, regions of the Black Sea, etc.) have shown that the chronic excessive fluoride intake to the human organism is a cause of fluoride intoxication—fluorosis [4–7]. However, for the last decades due to increasing pollution of environment

the fluorosis has become a common phenomenon not only in the countries of hot climate, but also in the USA, Europe, China, Japan, Argentina. In the Russian territory the fluorosis is diagnosed in the Moskoskaya, Tverskaya, Tambovskaya, Ryazanskaya, Vologodskaya oblasts, in the Ural, Mordovia, and Kareliya. The most severe cases of fluorosis are observed in workers of the factories mining and processing phosphate ore, aluminum, chemical fertilizers, pesticides, and fuel, in the regions of intense use of phosphate fertilizers, in the regions where the coal is used for heating.

There are three types of fluorosis—tooth fluorosis, skeletal fluorosis (affecting bone tissue), and the associated with it fluorosis of soft tissues. The most widely distributed and primary form is the tooth fluorosis that can be separated from the skeletal fluorosis by the prolonged asymptomatic period of 10–30 years, during which the bone tissue continuously accumulates fluoride [77, 78]. On the whole, at the optimal water fluoride content of approximately 1 mg/l, the tooth fluorosis is observed in 1–2% of population, at 2 mg/l—in 10–12% of population, increasing the water fluoride concentration to 2.4–4.1 mg/l elevates frequency of this disease to 33% [77].

The symptoms of tooth fluorosis include enamel damage in the form of white, but at the later stages yellow or brown, stains. At the high fluoride concentrations the tooth structure is disturbed—the enamel becomes porous, the alterations in the dentin structure resulting in increased fragility, and erosion of teeth are observed. The fluoride also facilitates deposition of calcified material around tooth channels and inside the pulp camera, which narrows camera and decreases the tooth nutrition. The tooth fluorosis is irreversible. Predominantly damaged are the permanent teeth, therefore the excessive fluoride consumption is especially dangerous during the period of formation and calcification of the permanent teeth, i.e., during the first 3–8 years of life [78].

The basic process of tooth fluorosis is a hematogenic toxic action of fluoride on ameloblasts (the enamel-secreting cells) for the period of tooth development [78, 79]. At the secretory stage of enamel formation, fluoride decreases production of matrix and changes its composition, alters mechanisms of ion transport, while at the stage of enamel maturation—delays removal of proteins and water. Besides, fluoride affects nucleation and growth of enamel crystals at all stages of enamel formation and calcium homeostasis. This results in diffuse hypomineralization, dystrophic alterations in ameloblasts, improper formation and reduced thickness of enamel layer. After the completion of tooth growth and their calcification, sensitivity to fluoride decreases.

The skeletal fluorosis is characterized by alterations in the bone structure and excessive calcification leading to deformation of the skeleton, a rise in the bone mass, development of osteosclerosis, osteoporosis or osteomalatia [4–7, 77]. These processes are accompanied by pain and reduced joint motility, ligament calcification, decreased muscle mass, and neurological defects. The skeletal fluorosis is observed at the water fluoride concentration within 5–13 mg/l, but under combination of unfavorable factors—at 0.7–3 mg/l. The development of fluorosis is facilitated by hot climate leading to increase in the water consumption and deposition of fluoride in bones, hard physical activity, deficient nutrition—insufficiency of proteins, vitamins C and D, calcium, as well as insufficient consumption of milk and vegetables. The skeletal fluorosis as a rule develops for 15–20 years at the water fluoride content of about 10 mg/l and for 30 years at a concentration of 5–6 mg/l. The age and kidney function also affects development of fluorosis. The patients with altered renal functions or with diabetes are more sensitive to fluoride due to decreased fluoride clearance from the organism [12, 13].

The precise mechanism of the skeletal fluorosis development is not completely studied, but fluoride is suggested to be incorporated into hydroxyapatites of bone tissues with formation of the less soluble fluoroapatite. Although an excessive deposition of fluoroapatite crystals increases stability of the crystal bone element, the bone mineralization is delayed or postponed, the bones become more fragile [80, 81]. At high concentrations, fluoride stimulates activity of osteoblasts, which leads to an increase in the density and mass of bones. The experiments on animals showed a two-phase character of the fluoride effect on the bone density: an increase of the bone fluoride concentration to 1200 μg/g (as compared to 500–1000 μg/g of bone

in norm) elevates density of bone tissue without a rise of bone mass, an increase of fluoride concentration to 3500–5500 μg/g leads to osteosclerosis, joint pain, low ligament calcification, while concentration above 8400 μg/g induced bone exostoses, significant ligaments calcification, and decreased joint motility.

Apart from the pathologies described above, the excessive fluoride intake under certain conditions leads to multiple structural alterations in the whole organism and affects virtually all human physiological functions, which is inevitably accompanied by development of associated diseases and disability [82]. The cardiovascular system shows vasotonic symptoms (bradicardia, hypotonia) and morphological alterations in the blood composition (leucopenia, lymphocytosis). The patients with chronic industrial fluorosis and severe endemic fluorosis have neurologic symptoms—a decrease in the pain and temperature sensitivity, radiculitis, and myelopathies appearing mainly due to mechanical compression of the spinal cord and nerve tracts. At the late stages of disease the changes of neurogenic character occur in muscle tissue [82, 83]. Liver and kidneys also are often damaged; their pathologies include degeneration of renal tubules, necrosis of hepatocytes, inflammation, fibrosis or hyperplasia [84]. Fluoride at high concentrations is able to suppress functioning of human thyroid inducing hypoparathyroidism or provoking secondary hyperparathyroidism [85, 86]. Moreover, the increased fluoride content in the air of industrial factories often leads to development of chronic inflammatory reactions in workers—asthma, cough, chronic bronchitis with diffuse interstitial fibrosis and pulmonary emphysema, allergy, skin irritation [16, 17].

For the last few years, there appeared data that the prolonged excessive intake of fluoride to human organism is accompanied by a decrease in intellectual capacity of children and various psychic disorders in adults [87, 88]. Analysis of many-year clinical observations of children in China revealed a strong correlation between high fluoride content in drinking water and decreased coefficient of intelligence quotient (IQ). The mechanisms of toxic fluoride action on the intellectual capacities are poorly understood; probably, this is associated with the fluoride ability to suppress functioning of thyroid gland, as thyroid hormones play an important role in the brain development [88].

The data on fluoride effects on the reproductive human functions are contradictory. In the earlier works the association between fluoride consumption by mother and risk of miscarriages and pregnancy complications was not revealed [4, 77]. The recent investigations showed that water fluoridation could be associated with a rise of risk of premature delivery (<37 weeks of pregnancy), especially in women from the lower social layers [89].

Also contradictory are data on the possible association between the everyday excessive fluoride consumption and development of oncologic diseases, as often it is not possible to precisely establish the amount of consumed fluoride and its sources [13, 77]. A rise of incidence of tumor cases of lungs, liver, stomach, pancreas, lymph-hematopoietic system, prostate, and brain were observed in workers of aluminum industry; however, these workers also had a contact with other substances—aromatic compounds, quarts, etc.

CONCLUSION

As a results of analysis of numerous investigations, it has become clear that inorganic fluoride is toxic for majority of plant and animal species. However, the majority of experimental works were limited by time, while the used fluoride concentrations (50–120 mg/l) were much higher than those existing in natural biogeocenoses. A possibility of partial adaptation of animals to high fluoride concentration in some particular biocenosis also cannot be ruled out. At present, to establish the maximally admissible fluoride concentrations in environment, it is necessary to perform additional investigations of the chronic action of low fluoride concentrations on the living organisms and possible accumulation of fluoride in alimentary chains.

The studies focused on effects of fluoride on human health do not permit making unanimous conclusions. It is doubtless that fluoride at low concentrations produces a favorable effect on human health. On the other hand, risk of development of diseases associated with consumption of excessive fluoride amounts increases for the recent time and leads to necessity to control doses and sources of fluoride intake to the human organism.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research (project no. 09-04-01679).

REFERENCES

1. Pizzo, G., Piscopo, M.R., Pizzo, I., and Guiliana, G., Community Water Fluoridation and Caries Prevention: a Critical Review, *Clin. Oral Invest.*, 2007, vol. 11, pp. 189–193.
2. Espelid, I., Caries Preventive Effect of Fluoride in Milk, Salt and Tablets: a Literature Review, *Eur. Arch. Paediatr. Dent.*, 2009, vol. 10, pp. 149–156.
3. Vestergaard, P., Jorgensen, N.R., Schwarz, P., and Mosekilde, L., Effects of Treatment with Fluoride on Bone Mineral Density and Fracture Risk—a Meta-Analysis, *Osteoporos. Int.*, 2008, vol. 19, pp. 257–268.
4. Krishnamachari, K.A., Skeletal Fluorosis in Humans: a Review of Recent Progress in the Understanding of the Disease, *Prog. Food Nutr. Sci.*, 1986, vol. 10, pp. 279–314.
5. Fordyce, F.M., Vrana, K., Zhovinsky, E., Povoroznuk, V., Toth, G., Hope, B.C., Iljinsky, U., and Baker, J., A Health Risk Assessment for Fluoride in Central Europe, *Environ. Geochem. Health*, 2007, vol. 29, pp. 83–102.
6. Cronin, S.J., Manoharan, V., Hedley, M.J., and Loganathan, P., Fluoride—a Review of Its Fate and Hazard Potential in Grazed Pasture Systems, *N. Z. J. Agric. Res.*, 2000, vol. 43, pp. 295–321.
7. Qin, X., Wang, S., Yu, M., Zhang, L., Li, X., Zuo, Z., Zhang, X., and Wang, L., Child Skeletal Fluorosis from Indoor Burning of Coal in Southwestern China, *J. Environ. Public Health*, 2009, doi: 10.1155/2009/969764.
8. Muramoto, S., Nishizaki, H., and Aoyama, I., Effects of Fluorine Emissions on Agricultural Products Surrounding an Aluminum Factory, *J. Environ. Sci. Health B*, 1991, vol. 26, pp. 351–356.
9. Camargo, J.A., Fluoride Toxicity to Aquatic Organisms: a Review, *Chemosphere,* 2003, vol. 50, pp. 251–264.
10. Weinstein, L., Fluoride and Plant Life, *J. Occup. Med.*, 1977, vol. 9, pp. 49–78.
11. Sigler, W.F. and Neuhold, J.M., Fluoride Intoxication in Fish: a Review, *J. Wildl. Dis.*, 1972, vol. 8, pp. 252–254.
12. Whitford, G.M., Intake and Metabolism of Fluoride, *Adv. Dent. Res.*, 1994, vol. 8, pp. 5–14.
13. Dhar, V. and Bhatnagar, M., Physiology and Toxicity of Fluoride, *Indian J. Dent. Res.*, 2009, vol. 20, pp. 350–355.
14. Wong, M.H., Fung, K.F, and Carr, H.P., Aluminium and Fuoride Contents of Tea, with Emphasis on Brick Tea and Their Implication, *Toxicol. Lett.*, 2003, vol. 137, pp. 111–120.
15. Xu, R.H., Yuan, H.H., and Fan, A., Endemic Fluorosis in China from Ingestion of Food Immersed in Hot Spring Water, *Bull. Environ. Contam. Toxicol.*, 1995, vol. 54, pp. 337–341.
16. Sorgdrager, B., Pal, T.M., de Loof, A.J.A., Dubois, A.E.J., and de Monchy, J.G.R., Occupational Asthma in Aluminum Potroom Workers Related to Pre-Employment Eosinophil Count, *Eur. Respir. J.*, 1995, vol. 8, pp. 1520–1524.
17. Gubbay, A.D. and Fitzpatrick, R.I., Dermal Hydrofluoric Acid Burns Resulting in Death, *Aust. N. Z. J. Surg.*, 1997, vol. 67, pp. 304–306.
18. Shulman, E. and Vallejo, M., Effects of Gastric Contents on the Bioavailability of Fluoride in Humans, *Pediatr. Dent.*, 1990, vol. 12, pp. 237–240.
19. Ekstrand, J. and Ehrnebo, M., Influence of Milk Products on Fluoride Bioavailability in Man, *Eur. J. Clin. Pharmacol.*, 1979, vol. 16, pp. 211–215.
20. Whitford, G.M., Callan, R., and Wang, H., Fluoride Absorption through the Hamster Cheek Pouch: a pH-Dependent Event, *J. Appl. Toxicol.*, 1982, vol. 2, pp. 303–306.
21. McIvor, M.E., Acute Fluoride Toxicity, *Drug Safety*, 1990, vol. 5, pp. 79–85.
22. Gutnecht, J. and Walter, A., Hydrofluoric and Nitric Acid Transport through Lipid Bilayer Membranes, *Biochim. Biophys. Acta*, 1981, vol. 644, pp. 153–156.
23. He, H., Ganapathy, V., Isales, C.M., and Whitford, G.M., pH-Dependent Fluoride Transport in Intestinal Brush Border Membrane Vesicles, *Biochim. Biophys. Acta*, 1998, vol. 1372, pp. 244–254.
24. Lambert, A. and Lowe, A.G., Chloride/Bicarbonate Exchange in Human Erythrocytes, *J. Physiol.*, 1978, vol. 275, pp. 51–63.
25. Ekstrand, J., Relationship between Fluoride in the Drinking Water and the Plasma Fluoride Concentration in Man, *Caries Res.*, 1978, vol. 12, pp. 123–127.
26. Parkins, F.M., Tinaoff, N., Moutinho, M., Anstey, M.B., and Waziri, M.H., Relationship of Human Plasma Fluoride and Bone Fluoride to Age, *Calcif. Tissue Res.*, 1974, vol. 16, pp. 335–338.
27. Shen, Y.W. and Taves, D.R., Fluoride Concentrations in the Human Placenta and Maternal and Cord Blood, *Am. J. Obstet. Gynecol.*, 1974, vol. 119, pp. 205–207.

28. Whitford, G.M., Pashley, D., and Reynolds, K., Fluoride Tissue Distribution: Short-Term Kinetics, *Am. J. Physiol.*, 1979, vol. 236, pp. F141–F148.
20. Schiffl, H. and Binswanger, U., Human Urinary Fluoride Excretion as Influenced by Renal Functional Impairment, *Nephron*, 1980, vol. 26, pp. 69–72.
30. Taves, D.R., Evidence that there are Two Forms of Fluoride in Human Serum, *Nature*, 1968, vol. 217, pp. 1050–1051.
31. Ekstrand, J., Ericsson, Y., and Rosell, S., Absence of Protein Bound Fluoride from Human Blood Plasma, *Arch. Oral Biol.*, 1977, vol. 22, pp. 229–232.
32. Venkateswarlu, P., Determination of Fluorine in Biological Materials: a Review, *Adv. Dent. Res.*, 1994, vol. 8, pp. 80–86.
33. Christie, W.W., Hamilton, J.T.G., and Harper, D.B., Mass Spectrometry of Fluorinated Fatty Acids in the Seed Oil of *Dichapetalum toxicarium*, *Chem. Phys. Lipids*, 1998, vol. 97, pp. 41–47.
34. Deng, H., O'Hagan, D., and Schaffrath, C., Fluorometabolite Biosynthesis and the Fluorinase from *Streptomyces cattleya*, *Nat. Prod. Rep.*, 2004, vol. 21, pp. 773–784.
35. Zechel, D.L., Reid, S.P., Nashiru, O., Mayer, C., Stoll, D., Jakeman, D.L., Warren, R.A., and Withers, S.G., Enzymatic Synthesis of Carbon-Fluorine Bonds, *J. Am. Chem. Soc.*, 2001, vol. 123, pp. 4350–4351.
36. Marquis, R.E., Clock, S.A., and Mota-Meira, M., Fluoride and Organic Weal Acids as Modulators of Microbial Physiology, *FEMS Microbiol. Rev.*, 2003, vol. 26, pp. 493–510.
37. Langer, U. and Gunther, T., Effects of Alkaline Dust Deposits from Phosphate Fertilizer Production on Microbial Biomass and Enzyme Activities in Grassland Soils, *Environ. Pollut.*, 2001, vol. 112, pp. 321–327.
38. Bhatnagar, M. and Bhatnagar, A., Algal and Cyanobacterial Responses to Fluoride, *Fluoride,* 2000, vol. 33, pp. 55–65.
39. Oliveira, L., Antia, N.J., and Bisalputra, T., Culture Studies on the Effects from Fluoride Pollution on the Growth of Marine Phytoplankters, *J. Fish. Res. Board Can.*, 1978, vol. 35, pp. 1500–1504.
40. Perkins, D.F. and Millar, R.O., Effects of Airborne Fluoride Emissions near an Aluminium Works in Wales: Part 1—Corticolous Lichens Growing on Broadleaved Trees, *Environ. Pollut.*, 1987, vol. 47, pp. 63–78.
41. Reddy, M.P. and Kaur, M., Sodium Fluoride Induced Growth and Metabolic Changes in *Salicornia branchiata* Roxb, *Water Air Soil Pollut.*, 2008, vol. 188, pp. 171–179.
42. Hill, A. and Pack, M., Effects of Atmospheric Fluoride on Plant Growth, *Fluoride Effects on Vegetation, Animals and Humans*, Shupe, J., Peterson, H., and Leone, N., Eds., Salt Lake City, Utah, 1983, pp. 105–113.
43. McCune, D.C., Lauver, T.L., and Hansen, K.S., Relationship of Concentration of Gaseous Hydrogen Fluoride to Incidence and Severity of Foliar Lesions in Black Spruce and White Spruce, *Can. J. For. Res.*, 1991, vol. 21, pp. 756–761.
44. Sidhu, S.S. and Staniforth, R.J., Effects of Atmospheric Fluorides on Foliage, and Cone and Seed Production in Balsam Fir, Spruce and Larch, *Can. J. Bot.*, 1986, vol. 64, pp. 923–931.
45. Sands, M., Nicol, S., and McMinn, A., Fluoride in Antarctic Marine Crustaceans, *Mar. Biol.*, 1998, vol. 132, pp. 591–598.
46. Wright, D.A. and Davison, A.W., The Accumulation of Fluoride by Marine and Intertidal Animals, *Environ. Pollut.*, 1975, vol. 8, pp. 1–13.
47. Metcalfe-Smith, J.L., Holtze, K.E., Sirota, G.R., Reid, J.J., and de Solla, S.R., Toxicity of Aqueous and Sediment-Associated Fluoride to Freshwater Organisms, *Environ. Toxicol. Chem.*, 2003, vol. 22, pp. 161–166.
48. Connell, A.D. and Airey, D.D., The Chronic Effects of Fluoride on the Estuarine Amphipods *Grandidierella lutosa* and *G. lignorum*, *Water Res.*, 1982, vol. 16, pp. 1313–1317.
49. Buse, A., Fluoride Accumulation in Invertebrates Near an Aluminum Reduction Plant in Wales, *Environ. Pollut. A*, 1986, vol. 41, pp. 199–217.
50. Shi, X., Zhuang, P., Zhang, L., Feng, G., Chen, L., Liu, J., Qu, L., and Wang, R., The Bioaccumulation of Fluoride Ion (F(–)) in Siberian Sturgeon (*Acipenser baerii*) under Laboratory Conditions, *Chemosphere*, 2009, vol. 75, pp. 376–380.
51. Milhaud, G., El Bahri, L., and Dridi, A., The Effects of Fluoride on Fish in Gabes Gulf, *Fluoride*, 1981, vol. 14, pp. 161–168.
52. Neuhold, J.M. and Sigler, W.F., Effect of Sodium Fluoride on Carp and Rainbow Trout, *Trans. Am. Fish. Soc.*, 1960, vol. 89, pp. 358–370.
53. Bartlett, J.D., Dwyer, S.E., Beniash, E., Skobe, Z., and Payne-Ferreira, T.L., Fluorosis: a New Model and New Insights, *J. Dent. Res.*, 2005, vol. 84, pp. 832–836.
54. Damkaer, D.M. and Dey, D.B., Evidence for Fluoride Effects on Salmon Passage at John Day Dam, Columbia River, 1982–1986, *North Am. J. Fish Manag.*, 1989, vol. 9, pp. 154–162.
55. Pillai, K.S. and Mane, U.H., The Effect of Fluoride on Fertilized Eggs of a Freshwater Fish, *Catla*

catla (Hamilton), *Toxicol. Lett.*, 1984, vol. 22, pp. 139–144.
56. Mishra, P.C. and Mohapatra, A.K., Haematological Characteristics and Bone Fluoride Content in *Bufo melanostictus* from an Aluminium Industrial Site, *Environ. Pollut.*, 1998, vol. 99, pp. 421–423.
57. Kaplan, H.M., Yee, N., and Glaczenski, S.S., Toxicity of Fluoride for Frogs, *Lab. Anim. Care*, 1964, vol. 14, pp. 185–188.
58. Kuusisto, A.N. and Telkkä, A., The Effect of Sodium Fluoride on the Metamorphosis of Tadpoles, *Acta Odontol. Scand.*, 1961, vol. 19, pp. 121–127.
59. Hahn, P.H., and Guenter, W., Effect of Dietary Fluoride and Aluminium on Laying Hen Performance and Fluoride Concentration in Blood, Soft Tissue, Bone, and Egg, *Poult. Sci.*, 1986, vol. 65, pp. 1343–1349.
60. Coetzee, C.B., Casey, N.H., and Meyer, J.A., Fluoride Tolerance of Laying Hens, *Br. Poult. Sci.*, 1997, vol. 38, pp. 597–602.
61. Smith, R.E., Torgersen, J., Long, P.H., and Maurer, J.K., Lethality and Bone Alterations in Chicken Embryos and Newly Hatched Chickens Given Bone-Active Agents, *Am. J. Vet. Res.*, 1988, vol. 49, pp. 1376–1381.
62. Hoffman, D.J., Pattee, O.H., and Wiemeyer, S.N., Effects of Fluoride on Screech Owl Reproduction: Teratological Evaluation, Growth, and Blood Chemistry in Hatchlings, *Toxicol. Lett.*, 1985, vol. 26, pp. 19–24.
63. Walton, K.C., Tooth Damage in Field Voles, Wood Mice and Moles in Areas Polluted by Fluoride from an Aluminium Reduction Plant, *Sci. Total. Environ.*, 1987, vol. 65, pp. 257–260.
64. Boulton, I.C., Cooke, J.A., and Johnson, M.S., Fluoride Accumulation and Toxicity in Laboratory Populations of Wild Small Mammals and White Mice, *J. Appl. Toxicol.*, 1995, vol. 15, pp. 423–431.
65. Boulton, I., Cooke, J., and Johnson, M., Age-Accumulation of Fluoride in an Experimental Population of Short-Tailed Field Voles (*Microtus agrestis* L.), *Sci. Total Environ.*, 1994, vol. 154, pp. 29–37.
66. Krasowska, A., Wlostowskim, T., and Bonda, E., Zinc Protection from Fluoride-Induced Testicular Injury in the Bank Vole (*Clethrionomys glareolus*), *Toxicol. Lett.*, 2004, vol. 147, pp. 229–235.
67. Walton, K.C., Fluoride in Fox Bone Near an Aluminium Reduction Plant in Anglesey, Wales, and Elsewhere in the United Kingdom, *Environ. Pollut. B.*, 1984, vol. 7, pp. 273–280.
68. Aulerich, R.J., Napolitano, A.C., and Bursian, S.J., Chronic Toxicity of Dietary Fluorine to Mink, *J. Anim. Sci.*, 1987, vol. 65, pp. 1759–1767.
69. Eckerlin, R.H., Krook, L., Maylin, G.A., and Carmichael, D., Toxic Effects of Food-Borne Fluoride in Silver Foxes, *Cornell Vet.*, 1986, vol. 76, pp. 395–402.
70. Kierdorf, U. and Kierdorf, H., Assessing Regional Variation of Environmental Fluoride Concentrations in Western Germany by Analysis of Antler Fluoride Content in Roe Deer (*Capreolus capreolus*), *Arch. Environ. Contam. Toxicol.*, 2002, vol. 42, pp. 99–104.
71. Zemek, F., Herman, M., Kierdorf, H., Kierdorf, U., and Sedlacek, F., Spatial Distribution of Dental Fluorosis in Roe Deer (*Capreolus capreolus*) from North Bohemia (Czech Republic) and Its Relationships with Environmental Factors, *Sci. Total Environ.*, 2006, vol. 370, pp. 491– 505.
72. Suttie, J.W., Hamilton, R.J., Clay, A.C., Tobin, M.L., and Moore, W.G., Effects of Fuoride Ingestion on White-Tailed Deer (*Odocoileus virginianus*), *J. Wildl. Dis.*, 1985, vol. 21, pp. 283–288.
73. Adelung, D., Bossmann, K., and Rossler, D., The Distribution of Fluoride in Some Antarctic Seals, *Polar Biol.*, 1985, vol. 5, pp. 31–34.
74. Landy, R.B., Lambertsen, R.H., Palsson, P.A., Krook, L., Nevius, A., and Eckerlin, R., Fluoride in the Bone and Diet of Fin Whales, *Balaenoptera physalus*, *Mar. Environ. Res.*, 1991, vol. 31, pp. 241–247.
75. Kidd, E.A.M. and Fejerskov, O., What Constitutes Dental Caries? Histopathology of Carious Enamel and Dentin Related to the Action of Cariogenic Biofilms, *J. Dent. Res.*, 2004, vol. 83, pp. C35–C38.
76. Robinson, C., Fluoride and the Caries Lesions: Interactions and Mechanism of Action, *Eur. Arch. Paediatr. Dent.*, 2009, vol. 10, pp. 136–140.
77. Kaminsky, L., Mahoney, M., Leach, J., Melius, J., and Miller, M., Fluoride: Benefits and Risks of Exposure, *Crit. Rev. Oral Biol. Med.*, 1990, vol. 1, pp. 261–281.
78. Aoba, T. and Fejerskov, O., Dental Fluorosis: Chemistry and Biology, *Crit. Rev. Oral Biol. Med.*, 2002, vol. 13, pp. 155–170.
79. Bronckers, A.L.J.J., Lyaruu, D.M., and DenBesten, P.K., The Impact of Fluoride on Ameloblasts and the Mechanisms of Enamel Fluorosis, *J. Dent. Res.*, 2009, vol. 88, pp. 877–893.
80. Chachra, W., Vieira, A.P., and Grynpas, M.D., Fluoride and Mineralized Tissues, *Crit. Rev. Biomed. Eng.*, 2008, vol. 36, pp. 183–223.
81. Allolio, B. and Lehmann, R., Drinking Water Fluoridation and Bone, *Exp. Clin. Endocrinol. Diabetes*, 1999, vol. 107, pp. 12–20.

82. Reddy, D.R., Neurology of Endemic Skeletal Fluorosis, *Neurol. India*, 2009, vol. 57, pp. 7–12.
83. Kumar, H., Boban, M., and Tiwari, M., Skeletal Fluororis Causing High Cervical Myelopathy, *J. Clin. Neurosci.*, 2009, vol. 16, pp. 828–830.
84. Xiong, X., Liu, J., He, W., Xia, T., He, P., Chen, X., Yang, K., and Wang, A., Dose-Effect Relationship Between Drinking Water Fluoride Levels and Damage to Liver and Kidney Functions in Children, *Environ Res.*, 2007, vol. 103, pp. 112–116.
85. Tamer, M.N., Kale Koroglu, B., Arslan, C., Akdogan, M., Koroglu, M., Cam, H., and Yildiz, M., Osteosclerosis due to Endemic Fluorosis, *Sci. Total Environ.*, 2007, vol. 373, pp. 43–48.
86. Zachariassen, K.E. and Flaten, T.P., Is Fluoride-Induced Hyperthyroidism a Cause of Psychosis among East African Immigrants to Scandinavia?, *Med. Hypotheses*, 2009, vol. 72, pp. 501–503.
87. Tang, Q.-Q., Du, J., Ma, H.-H., Jiang, S.-J., and Zhou, X.-J., Fluoride and Children's Intelligence: a Meta Analysis, *Biol. Trace Elem. Res.*, 2008, vol. 126, pp. 115–120.
88. Trivedi, M.H., Verma, R.J., Chinoy, N.J., et al., Effect of High Fluoride Water on Intelligence of School Children in India, *Fluoride*, 2007, vol. 40, pp. 178–183.
89. Hart, R., Feelymyer, J., Gray, C., Lodise, T., Patel, N., Wymer, S., and McNutt, L.-A., Relationship Between Municipal Water Fluoridation and Preterm Birth in Upstate New York, *Abstracts of APHA 137th Annual Meeting*, 2009, Paper 197468.