Please note that this document, published on 7 June 2006, replaces the earlier version which contained an error in pages 1, 12, 13, 44 and 58.

Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Fluoride

(Request N° EFSA-Q-2003-018)

(adopted on 22 February 2005)

SUMMARY

Fluoride is not essential for human growth and development but is beneficial in the prevention of dental caries (tooth decay) when ingested in amounts of about 0.05 mg/kg body weight per day and when applied topically with dental products such as toothpaste. Dental enamel which contains fluoride is less likely to develop caries, because of greater resistance to ingested acids or to acids generated from ingested sugars by the oral bacteria. In addition, fluoride inhibits sugar metabolism by oral bacteria.

Fluoride content of the body is not under physiological control. Absorbed fluoride is partly retained in bone and partly excreted, predominantly via the kidney. In infants retention in bone can be as high as 90% of the absorbed amount, whereas in adults retention is 50% or less. Fluoride is also incorporated into dental enamel during tooth formation.

Excessive intake of fluoride during enamel maturation before tooth eruption from birth to eight years of age, when enamel formation is complete, can lead to reduced mineral content of enamel and to dental fluorosis of deciduous but predominantly of permanent teeth. The incidence and severity of dental fluorosis is dose-dependent. Mild dental fluorosis is not readily apparent and is associated with increased resistance to caries. The Panel considered moderate dental fluorosis, which is characterised by staining and minute pitting of teeth, to be an adverse effect. On the basis that the prevalence of moderate dental fluorosis of permanent teeth is less than 5% in populations ingesting 0.08-0.12 mg fluoride/kg body weight/day, the Panel considered that the upper level (UL) for fluoride is 0.1 mg fluoride/kg/day in children aged 1-8 years. This is equivalent to 1.5 and 2.5 mg fluoride per day in children aged 1-3 years and 4-8 years, respectively.

Fluoride accretion in bone increases bone density but excessive long term intake reduces bone strength and increases risk of fracture and skeletal fluorosis (stiffness of joints, skeletal deformities). Studies with therapeutic oral administration of fluoride in amounts of 0.6 mg/kg body weight/day in postmenopausal women over several years increased the risk for non-vertebral bone fractures significantly. The Panel applied an uncertainty factor of 5 to derive an UL of 0.12 mg/kg body weight/day. This is equivalent to an UL of 5 mg/day in children aged 9-14 years and 7 mg/day for age 15 years and older, including pregnant and lactating women.

The UL for fluoride applies to intake from water, beverages, foodstuffs, including fluoridated salt, dental health products and fluoride tablets for caries prevention.

Children aged 1-8 years have fluoride intakes from food and water well below the UL provided the fluoride content of their drinking water is not higher than 1.0 mg/L. An increase

in the prevalence of mild dental fluorosis observed in some countries has been attributed to the inappropriate use of dental care products, particularly of fluoridated toothpaste.

The Panel did not establish an UL for infants. Breast-fed infants have very low fluoride intakes from human milk (2-40 μg/day) and are not at risk of developing enamel fluorosis even when given fluoride supplements of 0.25 mg/day. The Panel notes that the Scientific Committee on Food has recommended a maximum fluoride level of 0.6-0.7 mg/L in infant formula and follow on formula, equivalent to an intake of about 0.1 mg/kg body weight per day in infants during the first six months of life (body weight 5 kg). For powdered formula, this maximum will be exceeded if water containing more than 0.7 mg/L is used for its preparation.

For children older than eight years and adults the probability of exceeding the UL of 5/7 mg fluoride/day on a normal diet is generally estimated to be low. However, consumption of water with a high fluoride content, e.g. more than 2-3 mg/L, predisposes to exceeding the UL.

KEY WORDS

Fluorine, fluoride, fluorosis, bone, teeth, drinking water, food, supplement, dental product.

BACKGROUND

In 2002, the European Parliament and the Council adopted Directive 2002/46/EC[1] related to food supplements containing vitamins and minerals.

In addition, and as announced in its White Paper on Food Safety, the Commission aims to put forward a proposal for harmonising legislation concerning the addition of vitamins and minerals to foods.

With a view to provide scientific support to the European Commission's legislative work in this field, the Scientific Committee on Food (SCF) issued, from October 2000 to April 2003, a series of opinions on tolerable upper intake levels of individual vitamins and minerals and safety factors in relation to their use in fortified foods and food supplements (available on the Internet at: http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).

The SCF opinions covered 22 out of the 29 nutrients, which were considered to be within their mandate for this task. In addition, during the decision making process for the adoption of Directive 2000/46/EC on food supplements the Parliament requested the inclusion of boron, nickel, silicon, vanadium and tin in the proposal. The Commission did not accept the Parliament's request in the absence of a positive safety evaluation by the SCF. Therefore, the European Food Safety Authority is asked to provide scientific opinions on the remaining 12 vitamins and minerals in accordance with the present terms of reference.

[1] Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements. OJ L 183. 12.7.2002, p. 51.

TERMS OF REFERENCE

With respect to the outstanding 12 vitamins and minerals, the European Food Safety Authority is asked 1) to review the upper levels of daily intakes that are unlikely to pose a risk of adverse health effects; 2) to provide the basis for the establishment of safety factors, where necessary, which would ensure the safety of fortified foods and food supplements containing the aforementioned nutrients.

ASSESSMENT

1. INTRODUCTION

Fluorine is a gaseous halogen with an atomic mass of 18.998. It is the most electronegative and reactive of all elements, therefore it occurs naturally only in ionic forms, fluorides, after reaction with metallic elements or with hydrogen. Fluorides are ubiquitous in air, water and the lithosphere, where they are seventeenth in the order of frequency of occurrence (0.06-0.09% of the earth's crust) (WHO, 1994). Fluorides occur in rocks and soil as fluorspar (CaF_2), cryolite ($3NaFxAlF_3$) or apatite ($3Ca_3(PO4)2xCa(F,OH,Cl)_2$, in mica, hornblende, or as pegmatites like topaz and tourmaline. Cryolite used for the production of aluminium and rock phosphates used for the production of fertilisers can have fluoride contents up to 4.2%. Most of this fluoride is firmly bound and not biologically available. Availability of fluoride from soil depends on the solubility of the fluoride compound, the acidity of the soil and the presence of water.

All water contains fluorides, sea water between 1.2 and 1.5 mg/L. Waters with high fluoride content are usually found at the foot of high mountains. Ground water with fluoride concentrations as high as 25 mg/L have been found. Surface water usually has lower fluoride content below 0.5 mg/L, but very high fluoride levels have been found in lakes in Tanzania (95 mg/L) and Kenya (2800 mg/L) (WHO, 2000).

Fluoride in air exists in gaseous or particulate forms and arises from fluoride containing soils, industry, coal fires and especially volcanoes. In non-industrial areas it ranges between 0.05-1.9 $\mu g/m^3$. Hydrogen fluoride, a highly corrosive gas or liquid at room temperature is used extensively by industry. It readily dissolves in water to hydrofluoric acid, which though a weak acid, etches glass and because of its industrial use is the most important atmosphere contaminant. It is rapidly converted to fluoride salts.

The most important fluorides for human use are sodium and potassium fluoride, which are highly soluble in water. They are used for addition to foods (e.g. salt), dental products and fluoridation of water. They are permitted for use in foods for particular nutritional uses (FPNU) and food supplements (Commission Directive 2001/15/EC; Directive 2002/46/EC).

In Annex III part 1 of the amended Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products, 20 fluoride compounds are listed which may be used in oral hygiene products up to a maximum concentration in the finished products of 0.15% (1500 ppm), calculated as fluorine.

Fluorosilicic acid or hydrofluorosilicic acid (H_2SiF_6) or sodiumhexafluorosilicate (Na_2SiF_6) are used for drinking water fluoridation.

2. NUTRITIONAL BACKGROUND, FUNCTION, METABOLISM AND INTAKE

2.1 Function of fluoride

There is insufficient evidence for the indispensability of fluoride for human health. Because of the ubiquity of fluoride it is virtually impossible to create an experimental situation free of fluoride.

Schwarz and Milne (1972) reared several generations of F344 rats in isolators on a fluoride-deficient diet (0.002-0.023 mg/kg/day). Rats on this diet showed decreased gain in weight and bleached incisors. Weight gain was improved by fluoride supplementation of the diet (2.5 mg/kg), tooth pigmentation was not. Rats in both the group on the fluoride-deficient and the fluoride-supplemented diet had shaggy fur, loss of hair and seborrhoea, indicative of a probable deficiency of other nutrients in the synthetic diet as well.

In a cohort study of 109 infants exclusively breast-fed for at least four months (fluoride in breast-milk 0.003 mg/L) and living in an area with low fluoride content of the drinking water (0.018-0.166 mg/L), those receiving a fluoride supplement from the 6^{th} day of life onwards in addition to their fluoride intake of less than 0.003 mg/day from human milk, showed a significantly greater increase in length and weight, especially when the mother had taken fluoride supplements during pregnancy, and a significantly (by 12 days) earlier eruption of the first tooth in boys, than those who did not receive a fluoride supplement during the first six months of life (Bergmann, 1994). Although suggestive, these results do not prove an essential role of fluoride in human development and growth.

In vitro, fluoride (0.02-0.1 mg/L) addition to a supersaturated solution of calcium phosphate initiates the formation of hydroxylapatite ($Ca_3(PO_4)_2 \cdot Ca(OH)_2$) which is the mineral substance of bone and teeth. With increasing fluoride concentrations fluoroapatite ($Ca_3(PO_4)_2 \cdot CaF_2$) is formed and results in more regular and bigger apatite crystals which are less acid soluble (Featherstone *et al.*, 1983; Newesely, 1961; Okazaki *et al.*, 1985).

Fluoride in the body is mainly associated with calcified tissue (bone and teeth) due to its high affinity for calcium. In bone the substitution of fluoride for hydroxyl groups in apatite alters the mineral structure of the bone. This is electrostatically more stable and more compact and results in increased density and hardness, but not increased mechanical strength in rabbit (Chachra *et al.*, 1999). Both in rats and in humans there is evidence for a biphasic effect of fluoride on bone strength, with increases in both bone strength and bone fluoride content at moderately high fluoride intake (16 mg/L in drinking water of rats during 16 weeks) leading to a bone fluoride content of up to 1200 mg/kg and a decrease with higher fluoride intake (up to 128 mg/L in drinking water) and bone fluoride content up to 10,000 mg/kg (Turner *et al.*, 1992).

Besides the physicochemical effects of fluoride on the bone, fluoride in high doses (0.02-0.2 mg/L) was found to be mitogenic in osteoblasts and inhibitory to osteoclasts of chicken embryos *in vitro* (Farley *et al.*, 1983; 1988; Gruber and Baylink, 1991). The mitogenic effect is restricted to osteoblastic precursors (Bonjour *et al.*, 1993) and the same fluoride dose can be toxic to individual osteoblasts (Chachra *et al.*, 1999). Fluoride can activate thyroid adenylate cyclase (ATP pyrophosphate-lyase (cyclizing)) *in vitro* at very high concentrations (10 mg/L or 190 mg/L) (Goldhammer and Wolff, 1982).

Fluoride has a cariostatic effect on erupted teeth of both children and adults. A pre-eruptive effect of fluoride through increasing fluoridation of the developing enamel is supported by evidence (Groeneveld *et al.*, 1990; Murray, 1993), but difficult to differentiate from the cariostatic effect of fluoride on erupted teeth. The prevalence of dental caries in a population was not inversely related to the concentration of fluoride in enamel (Clarkson *et al.*, 1996), which apart from the outmost surface is accumulated through pre-eruptive enamel development (Richards *et al.*, 1992; Weatherall and Robinson, 1988). Fluoridated enamel is less acid soluble (Beltran and Burt, 1988). It was also demonstrated that the positive effect on reduction of caries in both deciduous and permanent teeth was more marked the earlier children were exposed to fluoridated water or fluoride supplements (Groeneveld *et al.*, 1990; Stephen *et al.*, 1987). Comparisons of caries prevalence between two communities in England with water fluoride concentrations of 0.2 and 1.5-2.0 mg/L showed that in all age groups (from 15 to >44 years) caries experience of all teeth was significantly lower in the community with high fluoride water concentration (44% less in persons over 45 years) (Jackson *et al.*, 1973). A similar study in Sweden compared caries prevalence in 30 to 40-years old life-long residents of Uppsala (n=260; water fluoride concentration 1.0 mg/L) with those of Enköping (n=236; fluoride in water 0.3 mg/L) and found 21% units less decayed and filled surfaces in Uppsala. Caries prevalence in that study was not influenced by other topical fluoride sources (Wiktorsson *et al.*, 1992).

The cariostatic effect of fluoride in saliva or plaque on erupted teeth is due to an inhibition of the demineralisation of sound enamel by ingested acid foods or acid formed by cariogenic bacteria in the dental plaque and by enhancing remineralisation of demineralised enamel. Demineralised enamel takes up more fluoride than sound enamel and the resultant structure is more acid resistant and contains more fluoride (Featherstone, 1999; White and Nancollas, 1990). Moreover, fluoride affects the metabolism of carbohydrates and the production of adhesive polysaccharides by cariogenic bacteria (Hamilton, 1990). However, caries is not a fluoride deficiency disease and no specific fluoride deficiency syndrome has been found.

2.2 Fluoride homeostasis

Ninety-nine percent of the total fluoride content of the body is concentrated in calcified tissue. Body fluid and soft tissue fluoride concentrations are not under homeostatic control and reflect the recent intake (Ekstrand *et al.*, 1977). In blood the fluoride ion concentration in plasma is twice that in blood cells (Whitford, 1996). Via the plasma fluoride is distributed to all tissues. The ratio fluoride in soft tissue to fluoride in plasma is between 0.4 and 0.9. Exceptions are the kidney, pineal gland, brain and adipose tissue. The kidney can accumulate fluoride to higher concentrations than in plasma (Taves *et al.*, 1983). Experiments with radioactive fluoride have shown that it is not actively transported into the thyroid gland of humans or rats. Nonetheless, after long-term exposure to a high fluoride content in feed or water, the thyroid glands of some animals (cows and rats) have been found to contain increased fluoride levels compared to their non-exposed controls (Bürgi *et al.*, 1984).

2.2.1 Intestinal fluoride absorption

Inhalation of fluoride from the air, as a rule, does not contribute more than 0.01 mg/day to the total intake, except in occupational settings where intake by that route can be several milligrams (Hodge and Smith, 1977). For the purpose of setting an UL for oral exposure to fluoride, exposure via inhalation is not relevant and shall not be taken into account.

Readily soluble fluorides (sodium, hydrogen, fluorosilicic, sodium monophosphate) are rapidly almost completely absorbed with a plasma peak level occurring after 30 minutes (70, 130, 300, 450 μg/L after single doses of 1.5, 3, 6, 10 mg of fluoride as the sodium salt, respectively), in contrast to the low-soluble fluoride compounds calcium fluoride, magnesium fluoride and aluminium fluoride. Fluoride from toothpaste is also absorbed. Sodium monofluorophosphate from toothpaste needs dephosphorylation before absorption in the lower intestine. There is variability in the bioavailability of fluoride from different foods (Trautner and Siebert, 1983).

Most of fluoride is absorbed as undissociated hydrogen fluoride and absorption occurs by passive diffusion in both the stomach and the small intestine. Higher acidity of the stomach increases absorption. The presence of calcium, magnesium, phosphorus and aluminium decreases the absorption of fluoride (Cerklewski, 1997; Harrison *et al.*, 1984; Kuhr *et al.*, 1987; McClure *et al.*, 1945; Spencer *et al.*, 1981). In the case of calcium the inhibitory effect depends on the presence of food. Sodium fluoride tablets given in water on an empty stomach were almost 100% absorbed. The same doses given together with milk were 70% absorbed, and were 60% absorbed when given with a meal (Ekstrand and Ehrnebo, 1979; Shulman and Vallejo, 1990; Trautner and Einwag, 1987). Consecutively faecal fluoride excretion is increased.

2.2.2 Fluoride distribution and storage in the body

Absorbed fluoride is rapidly distributed by the circulation to the intracellular and extracellular fluid but is retained only in calcified tissues. The fluoride plasma concentration is dependent on the fluoride dose ingested, dose frequency and the plasma half-life, which was determined to be 3-9 hours after giving doses of 3 to 10 mg as tablets orally. The plasma clearance of fluoride ranged between 0.12 and 0.2 L/kg/h independent on the dose (Ekstrand *et al.*, 1977). Plasma fluoride occurs in both ionic and non-ionic forms. The non-ionic fluoride in plasma consists mostly of fat-soluble fluorocompounds. Ionic fluoride is not bound to plasma proteins or other compounds. Its level (μmol) reflects the recent fluoride intake and the fluoride content of drinking water (in mg/L) when water is the predominant fluoride source (WHO, 1994). Plasma fluoride levels increase with age and with increasing fluoride content of bone, and as a consequence of renal insufficiency (Ekstrand and Whitford, 1988; Ekstrand *et al.*, 1978; Singer and Ophaug, 1979).

Fluoride concentrations in plasma ranging from 0.4-2.4 μmol/L (7.6-45.6 μg/L) have been reported in healthy adults (IPCS, 2002). Concentrations are lower (<10μg/L) in persons living in areas with a low fluoride content in the drinking water (<0.2 mg/L) and the diet (Ekstrand *et al.*, 1977; Fuchs *et al.*, 1975; Schiffl and Binswanger, 1980), somewhat higher (13 μg/L) in those whose drinking water is fluoridated (1 mg/L) (Taves, 1966), and can be twenty-fold elevated in patients with both skeletal and dental fluorosis due to high fluoride levels in drinking water (>8 mg/L) (Jha *et al.*, 1982). Circulating fluoride passes the placenta and reaches the fetus. The level of fluoride in cord blood is about 75% of the level in maternal blood. The fluoride concentration in the placenta can be higher than in maternal blood. Use of 1.5 mg fluoride supplements during pregnancy markedly increased placental fluoride levels and to a lesser extent fetal blood levels (Caldera *et al.*, 1988; Shen and Taves, 1974).

Fluoride concentrations in ductal and glandular saliva closely follow the plasma concentration but at a lower level (about two-thirds of the plasma level (Ekstrand, 1977; Whitford *et al.*, 1999a). Apart from the intake via water and diet the fluoride concentration in saliva and

dental plaque is dependent on topical fluoride application via dental care products (Oliveby *et al.*, 1990; Ekstrand, 1997; Ekstrand, 1977; Ekstrand *et al*., 1977; Featherstone, 1999; Hetzer, 1997; Sjögren *et al.*, 1993; Twetman *et al.*, 1998). Children with no caries experience were found to have higher salivary fluoride concentrations than children highly affected by caries (40 versus 20 μg/L) (Shields *et al.*, 1987).

Fluoride retention in bone (and dentine) is proportional to the long-term fluoride exposure and, moreover, dependent on the turnover rate of bone, on age, gender and the type of bone (Caraccio *et al.*, 1983). Infants and young children will retain up to 75% of the absorbed dose in skeletal tissue. Exclusively breast-fed infants not receiving a fluoride supplement showed negative fluoride balances up to the age of four months and excreted more fluoride than they ingested (Bergmann, 1994).

Fluoride is primarily taken up on the surface of bone crystallites via isoionic and heteroionic exchange. It is later incorporated into the crystal lattice structure of teeth and bone by replacing hydroxyl ions and producing partially fluoridated hydroxyapatite (WHO, 1994).

Fluoride is not irreversibly bound to bone, as has been demonstrated in persons who after having lived in areas with a high fluoride concentration in drinking water moved to an area with low fluoride levels in water. Their urinary fluoride excretion fell slowly over many years and their plasma fluoride levels remained high, indicating release of fluoride from remodelling of bone (WHO, 1994; Khandare *et al.*, 2004).

A linear relationship between the fluoride content of drinking water and bone fluoride content was reported by Zipkin (1958). Fluoride increases with age in bone, more rapidly in women than in men and preferably in cancelleous bone (Alhava *et al.*, 1980; Eble *et al.*, 1992). The fluoride concentration in bone ash from 28 stillborn infants and of infants dying during the first days of life was around 70 mg/kg and not related to gestational age, weight or length (Bergmann, 1994).

In contrast to skeletal bone and dentine which accumulate fluoride throughout life and in proportion to the absorbed dose of fluoride, enamel of teeth reflects the biologically available fluoride at the time of tooth formation. Enamel maturation of deciduous teeth is completed between the age of 2 to 12 months. In permanent teeth enamel maturation is completed at the age of 7-8 years, except in the third molars, in which it continues until the age of 12-16 years. Post-eruptive fluoride uptake of enamel is expressed only in the outer layer and depends on fluoride in saliva, food, dental plaque and dental products (WHO, 1994). In areas with low fluoride concentrations in drinking water (≤0.1 mg/L) the fluoride concentration at 2 micrometer depth of enamel averages 1700 mg/kg, with fluoride concentrations in water of 1 mg/L it is 2200-3200 mg/kg. When water contains 5-7 mg/L of fluoride the concentration in enamel has been 4800 mg/kg. Such concentrations usually are accompanied by dental fluorosis (NRC, 1993).

2.2.3 Excretion of fluoride

Absorbed fluoride which is not deposited in calcified tissue is excreted almost exclusively via the kidney. The percentage of absorbed fluoride excreted via the kidney is about 50% in healthy young and middle-aged adults, in young infants and children it can be only 10-20%, in elderly persons higher than 50%. Fluoride is filtered in the renal glomeruli and reabsorbed in the renal tubuli (10-90%), dependent on the pH of the tubular fluid. The renal clearance of

fluoride is 30-50 mL/min in adults (Ekstrand *et al.*, 1982; Schiffl and Binswanger, 1982). Fluoride excretion is reduced with impaired renal function (Schiffl and Binswanger, 1980; Spak *et al.*, 1985; Torra *et al.*, 1998).

About 10-25% of the daily intake of fluoride is excreted via the faeces (WHO, 1994).

Fluoride concentration in human milk is reported to range between 2 and 95 μg/L (IPCS, 2002), which wide range is probably due to analytical difficulties. Whereas Spak *et al.* (1983) found no correlation between the fluoride content of drinking water (0.2 to 1 mg/L) and fluoride content of human milk (7.6 μg/L), Dabeka *et al.* (1986) could show a relationship: 32 mothers in an area with fluoride in drinking water of <0.16 mg/L secreted milk with an average fluoride concentration of 4.4 μg/L, while 112 mothers in an area with drinking water fluoride concentrations of 1 mg/L had fluoride concentrations in their milk of 9.1 μg/L. Ekstrand *et al.* (1981) have shown that fluoride supplements of 1.5 mg given to the mothers did not increase the fluoride concentration in milk. Very variable fluoride concentrations in human milk were reported also from Finland (1.9-51.3 μg/L) (Esala *et al.*, 1982) and very low concentrations from Germany 3-4 μg/L in areas with low fluoride in drinking water (<0.2 mg/L). There was no change in the fluoride concentration with progression of lactation (Bergmann, 1994).

2.2.4 Biomarkers for fluoride exposure and status

The determination of the fluoride concentration in body fluids (urine, plasma, saliva) gives some indication of recent fluoride intake and does not well reflect the fluoride body burden. Renal fluoride excretion varies, moreover, with urinary flow and pH. There is no clear-cut relationship between fluoride content in bone and extracellular fluids. The concentration of fluoride in nails (50% higher in finger than in toenails) and hair appears to be proportional to the exposure over longer periods of time taking into account their growth rate (Czarnowski and Krechniak, 1990; Schamschula *et al.*, 1985; Kono *et al.*, 1990; Whitford *et al.*, 1999b). An additional daily intake of 3.0 mg fluoride over 30 days resulted immediately in a 90% increase of the basal urinary fluoride excretion and three months later in an increase of the fluoride content of fingernails (Whitford *et al.*, 1999b). Subjects living in areas with high fluoride content in water (1.6-3.1 mg/L) had 1.8 and 2.9 times higher fluoride contents in fingernails than subjects from areas with intermediate (0.5-1.1 mg/L) and low (<0.11 mg/L) fluoride content in the water, respectively (Schamschula *et al.*, 1985).

Fluoride concentrations in calcified tissues reflect the historical body burden. This concerns especially the skeleton, taking into account that fluoride is not evenly distributed and is for example higher in cancellous than in cortical bone (Alhava *et al.*, 1980). The fluoride content in enamel is indicative of the amount taken up during tooth formation, whereas the surface layers of enamel of erupted teeth is affected by the fluoride concentrations in the mouth. The fluoride content in enamel biopsies from 137 children aged 14 years at 0.44-0.48 μm and 2.4-2.6 μm depth was proportional to the fluoride content of the drinking water (0.09 versus 1.9 mg/L: 1549 and 641 versus 3790 and 2110 mg/kg, respectively) (Schamschula *et al.*, 1985). Dentine, which like bone slowly increases in fluoride content throughout life and, unlike bone, does not undergo resorption, is probably the most suitable indicator of chronic fluoride intake.

The incidence of dental fluorosis in a population is related to the concentration of fluoride in drinking water (Dean, 1942) and from food (Liang *et al.*, 1997). It can be considered as a

biomarker for total exposure during the time of life when enamel is formed (up to age 7-8 years) (WHO, 1994).

2.3 Recommended dietary intakes for functional effects and typical intakes

2.3.1 Adequate intakes

The SCF did not define adequate or recommended fluoride intakes (SCF, 1993). Other bodies defined adequate fluoride intakes on the basis of the negative relationship between caries prevalence and fluoride intake (FNB, 1997; D-A-CH, 2000).

There is no convincing evidence that health and development of humans depend on the intake of fluoride, however, due to the ubiquitous presence of fluoride in the environment a zero exposure is not possible under normal circumstances.

Based on epidemiological studies of the inverse relationship between dental caries and the concentration of fluoride in drinking water in the 1940s it was concluded that fluoride has a beneficial effect in increasing the resistance to dental caries in children (Dean *et al.*, 1942) and at all ages (Russell and Elvove, 1951). In communities with water fluoride concentrations (0.7 to 1.2 mg/L, depending on the average regional temperature) the caries prevalence was 40-60% lower than in communities with low water fluoride concentrations. The studies of Dean (1942) had also shown that a positive relationship existed between water fluoride concentration and the prevalence of dental fluorosis. A concentration of about 1 mg fluoride/L in drinking water was identified as being "optimal" both in reducing caries prevalence and keeping dental fluorosis prevalence below 10% in the population. This fluorosis was of the mild to very mild type (see Annex 2) and practically none of the moderate to severe type.

From this "optimal" water fluoride concentration derives the estimated adequate fluoride intake of infants and children above the age of 6 months of 0.05 mg/kg body weight/day (Burt, 1992; Singer and Ophaug, 1979): age 7-12 months 0.5 mg/day; age 1-3 years 0.7 mg/day; age 4-8 years 1 mg/day; age 9-13 years 2 mg/day; age 14-18 years 3 mg/day; for females and males of 19 years and above 3 and 4 mg/day, respectively (FNB, 1997). The guidance reference values of the Austrian, German and Swiss Nutritional societies are based on the same calculation (D-A-CH, 2000).There is a difference in the adequate intake or guidance value for fluoride below the age of six months defined by the FNB and by D-A-CH. The very low fluoride intake of breast-fed infants which is about 0.01 mg/day is defined as the adequate intake for age 0-6 months by the FNB. Assuming an average body weight of 5 kg for an infant of that age group and a guidance value of 0.05 mg/kg body weight/day a guidance value of 0.25 mg fluoride/day has been calculated (D-A-CH, 2000).

2.3.2 Fluoride intake (exposure)

Fluoride exposure via inhalation and the skin will not be considered, because in normal circumstances they contribute little to the total intake. However, the fluoride content of food dried over high-fluoride coal fires can increase considerably (from 5- to 50-fold) and be a significant source of oral ingestion, as shown in China (Liang *et al.*, 1997).

Exposure by oral ingestion of fluoride is by water, food (including fluoridated salt available in Austria, Belgium, Czech Republic, France, Germany, Spain and Switzerland), cosmetic dental

products and fluoride supplements. Fluoride supplements are considered to be drugs in most countries of the European Community.

2.3.2.1 Water

Among the main sources of total fluoride intake in Europe are drinking and mineral waters with more than 0.3 mg/L of fluoride. From U.S. and Canadian studies the total fluoride intake of adults in areas with different fluoride content of drinking water was estimated: 0.3-1 mg/day, 1.4-3.4 mg/day with water fluoride content <0.3 mg/L and 1.0 mg/L, respectively (FNB, 1997).

Fluoride concentrations in drinking water in Europe differ between countries and within countries dependent on natural circumstances and on water fluoridation (United Kingdom, Ireland, Spain). In Ireland, the recommended fluoride content of public drinking water was recently reduced from 0.8-1.0 mg/L to 0.6-to 0.8 mg/L (Government of Ireland, 2002). Water fluoridation which had been practiced in Basel, Switzerland since 1962 (0.7-0.9 mg/L) was terminated in 2003 and fluoride content in water has returned to its natural low level of 0.1-0.2 mg/L (KL BS, 2003).

In Germany the fluoride concentration in groundwater is generally low. A survey based on 1040 sample points measured a mean fluoride concentration of 0.1 mg/L with a minimum of less than 0.1 mg/L, and a maximum value of 1.1 mg/L (Schleyer and Kerndorff, 1992).

Fluoride concentrations in drinking water collected during 1985 from public water plants in the Netherlands was 0.04-0.23 mg/L (Sloof *et al.*, 1989). The range of fluoride concentrations in 5900 groundwater samples from Finland was reported to be <0.1-3.0 mg/L (Lahermo *et al.*, 1990). Fluoride concentration in 4000 drinking water samples from 36 districts in the Czech Republic ranged between 0.05 and 3.0 mg/L (NIPH, 1996) and it was 0.02-3.0 mg/L in drinking water from 94 locations in Poland (Czarnowski *et al.*, 1996). The highest fluoride content in drinking water of the canton Valois, Switzerland was found to be 0.9 mg/L, whereas about half of the cantonal area was served with drinking water containing less than 0.1 mg/L (Rapport Annuel, 1999).

Total tap water intake of adolescents in the UK and in Germany was 676 g/day and 718 g/day, respectively (Sichert-Hellert *et al.*, 2001; Zohouri *et al.*, 2004). Total fluoride intake from all kind of drinks in British adolescents was estimated to be 0.47 mg/day.

Drinking tap water, however, is increasingly replaced by the use of bottled water. Whereas drinking water for human consumption according to Council Directive 98/83/EC, following the advice of the Scientific Committee on Food (SCF, 1998), may not contain more than 1.5 mg fluoride/L, bottled natural mineral waters can have higher fluoride levels. Natural mineral waters which contain more than 1 mg fluoride/L can be labelled as “contains fluoride”. According to Council Directive 88/777/EEC on the approximation of the laws of the Member States relating to the exploitation and marketing of natural mineral waters, Member States can make national provisions for labelling a natural mineral water as suitable for the use in infant nutrition. According to Directive 2003/40/EEC the fluoride content of natural mineral waters must be not more than 5 mg/L by 1 January 2008. Mineral waters exceeding 1.5 mg fluoride/L shall bear on the label the words “contains more than 1.5 mg/L of fluoride: not suitable for regular consumption by infants and children under 7 years of age” and shall indicate the actual fluoride content.

A survey of 150 mineral and table waters from the German market measured an average fluoride concentration of 0.58 ± 0.71 mg/L: 24% had a fluoride concentration below 0.1 mg/L, 43% equal to or below 0.3 mg/L, 31% between 0.3 and 0.6 mg/L, and 8 (5%) waters had a fluoride concentration above 1.5 mg/L with a maximum value of 4.5 mg/L. The average consumption of bottled water in Germany at the time of the survey was estimated to be 104 L per year (Schulte *et al.*, 1996). In a similar survey of 33 bottled waters from the Swedish market a median fluoride concentration of 0.19 mg/L with a range of 0-3.05 mg/L was determined (Rosborg, 2002). The fluoride concentration in 25 commercial brands of bottled water (spring, mineral or distilled) available in the UK was 0.08 (± 0.08) mg/L with a range from 0.01-0.37 mg/L. The average bottled water intake was estimated to be 108 mL/day in adults (Zohouri *et al.*, 2003) and only 20 mL/day in adolescents (Zohouri *et al.*, 2004). Twenty-four mineral waters available in Belgium had fluoride concentrations below 1 mg/L in 16 cases, but the highest value found was 5.5 mg/L. A case of dental fluorosis in an eight-year old girl was attributed to the preparation of her infant formula with mineral water containing 1.2 mg fluoride/L. Her fluoride intake from age three months to age 12 months was well above 0.1 mg/kg body weight/day (Bottenberg, 2004)

2.3.2.2 Food

Fluoride intake from food is generally low except when food is prepared with fluoridated water. Exceptions are tea which can contain considerable amounts of fluoride (0.34-5.2 mg/L) (Schmidt and Funke, 1984; Wei *et al.*, 1989; Chan and Koh, 1996), dependent on type, brewing and fluoride content of water. Some brands of instant teas were reported to be another significant source of fluoride intake (up to 6.5 mg/L when prepared with distilled water) (Whyte *et al.*, 2005).

Vegetables and fruit, except when grown near fluoride emitting industrial plants, contain between 0.02 and 0.2 mg/kg fresh weight, milk and milk products 0.05-0.15 mg/kg, bread, cereals and meals 0.1-0.29 mg/kg, meat and meat products 0.15-0.29 mg/kg, eggs 0.18 mg/kg, fish and fish sticks 0.48-1.91 mg/kg (Bergmann, 1994; EGVM, 2001). The fluoride content of both fish and meat depends on the care taken with deboning, and can be as high as 5 mg/kg. (Bergmann, 1994). Dried herbs contain up to 2.0 mg/kg fluoride. Table 1 summarises the fluoride content in various types of foods from various parts of the world compiled by IPCS (2002) as well as Chinese data on corn and vegetables dried naturally or over high-fluoride coal fires (Liang *et al.*, 1997).

The fluoride content of the water used in industrial production and home cooking affects the fluoride content of the prepared food. The use of water containing 1 mg/L has been estimated to increase the fluoride content of the food by 0.5 mg/kg compared to low-fluoride water (Becker and Bruce, 1981; Marier and Rose, 1966).

Breast-fed infants receive very little fluoride, because human milk contains between 2-10 µg/L. An intake of 800 mL human milk corresponds to 1.6-8 µg/day or approximately 0.3-1.6 µg/kg/day (Bergmann, 1994; Fomon *et al.*, 2000). Infant formula, with the exception of soy protein based formula, has a low fluoride content when the powder is prepared with distilled water (0.01 to 0.05 mg/L). If these formulas were prepared with water containing 0.3 mg fluoride/L and a 5-kg infant drinks 800 mL, fluoride intakes of 60 µg fluoride/kg body weight/day or less would result. The use of fluoridated drinking water (1 mg/L) would considerably increase the fluoride intake threefold (Bergmann, 1994; Kramb *et al.*, 2001).

Table 1. Fluoride contents in some food categories (from IPCS, 2002)

Food	Fluoride (mg/kg)	Country of origin
Milk and milk products	0.01 - 0.8	Canada, Hungary, Germany
Meat and poultry	0.01 - 1.7	Canada, Hungary, Germany
Fish	0.06 - 4.57	Canada, USA
Soups	0.41 - 0.94	Canada, Hungary
Baked goods and cereals	0.04 - 1.85	Canada, China, Hungary, Germany
Vegetables	0.01 - 1.34	Canada, China, Hungary, Germany
Fruits and fruit juices	0.01 - 2.8	Canada, Hungary, Germany, USA
Fats and oils	0.05 - 0.13	Canada
Sugars and candies	0.05 - 0.13	Canada
Beverages	0.003 - 1.28	Canada, Hungary, Germany, USA
Tea leaves	82 - 371	China, Hungary, Hong Kong
brewed	0.05 - 4.97	Canada, Germany
Corn, dried naturally	0.55 - 5.48	China
Corn, dried over coal fire	3.25 - 246.1	China
Vegetables, fresh	0.31 - 9.25	China
Vegetables, dried over coal fire	8.0 - 52.0	China

Similar differences in fluoride content of infant formulas prepared with low-fluoride (0.2 mg/L) and high-fluoride (1 mg/L) water and in intakes from such formulas were calculated by Fomon *et al.* (2000). With increasing percentages of the population receiving fluoridated drinking water in the United States a parallel increase of the percentage of infants receiving more than 70 μg fluoride/kg body weight/day has been reported. Not all of this increase in fluoride intake was due to the increase in drinking water fluoridation, but to fluoride supplements (Fomon *et al.*, 2000). Since 1979, liquid ready-to-feed infant formulas in the United States and Canada contain 200 μg fluoride/L.

In a recent study from the United States a mathematical model to estimate the average daily fluoride intake from all dietary sources was applied. The average or central tendency exposure (CTE) and the high-end or reasonable maximum exposure (RME) of infants in areas without fluoridation of the drinking water was 0.074 and 0.11 mg/kg/day, respectively, whereas in areas with fluoridated drinking water the CTE and RME were 0.11 and 0.21 mg/kg/day. For children between the age of three and five years the same model calculations estimated the CTE and RME in areas without fluoridation to be 0.025 and 0.04 mg/kg/day, while in areas with fluoridation of the drinking water the values were 0.05 and 0.09 mg/kg/day, respectively (Erdal and Buchanan, 2005).

The fluoride intake of German children between 1 and 14.9 years of age and of adults was estimated from analysed fluoride concentrations in food and consumption data (Bergmann, 1994) (Table 2).

This model calculation demonstrates the importance of the fluoride content of drinking water for the total dietary fluoride intake and permits to estimate the effect of any additional intake of fluoride from supplements and drugs.

The average total dietary fluoride intake, including tea but excluding drinking water, of the adult population in the UK was estimated from the 1997 Total Diet Study to be 1.2 mg/day

(EGVM, 2001). In Sweden the fluoride intake from food and drink of adults in areas with low fluoride levels in drinking water (<0.4 mg/L) has been estimated to be 0.4-1.0 mg/day, while in areas with fluoride concentrations in the water of 1 mg/L the mean intake was estimated to be 2.1-4.4 mg/day (Becker and Bruce, 1981).

Table 2. Estimated fluoride intake of young children, adolescents and adults

Fluoride intake (mg/day)	age 1-1.9 years	age 12-14.9 years	adults
(1) Milk, meat, fish, eggs, cereals, vegetables, potatoes, fruit	0.042	0.114	0.120
(2) Fruit juice, soft drinks, mineral water, tea (adults)	0.011	0.065	0.259
(3) Sum fluoride from food and beverages (1)+(2)	**0.052**	**0.191**	**0.379**
(4) Drinking water (0.13 mg fluoride /L)	0.060	0.073	0.065
(5) Total fluoride intake ((3)+(4)	**0.112**	**0.264**	**0.444**
(6) Drinking water (1.0 mg fluoride /L)	0.458	0.560	0.500
(7) Total fluoride intake (3)+(6)	**0.510**	**0.751**	**0.879**
(8) Drinking water (2.0 mg/l)	0.916	1.120	1.000
(9) Total fluoride intake (3)+(8)	**0.968**	**1.311**	**1.379**
(10) Fluoridated salt, 3 g/day, 250 mg fluoride/kg		0.750	0.750
(11) Total fluoride intake (5)+(10)		**1.014**	**1.194**
(12) Total fluoride intake (7)+(10)		**1.501**	**1.629**
(13) Total fluoride intake (9)+(10)		**2.061**	**2.129**

Another dietary source of fluoride is fluoridated salt which contains 200-250 mg fluoride/kg of salt, mostly in the form of potassium fluoride. One gram of salt provides 0.2 to 0.25 mg of fluoride. The use of fluoridated salt may be restricted to use at home, like in Germany, where 75% of such salt is fluoridated, or it can be used in the preparation/production of meals and foods as well (Switzerland, France). The amount of fluoridated salt ingested per person per day is estimated to be 3 g in France, were 35% of salt is fluoridated, and 2 g in Germany corresponding to an additional fluoride intake of 0.50-0.75 mg/day (AFSSA, 2003). Fluoride from salt is well absorbed as demonstrated by Marthaler *et al.* (1995).

2.3.2.3 *Fluoride-containing dental products*

Dental products (toothpaste, rinses and gels) which contain fluoride can, especially when inappropriately used, increase the total intake of fluoride considerably (Burt, 1992). This happens particularly in young children below the age of 7 years who swallow between ten to nearly 100% of the toothpaste (Barnhart *et al.*, 1974; Hargreaves *et al.*, 1972; Naccache *et al.*, 1990, 1992; Salama *et al.*, 1989; Simard *et al.*, 1989). Depending on the amount of toothpaste used per brushing and on the fluoride content significant amounts of fluoride are swallowed and absorbed (up to 0.3 mg per brushing), as demonstrated by peak increases of fluoride in plasma of 3-4 year old children within thirty minutes after brushing with 0.6 g each of a toothpaste with 1000 mg fluoride/kg. The observed peak plasma level almost reached the same height as after the ingestion of a 0.5 mg fluoride tablet (75 to 85 µg/L) (Ekstrand *et al.*, 1983). Fluoride from toothpaste swallowed by a four-year old child was found to contribute up to one third to one half of total daily fluoride intakes of 3.6 and 2.3 mg, respectively

(Richards and Banting, 1996). In the European Communities about 90% of all toothpastes are fluoridated with a maximum level of 1500 mg/kg.

The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP, 2003) states that the amount of toothpaste applied to the toothbrush of a child below the age of 6 years can vary between 0.05 and 0.8 g. The recommended "pea size" amount is taken to be 0.25 g. In a model calculation with amounts of toothpaste of either 0.1 or 0.25 g which would correspond to fluoride doses between 0.1 and 0.37 mg if the toothpaste contained 1000 or 1500 mg/kg, swallowing of either 20% or 40% of the toothpaste was assumed and absorption of either 80 or 100% of the fluoride dose. The amount of absorbed fluoride would then range between 0.016 mg and 0.15 mg fluoride. The SCCNFP considered this amount as the sole source of fluoride exposure - even if applied three times per day - to not pose a safety concern when used by children under the age of six years and not likely to cause fluorosis. If compared to the "adequate" fluoride intake (FNB, 1997; D-A-CH, 2000) of children at that age (0.7-1 mg/day), fluoride ingestion from such toothpastes could amount to up to 50% of that amount.

In the model calculation for 3-5 year old children in the USA the fluoride intake from ingested toothpaste was estimated to be 30-60% of the dietary CTE and to be higher than the dietary RME (Erdal and Buchanan, 2005).

2.3.2.4 Fluoride supplements

Fluoride supplements are recommended by medical societies in some countries (e.g. DAKJ, 2000) for caries prevention, especially if the fluoride concentration from drinking water is low. The various recommended regimens differ considerably with regard to the starting time (birth or 6 months of age), amounts in relation to age, and restrictions in the presence of salt fluoridation or in dependence on the fluoride concentration in drinking water (D-A-CH, 2000; FNB 1997). However, these recommendations are formulated as a public health measure and the supplements are regulated as drugs and available on prescription. The EGVM has concluded that comments on fluoride with regard to food fortification, therefore, are inappropriate (EGVM, 2003). The assessment of the need or the usefulness and safety of fluoride containing drugs is not in the terms of reference of the Panel. Their potential contribution to the total daily intake, however, has to be taken into account in the risk assessment of fluoride. This contribution can amount up to 70% of the estimated reasonable maximum dietary exposure value in both infants and young children (Erdal and Buchanan, 2005).

2.3.2.5 Summary

The total daily intake of fluoride from all sources can range from the low intake of 0.5 mg/day from solid foods, milk, beverages and low-fluoride water reported for Germany (Bergmann, 1994), when no fluoridated salt is used, no fluoride containing dentifrice is used and no supplements are taken, to the moderate amount of 1.2 mg/day reported for the United Kingdom (EGVM, 2001). If fluoridated salt would be used 0.5-0.75 mg fluoride would be added, if fluoridated water was drunk (1 mg/L) and used for the preparation of food and tea (1-2 L of water/day; 500 mL of tea with a fluoride concentration of 5 mg/L) 3.5 to 4.0 mg fluoride would be added. The sum could be 6.0 mg fluoride per day, without fluoride from toothpaste taken into account. Even more extreme scenarios are possible and not completely

unrealistic, when fluoridated drinking water is replaced by the regular use of mineral water with fluoride concentrations above 1 mg/L.

For infants and children between the age of three and five years in the USA total daily intakes from all sources (drinking water, beverages, infant formula, cows' milk, food, soil, supplements and toothpaste) have been estimated using defined assumptions for intake, concentration in source, absorption and body weight. Cumulative CTE and RME for infants in non-fluoridated areas were 0.08 and 0.11 mg/kg/day, respectively, and 0.11 and 0.2 mg/kg/day, respectively for fluoridated areas. For young children the CTE and RME for non-fluoridated areas were 0.06 and 0.21 mg/kg/day and for fluoridated areas 0.06 and 0.23 mg/kg/day (Erdal and Buchanan, 2005). The assumptions used in that study are perhaps not applicable for all European countries, but the results illustrate well the range of potential exposure to fluoride via oral ingestion in infants and young children under variable conditions.

3. HAZARD IDENTIFICATION

3.1 *In vitro* and animal toxicity

Animal studies are considered in the risk assessment of fluoride insofar they support the multitude of human studies investigating both toxic effects and beneficial effects of fluoride in varying doses.

3.1.1 Acute toxicity

The LD_{50} for oral administration of sodium fluoride, sodiummonofluorophosphate and stannous fluoride in rats was reported to be 31-101, 75-102 and 45.7 mg fluoride/kg body weight, respectively (ATSDR, 1993; IARC, 1982). The LD_{50} for the same fluoride compounds in mice was found to be 44.3 and 58 mg fluoride/kg body weight, 54 and 94 mg fluoride/kg body weight and 25.5 and 31.2 mg fluoride/kg body weight, respectively (IARC, 1982; Whitford, 1990).

Symptoms of acute oral exposure included salivation, lacrimation, vomiting, diarrhoea, respiratory arrest and cardiac depression. Depending on the age of the animals nephrotoxic effects were observed. Gastric mucosal changes following the administration of acutely toxic doses of sodium fluoride by gavage to Holtzman rats (17.8 mg fluoride/kg body weight) occurred within 30 minutes of exposure and showed signs of recovery after 48 hours (Easmann *et al.*, 1985).

3.1.2 Short- and medium-term toxicity

3.1.2.1 Short-term studies

In a 14-day study five weeks old male and female F344/N rats and $B6C3F_1$ mice received a low-fluoride semisynthetic diet and drinking water ad libitum. Fluoride concentration in drinking water was zero, 22.5, 45, 90, 180 or 360 mg/L (as sodium fluoride). All rats on drinking water with a fluoride level of 360 mg/L died by day seven (male) and day ten (female). All rats receiving 180 or 360 mg/Lppm fluoride in drinking water showed

dehydration and lethargy and reduced water consumption. There were no gross lesions seen on necropsy after 14 days.

Mice on the same regimen survived the 14-day study period except for two male animals in the highest dose group. Weight losses occurred only in the highest dose group. There were no gross abnormalities on necropsy (NTP, 1990).

Male Holtzman rats which received drinking water with a fluoride content of either 38 or 85.5 mg/L during 21 days showed an increase in cortical and trabecular bone with the lower fluoride dose and an inhibition of endosteal bone formation and reductions of cancelleous bone volume with the higher dose (Turner *et al.*, 1989). Uslu (1983) observed a delay in fracture healing and a reduced collagen synthesis in male albino rats receiving 14 mg fluoride/kg body weight/day over 30 days.

Female Wistar rats administered fluoridated drinking water (113.5 or 136.2 mg fluoride/L) over five weeks showed signs of reduced trabecular bone mineralisation, particularly if the feed was deficient in calcium (Harrison *et al.*, 1984).

An increase of dermatan sulphate and chondroitin-6-sulphate in the tibia of male Sprague-Dawley rats which were dosed with 17.5 mg fluoride/kg body weight per day during one to two months was observed (Prince and Navia, 1983). An increase in bone matrix formation (by 20%) was also observed in male C57BL/6 mice receiving only 0.8 mg fluoride/kg body weight/day over a period of four weeks (Marie and Hott, 1986).

Male Swiss mice administered orally 5.2 mg fluoride/kg body weight/day over 35 days were reported to have reduced erythrocyte and lymphocyte numbers in blood and increases in monocytes, eosinophils and basophils when compared to controls (Pillai *et al.*, 1988).

3.1.2.2 Medium-term studies

In a 90-day study with female Wistar rats which received drinking water with either 100 or 150 mg fluoride/L vertebral bone quality, as measured by compression resistance related to ash content, was reduced (Søgaard *et al.*, 1995).

Whereas adult rats receiving drinking water with 16 mg fluoride/L over a period of 16 weeks showed an increase in femoral bone bending strength (by 38%), there was a decrease (by 20%) in rats with drinking water containing 64-128 mg fluoride/L for the same period (Turner *et al.*, 1992).

In a six-month study male and female four to six-week old F344/N rats and $B6C3F_1$ mice on a low-fluoride semisynthetic diet were administered water without fluoride or water containing 4.5, 13.5, 45 or 135 mg fluoride/L (rats) or 4.5, 22.5, 45, 90, 135 or 270 mg fluoride/L (mice). Body weight reduction and dental fluorosis occurred in the high-dose animals. The fluoride content of bone increased in bone in relation to fluoride content of drinking water.

Nine female mice in the high-fluoride (270 mg/L) group and one male in the 135 mg fluoride/L-group and four males in the highest-fluoride dose group died. Histological changes were identified in the kidney, liver, testes and myocardium of spontaneously dying mice. There was acute nephrosis with multifocal degeneration and tubular necrosis. Multifocal myocardial degeneration and scattered accumulation of mineral was seen. Livers showed

sparse enlarged multinucleated cells. Changes indicative of altered rates of bone deposition and remodelling were seen especially in the femur of nearly all mice receiving water with and above 45 mg fluoride/L and in half of male mice receiving water with 22.5 mg fluoride/L (NTP, 1990).

The administration of 13.6 mg fluoride/kg body weight/day in distilled water by gavage over ten weeks in C57BL/6N mice increased T-cell mitogenesis (by 84%) and reduced B-cell activity (antibody production, by 10%) (Sein, 1988). Antibody production was also inhibited in female rabbits which received over 6-9 months 4.5 mg fluoride/kg body weight/day (Jain and Susheela, 1987b).

Fluoride was reported to affect negatively some endocrine organs, particularly the thyroid, in animal studies (ATSDR, 2001). Rats administered 0.5 mg fluoride/kg/day via drinking water during two months showed decreased thyroxine levels and an increased T_3-resin uptake ratio (Bobek *et al*., 1976). However, when three-months old iodine depleted Wistar rats were administered fluoride in drinking water (60 and 200 mg fluoride/L) during a six-day repletion period with ^{125}I-labelled iodine, no antithyroid effect of fluoride was observed. Neither organification of iodine nor any subsequent step of thyroid hormone biosynthesis were affected. Fluoride had no effect on thyroglobulin content of the thyroid gland or on the degree of iodination of thyroglobulin (Siebenhüner *et al*., 1984).

Male Kunmin mice divided into nine groups which received for 150 days drinking waters deficient, normal or excessive (2.5 mg iodine/L and/or 30 mg fluoride/L) in iodine and fluoride showed goiter induced by both iodine deficiency and iodine excess. Fluoride excess induced dental fluorosis and increased fluoride content in bone. Fluoride excess also affected the thyroid changes due to both iodine deficiency and excess, After 100 days the effect of excess fluoride on the thyroid (weight, colloid goiter, T_3 and T_4 levels) was stimulatory in iodine deficiency and it was inhibitory in iodine excess, while after 150 days of fluoride excess these changes reversed or were no longer influenced by fluoride. Radioiodine uptake was inhibited by fluoride excess both in iodine deficiency and iodine sufficiency, while no such effect of fluoride could be observed in iodine excess (Zhao *et al*., 1998).

3.1.3 Long-term toxicity

3.1.3.1 Growth, survival, effects on bone and teeth and other organs

Several comprehensive studies of the carcinogenicity of sodium fluoride were conducted over a period of two years in male and female F344/N rats, Sprague-Dawley rats and B6C3F$_1$ and CD-1 mice (NTP, 1990: Maurer *et al.*, 1990; Maurer *et al.*, 1993; NRC, 1993). Sodium fluoride was administered either in drinking water *ad libitum* or in feed. The fluoride doses [mg /kg body weight/day] in rats were 0.1 and 0.2 (controls); 0.8; 1.8; 2.5 or 2.7; 4.1 or 4.5; 11.3; the fluoride doses in mice were 0.6 (control); 1.7 to 1.9; 4.5to 5.7; 8.1 or 9.1; and 11.3.

The administration of sodium fluoride, with the exception of the highest dose in rats, had no effect on organ and body weights compared to controls in both rats and mice, no effect on feed and water consumption and no effect on survival. White discoloration of teeth occurred in all groups to a certain extent, but its incidence was higher and it occurred earlier in the highest dose groups (80-100% of animals). Fluoride content of bone was age and dose related.

Rats which had received 4.5 or 11.3 mg fluoride/kg/day had an increased incidence of hyperostosis in the skull and showed hyperkeratosis and acanthosis of the stomach mucosa when compared to the control group with 0.1 mg fluoride/kg body weight/day.

Bone matrix synthesis and mineralisation was inhibited in male and female rats which received drinking water with sodium fluoride in concentrations of 22.7 and 36.3 mg fluoride/L for 250 days (Quiu *et al.*, 1987). Sprague-Dawley rats which had been administered drinking water with 50 mg fluoride/L during 18 months showed reduced femoral bone strength. Regression analysis indicated that older rats lost 36% of femoral bone strength when bone fluoride content increased from zero to 10,000 ppm (Turner *et al.*, 1995).

Rabbits which received daily single oral doses of 4.5 mg fluoride/kg body weight/day for six to 24 months showed in comparison to controls a multitude of changes in blood chemistry, composition of bone, morphology of organs and signs of a disturbed collagen biosynthesis (Bhatnagar and Susheela, 1998; Jain and Susheela, 1987a, 1987b; Jha *et al.*, 1982; Sharma and Susheela, 1988a; Sharma and Susheela, 1988b; Sharma, 1982; Susheela and Das, 1988; Susheela and Jain, 1983; Susheela and Kharb, 1990; Susheela and Sharma, 1982).

Alterations in trabecular and cortical bone remodelling (both resorptive and formative) were also observed in growing pigs receiving 2 mg fluoride/kg body weight/day (as sodium fluoride) orally during six months. The animals remained healthy and gained weight like control pigs. There was an increase in bone density (by 17%) and in ash weight density (by 3%) of vertebral trabecular bone, however the maximum compressive strength normalised for ash density was decreased (Kragstrup *et al.*, 1989; Mosekilde *et al.*, 1987). Beagle dogs ingesting 0.32 mg fluoride/kg body weight/day from drinking water over periods of six months remained healthy and showed increased trabecular bone remodelling activity, but also evidence of disturbed bone cell differentiation (Snow and Anderson, 1986).

3.1.3.2 Carcinogenicity

In male F344/N rats receiving 0.2 (control), 0.8, 2.5 or 4.1 mg fluoride/kg body weight/day in drinking water the incidence of osteosarcoma (three in the vertebra and one in the humerus) was 0/80 in the control group and 0/51, 1/50 and 3/80 in the low-, medium- and high-fluoride groups, respectively. Another osteosarcoma of subcutaneous origin occurred in a fourth high-dose male rat. No osteosarcomas were observed in female rats. The historical incidence of osteosarcomas in control male rats from dosed feed or water studies was 10/2,106 (0.47%) and 37/6,131 (0.6%) in male control rats from studies including all routes of administration. The four osteosarcomas of bone occurred with a statistically significant dose-response trend by the logistic regression test (p=0.027). The pair wise comparison of the incidence in the high-dose group versus that in controls was not statistically significant (p=0.099) and remained so when the subcutaneous osteosarcoma was included (p=0.057).

Other types of tumours, namely squameous papillomas or squameous cell carcinomas of the oral cavity, thyroid gland follicular cell tumours (adenomas and carcinomas) did not show differences in incidence in relation to the fluoride intake (NTP, 1990).

A total of three osteosarcomas and one osteoma occurred in male and female $B6C3F_1$ mice receiving 0.6 (control), 1.7, 4.9 or 8.1 mg fluoride/kg body weight/day (male) and 0.6, 1.9, 5.7 and 9.1 mg fluoride/kg body weight/day (female). An osteosarcoma occurred in one low-dose male mouse, in one low-dose female mouse and one osteosarcoma and one osteoma were

observed in female control mice. No osteosarcoma occurred in the medium- or high-dose mice. The incidence of hepatic neoplasms (adenoma, carcinoma, hepatoblastoma) was similar in male and female mice of control and fluoride exposed groups. The incidence of malignant lymphoma in female mice was 11/80, 5/52, 11/50 and 19/80, respectively (NTP, 1990).

On the basis of these studies NTP concluded that there was "equivocal evidence of carcinogenic activity of sodium fluoride in male F344/N rats".

In the carcinogenicity study with Sprague-Dawley rats receiving 0.1, 1.8, 4.5 or 11.3 mg fluoride/kg/day in their feed the incidence of bone tumours was 0/70, 0/58, 2/70 (one chordoma and one chondroma) and 1/70 (fibroblastic sarcoma) in male rats and 0/70, 2/52 (one osteosarcoma and one chondroma), 0/70 and 0/70 in female rats. From this study fluoride was considered to be not carcinogenic for rats. In contrast to the NTP (1990) study not all bones were investigated microscopically in this study. It should be noted that the bone ash concentration of fluoride in the NTP study with the highest fluoride dose administered was approximately one third of that observed in the study with Sprague-Dawley rats (Maurer *et al.*, 1990).

In a carcinogenicity bioassay with male and female CD-1 mice over a period of 95 and 97 weeks, respectively, which were administered 1.8, 4.5 or 11.3 mg fluoride/kg body weight/day in the feed in groups of 60 animals per gender and dose, the incidence of osteomas in male control and dosed mice was 1/50, 0/42, 2/44 and 13/50, whereas it was 2/50, 4/42, 2/44 and 13/50 in female mice. These animals were infected with a Type C retrovirus; moreover, there is controversy if these types of tumour should be classified as neoplasms (Maurer *et al.*, 1993; NRC, 1993). In this context it should be noted that fluoride concentration in bone ash of the mice in the highest dose group of the NTP (1990) study was less than 50% of the fluoride concentration measured in the highest dose group of this study (NRC, 1993).

Overall, based on the results of the most adequate long-term carcinogenicity studies, there is equivocal evidence of carcinogenicity in male rats and no evidence of carcinogenicity in mice.

3.1.4 Genotoxicity

3.1.4.1 In vitro studies

In general fluoride is not mutagenic in prokaryotic cells. Sodium fluoride did not induce gene mutations in Salmonella typhimurium at doses of 100 to 10,000 μg/plate in strains TA98, TA100, TA1535, TA 1537, TA 1538 and TA1597 and when tested with and without Aroclor1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Martin *et al.*, 1979; Haworth *et al.*, 1983). However, fluoride is not taken up significantly by strain TA98 cells (Ahn and Jeffery, 1994).

Both sodium and potassium fluoride (500-700 μg/mL) increased the frequency of mutations at the thymidine kinase locus in cultured mouse lymphoma and human lymphoblastoid cells (Caspary *et al.*, 1987; Cole *et al.*, 1986; Crespi *et al.*, 1990). At these fluoride levels in the medium growth and survival of cells were also reduced. Sodium fluoride (200-500 μg/mL) did not increase the frequency of mutations at the hypoxanthine-guanine phosphoribosyl

transferase locus in various cell systems exposed under neutral or acidic conditions (Oberly *et al.*, 1990; Slamenova *et al.*, 1992; 1996).

The frequency of chromosomal aberrations in many *in-vitro* assays was increased following exposure to sodium fluoride when compared to unexposed cells including human leukocytes, human peripheral blood lymphocytes, human fibroblasts, human amnion cells, human lymphoid cells and human keratinocytes (IPCS, 2002). The chromosomal aberrations consisted mostly of breaks/deletions and gaps with very few exchanges. Below sodium fluoride concentrations of 10 mg/L (4.52 mg fluoride/L) there were no significant increases in chromosome aberrations observed in human fibroblasts, Chinese hamster ovary cells or human diploid lung cells nor in Chinese hamster lung cells at concentrations at or below 500 mg/L (226 mg fluoride/L). The pattern observed was considered to be caused by effects of fluoride upon the synthesis of proteins involved in DNA synthesis and/or repair (IPCS, 2002). An increase in sister chromatid exchange (SCEs) was reported in Chinese hamster ovary cells at doses of 66.7 and 75 mg sodium fluoride/L without S9 and at doses greater than 1200 mg/L with S9 when harvesting time was extended (NTP, 1990).

Both negative and positive results on cytogenetic changes - mostly chromosomal aberrations - have been reported with sodium monofluorophosphate in human lymphocytes and leukocytes (Zeiger *et al.*, 1993).

3.1.4.2 In vivo studies

Positive genotoxicity findings occurred at doses which were highly toxic to cells and whole animals while lower doses were generally negative for genotoxicity.

Increases in the occurrence of chromosome aberrations were reported in Swiss mice bone marrow cells following acute oral, intraperitoneal or subcutaneous exposure to sodium fluoride (4.5-18 mg fluoride/kg body weight), with an increase in micronuclei after intraperitoneal administration only (Pati and Bunya, 1987). In other studies with Swiss Webster mice from colonies with oral exposure to sodium fluoride via water (50 mg fluoride/L) or feed (up to 50 mg fluoride/kg) for at least seven generations no difference in the occurrence of chromosome aberrations in bone marrow or testis cells was observed in comparison to animals from colonies maintained at low fluoride exposure (<0.05 mg/kg or L in feed or water) (Kram *et al.*, 1978; Martin *et al.*, 1979) and no change in SCEs occurrence was seen in bone marrow cells from mouse or Chinese hamster orally exposed to sodium fluoride (Kram *et al.*, 1978) or from Chinese hamsters orally exposed during 21 weeks to sodium fluoride in drinking water (1, 10, 50 or 75 mg/L) (Li *et al.*, 1989).

Chromosome aberrations were induced in a dose-dependent manner in spermatocytes from BALB/c mice given drinking water with 0, 1, 5, 10, 50, 100 or 200 mg fluoride/L for 3-6 weeks in the highest exposed animals (Mohamed and Chandler, 1982). Swiss Webster mice, on the contrary, who received fluoride in drinking water (1-100 mg/L) for six months or were maintained for several generations on drinking water with 50 mg fluoride/L did not develop chromosomal aberrations in mitotic or meiotic cells of testes (Martin *et al.*, 1979).

Sprague-Dawley rats which were fed either a normal diet low in fluoride (<0.12 mg/kg) or the same diet deficient in calcium (0.25 and 0.125%) or a low-fluoride diet containing 20% or 10% of protein or the 20%-protein diet in restricted amounts (deficiency in total nutrient and energy intake) over 48 weeks and which were further divided into groups administered

deionised water with no fluoride added or with fluoride concentrations of 5, 15 or 50 mg/L did not demonstrate changes in the occurrence of SCEs in bone marrow cells that could be attributed to fluoride. Malnourished energy-restricted rats showed an increase in SCE frequency compared to sufficiently nourished rats irrespective of the fluoride content of their drinking water (Dunipace *et al.*, 1998).

Sperm head morphologic abnormalities increased in Swiss mice which received doses of 10-40 mg sodium fluoride/kg body weight intraperitoneally over five days and were sampled 35 days later (Pati and Bhunya, 1987). No morphological abnormalities of sperm and no increase in the frequency of micronuclei occurrence were observed in mice which drank fluoridated water (up to 75 mg/L corresponding to 23 mg fluoride/kg body weight) during 21 weeks (Dunipace *et al.*, 1989) or were given fluoride up to 32 mg/kg by gavage over five days and killed after a further 30 days (Li *et al.*, 1987).

In summary, fluoride was unable to induce gene mutations in bacterial cells and in Chinese hamster cells. It was positive in the mouse lymphoma *in vitro* assay and in several cultured mammalian cells at chromosome level only at cytotoxic concentrations, probably by indirect mechanisms (e.g. effects on DNA synthesis/repair). Conflicting results were reported on the *in vivo* induction of chromosomal damage at highly toxic concentrations.

3.1.5 Reproductive toxicity

In a multigeneration study female Swiss Webster mice received a low-fluoride diet and drinking water with either zero, 50, 100 or 200 mg fluoride/L. Litter production, infertility proportions, age at delivery of first litter, time interval between litters and frequency of conception were comparable in the control group and in mice receiving water with up to 50 mg fluoride/L. At higher doses maternal toxicity and decreased reproduction were observed (Messer *et al.*, 1973). However, the feed in this study was marginal in iron content. Reproduction rate, litter size and weight were comparable in female Webster mice administered diets with less than 0.5 or 2 or 100 mg fluoride/kg for up to three generations (Tao and Suttie, 1976). However, mice which were administered ≥5.2 mg fluoride/kg body weight/day on days 6-15 after mating showed no signs of pregnancy or of implantation of embryos within the uterus (Pillai *et al.*, 1989). Reductions in fertility have been observed in male mice administered 4.5 mg fluoride/kg body weight/day and in male rabbits given 9.1 or 18.1 mg fluoride/kg body weight per day over 30 days (Chinoy *et al.*, 1991; Chinoy and Sharma, 1998).

Sperm motility and viability were reduced in both rats and mice after 30 days of oral administration of 4.5 or 9 mg fluoride/kg body weight/day, resulting in loss of fertility (Chinoy *et al.*, 1995; Chinoy and Sharma, 1998). Reversible histopathological and biochemical changes were observed in the testes of male mice administered 4.5 or 9 mg fluoride/kg body weight/day orally for 30 days (Chinoy and Sequeira, 1989a and b) and in the testes of male rabbits after the administration of 4.5 mg fluoride/kg body weight/day over 18-29 months (Susheela and Kumar, 1991).

Serum testosterone was found to increase in rats after drinking water with a fluoride content of 45 and 90 mg/L for two weeks. Thereafter, levels decreased and were not different from controls (0.3 mg/L) after six weeks (Zhao *et al.*, 1995). In rats which had received drinking water with either zero, 11.3, 45.2, 79.1 or 90.4 mg fluoride/L for 14 weeks no effects were observed on sperm count, testes weight, histopathology of testes, serum testosterone,

luteinising hormone and follicle stimulating hormone nor in the F_1 offspring exposed *in utero* and after birth to fluoride (Sprando *et al.*, 1997 and 1998).

No adverse effects on foetal development were found in Charles River rats when the dams ingested about 25 mg fluoride/kg body weight/day from drinking water on days 0-20 of gestation, despite signs of maternal toxicity (decreased fluid and feed consumption and reduced body weight) (Collins *et al.*, 1995). Foetal development was not impaired when pregnant CD rats and New Zealand White rabbits were administered approximately 13.2 and 13.7 mg fluoride/kg body weight/day from both feed and water during days 6 through 15 and 6 through 19 of gestation, respectively. The NOAEL for maternal toxicity from fluoride in drinking water was 8.1 mg/kg/day for both rats and rabbits. The NOAEL for developmental toxicity from fluoride in drinking water administered during organogenesis was 12.2 and 13.1 mg/kg body weight/day for rats and rabbits, respectively (Heindel *et al.*, 1996).

3.1.6 Interactions

In a study with male Sprague-Dawley rats lasting 48 weeks with half of the animals of each study group killed after 16 weeks the effects of nutritionally deficient diets (calcium, or protein or energy and total nutrients) on the manifestation of toxic fluoride effects outside the skeleton were investigated. All diets were low in fluoride (<0.12 mg/kg). The fluoride content of the drinking water was varied between zero, 5, 15 and 50 mg/L as sodium fluoride, to achieve plasma levels of fluoride comparable to humans with drinking water with fluoride contents of 1, 3 and 10 mg/L. There were 16-20 animals per group.

Average faecal fluoride excretion decreased with decreasing calcium content of the diet. Calcium deficient rats excreted more fluoride in their urine and calcium deficient rats retained significantly more fluoride (plasma, kidney, liver, femur, vertebra) when exposed to water containing 15 or 50 mg fluoride/L. On a body weight basis malnourished rats consumed and retained significantly more fluoride than rats fed *ad libitum* in proportion to fluoride intake. Fluoride bioavailability was influenced by diet: absorption was 92-94%, 76-78% and 58-64% with a calcium content of 0.125%, 0.25% and 0.5%, respectively. The protein content of the diet did not influence the percentage of fluoride absorbed (44-56%). Absorption in the malnourished rats was 73% of fluoride intake. The results of this study confirm the suggestion that nutritional deficiencies have an effect on both the metabolism of fluoride and on resulting tissue fluoride levels (Dunipace *et al.*, 1998).

3.2 Human toxicity

3.2.1 Acute toxicity

Acute high oral exposure to fluoride may lead to nausea, vomiting, abdominal pain, diarrhoea, drowsiness, headaches, polyuria and polydipsia, coma, convulsions cardiac arrest and death. Most cases resulted from accidental or suicidal ingestion of fluoride containing insecticides or dental products. Some occurred in consequence to improperly fluoridated drinking water.

The lethal dose for humans is reported to be 40-80 mg/kg bodyweight or 5-10 g of sodium fluoride. One thirteen month-old boy died from cardiac arrest within five hours after ingestion of fluoride with severe hypocalcaemia (Boink *et al.*, 1994). One three-year old child died who had swallowed sodium fluoride tablets amounting to 16 mg fluoride/kg bodyweight (Eichler *et al.*, 1982).

The minimum acute dose leading to gastrointestinal effects was described to be 0.4 to 5 mg/kg body weight (Eichler *et al.*, 1982; Whitford, 1996). The acute toxicity dose is lower for the more soluble salts of fluorine, which may be present in dental care products. The gastrointestinal effects arise from the action of hydrofluoric acid which is produced from fluoride salts in the stomach (Spak *et al.*, 1990).

Augenstein *et al.* (1991) reported on 87 cases of fluoride ingestion in children below the age of 12 years. Sixty-seven of these had ingested sodium fluoride tablets, fourteen fluoride containing drops, solutions or mouth rinses. Thirty percent of the children became symptomatic, most of them within one hour after ingestion, all of them within six hours. Eight children from 36 with a fluoride intake below 1 mg/kg body weight, 50% with an intake between 3 and 4 mg/kg and 100% with intakes above 4 mg/kg developed symptoms.

Several incidences of fluoride poisoning caused by accidentally overfluoridation of public water systems have been reported. In one incidence 200 pupils and 12 adults became ill with nausea and vomiting within minutes after ingestion of orange juice with a fluoride concentration of 270 mg/L (Infante, 1974).

Eight patients with renal insufficiency were dialysed with accidentally over fluoridated water (dose of 1 g fluoride) and became symptomatic because of virtually absent renal elimination of fluoride. One patient died from cardiac arrest. Postmortal fluoride concentration in blood was 4.9 mg/L (McIvor *et al.*, 1983; Waldbott, 1981).

3.2.2 Chronic toxicity

3.2.2.1 Epidemiological studies

3.2.2.1.1 Skeletal fluorosis

Skeletal fluorosis may arise from long-term excessive exposure to fluoride both by oral ingestion and by inhalation. In the preclinical stage of fluorosis the patient may be asymptomatic and only have an increase in bone density on radiography. With increasing fluoride incorporation into bone clinical stage I and II with pain and stiffness of joints, osteosclerosis of both cortical and cancelleous bone, osteophytes and calcification of ligaments develop. Crippling skeletal fluorosis (clinical stage III) may be associated with movement restriction of joints, skeletal deformities, severe calcification of ligaments, muscle wasting and neurological symptoms. All stages are accompanied by disturbed or deficient mineralisation of the bone, and osteomalacia may be present, particularly when calcium intake is insufficient. Crippling fluorosis is rare in non-tropical countries without occupational exposure to high airborne fluoride concentrations. A fluoride intake of at least 15-20 mg/day for periods of 20 years has been reported from epidemiological studies in these patients, via consumption of drinking water high in fluoride (>4 mg/L). Only five cases of crippling fluorosis have been reported in the USA during the last 40 years (NRC, 1993). One patient with a fluoride intake of 50 mg/day through drinking water with 25 mg fluoride/L over six years was reported from Canada (Boyle and Chagnon, 1995). Patients with renal insufficiency have an increased risk of developing skeletal fluorosis.

Parallel to higher fluoride concentrations in water and food the prevalence of skeletal fluorosis in the population increases (Liang *et al.*, 1997; Xu *et al.*, 1997). At fluoride

concentrations in water of 4 mg/L and higher, and a daily total fluoride intake of more than 14.0 mg/day, the prevalence of skeletal fluorosis in individuals with normal nutritional intake was 44%, and in individuals with deficient nutrition 69% and was associated with an even higher rate of dental fluorosis (88.3 and 95.0%, respectively) (Liang *et al.*, 1997). Skeletal fluorosis of stage I to III was associated with dental fluorosis in nine patients aged between 17 and 30 years living in Indian communities with fluoride concentrations of the drinking water of 8.1 to 8.6 mg/L (Jha *et al.*, 1982).

In the preclinical stage of skeletal fluorosis the fluoride concentration in bone ash is 3500-5500 mg/kg. In clinical stage I the fluoride content in bone ash is usually between 6000 and 7000 mg/kg; in stages II and III it exceeds 7500-8000 mg/kg (Hodge and Smith, 1977). Skeletal fluorosis, especially of trabecular bone, may be reversible to a certain degree when fluoride exposure is ended and fluoride balance becomes negative, dependent on the extent of bone remodelling (Grandjean and Thomsen, 1983).

Symptomatic skeletal fluorosis was chosen by the FNB (1997) as the critical endpoint for fluoride toxicity. The data base consisted of radiographic studies performed in children and adults in the 1950s and a study on bone mass measured by single photon absorptiometry in women. The results in communities with different fluoride concentrations in their drinking water were compared. The relevant studies are briefly described below.

McCauley and McClure (1954) found no differences in calcification of carpal bones of 2050 children 7 to 14 years of age living in Cumberland (Maryland) with a fluoride content of the drinking water of 0.12 mg/L (n=769), in Amarillo (Texas) with water fluoride of 3.3-6.2 mg/L (n=591) or in Lubbock (Texas) with water fluoride of 3.5-4.4 mg/L (n= 690). In the two high-fluoride communities enamel fluorosis (from very mild and worse, according to Dean, see Annex 2) occurred in 90.3 and 97.8% of children, respectively.

Schlesinger *et al.* (1956) reported radiographic findings from 1528 children first investigated at age 0-9 years in 1943 and from 905 of these children re-investigated in 1954/55. Children were either living in Newburgh with water fluoridation (1.2 mg/L) since 1940 or in Kingston with fluoride-"free" water. No differences in bone density and bone maturation were found.

A 10-year radiographic follow-up study of residents (>15 years; mean 38.2 and 36.7 years) was started in 1943 in two communities: Bartlett with water naturally containing 7.6-8 mg fluoride/L (n=116) and Cameron where water contained 0.4 mg fluoride/L (n=121). Apart from a higher incidence of dental fluorosis, coarse trabeculation of bone (5.4%), hypertrophic bone changes (10.8%) and fractures (15%) were more frequent in Bartlett than in Cameron (2.5%, 7.4%, 7.6%, respectively). However, these differences were statistically not significant. The authors concluded that roentgenographic evidence of bone changes can be produced by excessive fluoride in water, but in "only a select few (10 to 15% of those exposed)". These skeletal changes were not associated with other physical findings, even though the fluoride content in bone could be six times "normal" (Leone *et al.*, 1954 and 1955).

Stevenson and Watson (1957) reviewed 170 000 roentgenographs obtained in one hospital between 1943 and 1953 and identified 23 cases of osteosclerosis. These cases were life-long residents (aged 44-85 years) in areas with a fluoride content of the drinking water of >4 to 8 mg/L. However, even severe roentgenographic changes were not accompanied by clinical symptoms.

Sowers *et al.* (1986) investigated bone mass at mid-radius by single-photon absorptiometry and fracture rate in 827 women (age 20-80 years) from three communities with either water naturally high in fluoride (4 mg/L) or with fluoridated water (1 mg/L). They found a non-significant lower bone mass in participants older than 55 years from the high-fluoride community and an increased fracture incidence (p=0.0001). Estimated mean fluoride content from water was 5±2.1 mg/day in the high-fluoride community.

On the basis of these studies FNB (1997) identified a fluoride intake of 10 mg/day as likely not to cause skeletal fluorosis and therefore as NOAEL for North America. An uncertainty factor of 1 was chosen to define the UL, because the NOAEL was based on human studies and because the observed skeletal changes were non-symptomatic.

3.2.2.1.2 Dental fluorosis

Dental fluorosis is caused by excessive fluoride incorporation into dental enamel before eruption of teeth. Susceptibility to dental fluorosis ends around the age of eight years, when enamel maturation of permanent teeth is completed except for the third molars (see Annex 1 for timetable of dentition). Dental fluorosis is the result of hypomineralisation of the developing tooth with a disturbance of the normal loss of early-secreted matrix proteins and their excessive retention in the developing enamel in the presence of high fluoride concentrations. The most sensitive period for this adverse effect of fluoride is the pre-eruptive maturation stage of enamel development. For the maxillar central incisors, for example, the most critical phase of exposure to fluoride in drinking water was found to start at the age of 22 months and to last for about four months thereafter (Evans and Stamm, 1991). Hypomineralisation of both the surface and subsurface of the enamel means greater porosity. Increased porosity of the enamel makes it more vulnerable to mechanical stress and more accessible to fluoride. Therefore, fluorotic teeth have higher fluoride contents than normal teeth. The staining of fluorotic teeth in the more severe forms of dental fluorosis develops after tooth eruption. In general human dental fluorosis is more severe in teeth that mineralise later in life than in those mineralising early, and, therefore, it is primarily a condition of permanent teeth and in these increases in severity from the anterior to the posterior teeth (Thylstrup and Fejerskov, 1978). Extensive fluorosis of primary teeth, however, can be observed in areas of the world with high fluoride exposure through e.g. water (Thylstrup, 1978).

Dental fluorosis can be difficult to discriminate from other conditions in which amelogenesis in humans can be disturbed, such as calcium deficiency and generalised malnutrition. The likelihood of dental fluorosis increases in disorders of acid-base balance with reduction of the renal clearance of fluoride.

Milder forms of dental fluorosis, characterised by white spots and opaque striations on the surface of teeth are a cosmetic effect and do not impair function. On the contrary, it is associated with increased resistance against caries. Different classification or scoring systems have been developed for dental fluorosis. Three of the most commonly used systems are set out in Annex 2.

The scores from Dean's index are based on the two worst-affected teeth in the mouth and are derived from inspection of the non-dried whole tooth. Dean's index has been criticised for low sensitivity at both ends of the scale. Its category "severe" cannot, for example,

discriminate between the scores 5 to 9 of the Thylstrup-Fejerskov (TF) index (see below). Dean, using his scoring system, had recorded the occurrence of dental fluorosis in a population as the community index to permit comparison between different populations. This index is calculated as the sum of individual scores in an individual divided by the number of individuals examined. A community fluorosis index of 0.6 in a population was judged to represent a threshold for dental fluorosis as of public health significance. Community indices of 0.6 were observed in communities with fluoride contents of the drinking water between 1.6 and 1.8 mg/L (Dean, 1934; Dean *et al.*, 1941 and 1942). Per every increase in fluoride intake of 0.01 mg/kg body weight per day an increase in Dean's fluorosis community index by 0.2 has been predicted (Fejerskov *et al.*, 1996a).

The Thylstrup-Fejerskov (TF) index (Thylstrup and Fejerskov, 1978) with a 10-point scale on inspection of dried teeth is more sensitive both at low and high grade fluorosis compared with the Dean scale. It corresponds well with the fluoride content of enamel, except for the first three categories. It has been proposed that the prevalence and severity of dental fluorosis in a population should be presented as a cumulative distribution of severity of scores (ordinate percent of population, abscissa percent of teeth involved per person) (Fejerskov *et al.*, 1996b).

The Tooth Surface Index of Fluorosis (TSIF) (Horowitz *et al.*, 1984) determines a score on a seven-point scale to each unrestored surface of each non-dried tooth and also provides greater sensitivity than Dean's index. It has been criticised by including staining as a criterium, which is a post-eruptive phenomenon and dependent on a person's dietary and hygiene habits as well as on the degree of enamel porosity.

The application of the above scoring systems leads to results which are not directly comparable. Some investigators tend to modify them further, therefore, the evaluation of studies on the prevalence and severity of dental fluorosis in populations must take account of the chosen methodology.

The development of enamel fluorosis is dose dependent, irrespective of which scoring system is applied (Horowitz *et al.*, 1984; Fejerskov *et al.*, 1996a; McDonagh *et al.*, 2000). Even at low fluoride intakes from water, there will be a certain incidence of dental fluorosis.

From investigations in the 1930s and 1940s on the relationship between fluoride content of drinking water, dental fluorosis and caries occurrence, the dose dependency of occurrence and severity of dental fluorosis was already apparent: fluorosis classified as "moderate to severe" according to Dean appeared at fluoride concentrations in water of 1.9 mg/L (prevalence 2%) and increased in frequency with increasing fluoride content (2.2-2.6 mg/L: 10%; 3.9-4 mg/L: ≅ 40%; 4.4 mg/L and higher: >60%). In communities with a fluoride content in the water of 7.6 mg/L prevalence of fluorosis was 100%, 28% "very mild/mild" and 72% "moderate/severe". From the same investigation it appeared that the reduction in dental caries of children was nearly maximal in communities with a fluoride content of the water supply of 1 mg/L (Dean, 1942; Dean *et al.*, 1941 and 1942).

The results of an investigation of 4429 children aged 12 to 14 years from cities with different fluoride contents of drinking water are shown in Table 3. At the time of these examinations fluoride sources were water and food only. Looking at the prevalence of caries in these children it must be born in mind, that there has been a significant decline in caries incidence starting in the 1970s.

To achieve a balance between the water fluoride content that provided best prevention of caries and minimum occurrence of meaningful fluorosis ("mild/very mild" or worse) an "optimal" fluoride content in the water of 0.7-1.2 mg/L (depending on the mean temperature of the region) was established.

Consumption of water with an "optimal" fluoride content as the only source of dietary fluoride amounts to an intake of 0.4 to 1.7 mg fluoride/day in children between one and twelve years of age. On a body weight basis this is about 0.05 mg/kg/day. Later research confirmed that dental fluorosis of the three lowest categories of the TF index occurs even with fluoride intakes of 0.03-0.04 mg/kg/day. Fluorotic enamel of these three TF categories has a normal fluoride content.

Table 3. Incidence and distribution of dental fluorosis in 4429 children aged 12-14 years examined from 13 cities in relation to fluoride content of drinking water (Dean *et al.*, 1942)

		Fluoride content of drinking water (mg/L)											
		≤ 0.2		**0.3-0.4**		**0.6**		**0.9-1.2**		**1.9**		**2.5**	
Number examined		2142		717		614		275		273		404	
Nº fluorosis [n (%)]													
Normal	(0)	1912	(89.3)	533	(74)	444	(72)	121	(44)	69	(25)	26	(6.4)
Questionable	(0.5)	211	(9.9)	158	(22)	130	(21)	91	(33)	74	(27)	80	(19.8)
Fluorosis [n (%)]			(0.9)		(3.6)		(6.5)		(23)		(48)		(72)
Very mild	(1)	19	(0.9)	24	(3.3)	38	(6.2)	58	(21)	110	(40)	170	(42)
Mild	(2)	0		2	(0.3)	2	(0.3)	5	(1.8)	17	(6.2)	86	(21)
Moderate	(3)	0		0		0		0		3	(1.1)	36	(8.9)
Severe	(4)	0		0		0		0		0		0	
Caries in first molars		88%		77%		61%		52%		41%		40%	

In the following Table 4 the results of a cross-sectional survey performed 1980 in Illinois in seven communities with similar demographic characteristics on the incidence and severity of dental fluorosis assessed with both Dean's scoring system and the TSIF in 807 children aged eight to 16 years are presented. The study population was grouped according to the fluoride content of the drinking water which had been used throughout their lives into four groups: "optimal" and two-, three- or four-times "optimal" (1, 2, 3 and 4 mg fluoride /L) (Horowitz *et al.*, 1984).

A total prevalence of fluorosis ("very mild" and worse according to Dean) of 48% (95% CI: 40% to 57%) at a water fluoride concentration of 1 mg/L has been estimated in a recent meta-analysis. The prevalence of fluorosis of aesthetic concern ("mild" or worse, according to Dean's classification; TSIF two or more; TF three or more) was 12.5% (95% CI 7% to 21.5%) (McDonagh *et al.*, 2000). Sixt-nine per cent of 197 children between the ages of 7 and 11 years who had lived mostly in communities with fluoridated drinking water, demonstrated dental fluorosis, which was "very mild" according to the modified Dean's Index in 39% and "moderate to severe" in 13%. While there was no association between their history of total fluoride exposure and dental fluorosis, there was a significant association with the use of fluoride supplements below the age of three years (Morgan *et al.*, 1998).

Only 3% of six to 10-year old children (n=1249) in Germany in a region with less than 0.3 mg fluoride/L in drinking water were found to have dental fluorosis and 8.9% of 10 to 16-year old children (n=1298) living in areas with a fluoride concentration in the drinking water up to 1 mg/L. The fluorosis community index in these latter children was <0.35. The distribution of TF indices in the 6-10 years old children was as follows: TF1 1.2-1.5%; TF2 0.2-0.8%; TF3 0.6-1.2%; TF4 0-0.2%; TF5-9 0-0.3%. In the 10-16 years old the distribution was: TF1 2.8-3.4%; TF2 3.3-4.6%; TF3 1.1-1.9%; TF4 0.2-0.5%; TF5-9 zero. Data on the total fluoride intake of these children are not available (Hetzer, 1999; Hetzer *et al.*, 1997).

Table 4. Incidence and percentage distribution of severity of dental fluorosis assessed both with the TSIF and Dean's index and mean DMF surface scores for dental caries in 807 children aged 8-16 years in relation to the fluoride content of drinking water (Driscoll *et al.*, 1983; Horowitz *et al.*, 1984)

	Percentage distribution of TSIF scores									**Mean DMF surface scores (n)**
Water fluoride level	**n**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
optimal	336	84.5	12.4	2.0	1.1	0.0	0.0	0.0	0.0	3.14
2 x optimal	143	58.1	28.4	7.6	5.6	0.1	0.1	0.0	0.0	1.97
3 x optimal	192	50.4	25.7	13.2	9.3	0.4	0.8	0.0	0.2	1.41
4 x optimal	136	31.9	27.0	17.1	20.5	0.4	2.1	0.1	0.8	2.02

	Percentage distribution of Dean's score						
		Normal	Questionable	Very mild	Mild	Moderate	Severe
	n	**0**	**0.5**	**1**	**2**	**3**	**4**
optimal	336	56.0	29.5	7.4	4.8	1.8	0.6
2 x optimal	143	18.2	28.7	23.1	16.8	8.4	4.9
3 x optimal	192	22.9	26.0	15.1	19.8	7.8	8.3
4 x optimal	136	12.5	15.4	16.9	25.0	7.4	22.8

For total daily fluoride intakes of 1.7, 3.5 and 14.8 mg/day in well nourished subjects an incidence of dental fluorosis of 6.4, 10.5 and 88.3% was reported in China, whereas for intakes of 1.2, 2.6 and 15.3 mg fluoride/day in malnourished subjects the incidence was 4.8, 24.8 and 95%, respectively (Liang *et al.*, 1997).

While the intake of fluoride from water can be estimated with some certainty, e.g., by a formula which includes the variables body weight and average maximum air temperature (water intake [L/kg body weight]= 0.0025+0.0004xmean maximum temperature[^{o}F] (Galagan *et al.*, 1957), an estimation of fluoride intake from other sources is prone to the influence of a wide variety in individual habits (see Section 2.3.2). If the fluoride intake from water from Dean's data (Dean, 1942; Dean *et al.*, 1941; 1942) is calculated with the above formula on a body weight basis, it appears that an intake of 0.02 mg fluoride/kg/day is associated with a prevalence of dental fluorosis of 40-50% and of 15-25%, when the category "questionable" is excluded. The community index value at that intake is 0.3-0.4. The findings of Dean on the linear relationship between fluoride content of drinking water and the prevalence of dental fluorosis and/or the fluorosis community index were confirmed by two large studies performed in the USA 25 and 40 years later (Richards *et al.*, 1967; Butler *et al.*, 1985) and no upward shift of the dose-response curve was observed over that period (Fejerskov *et al.*, 1996a).

Similar dose-response relationships have been demonstrated between the fluoride intake from fluoride tablets and dental fluorosis. Fejerskov *et al.* (1996a) compared the prevalence of dental fluorosis, classified according to Dean, in American and Swedish children, who either lived in areas with fluoridated water (1-1.2 mg/L) or received fluoride tablets (Aasenden and Peebles, 1974; Granath *et al.*, 1985). While the prevalence was similar in the two groups of children receiving fluoridated water (total 63-67%; questionable plus very mild 30-31%; mild 8-9%; moderate 2-4%; severe zero), there was a significant difference in prevalence and severity of dental fluorosis between the American and Swedish children who had taken fluoride tablets. The total prevalence in the USA was 84% (questionable 16%; very mild 34%; mild 19%; moderate 14%) and it was 29% in Sweden (questionable 4%; very mild 14%; mild 10%; moderate zero). This difference is explained by the differing dosage regimes: USA 0.5 mg fluoride beginning from birth to 4 months of age until the age of three years and followed by 1 mg/day until the age of six years; Sweden 0.25 mg fluoride from 6 to 18 months and followed by 0.5 mg/ day until the age of six years. On a body weight basis American children received twice as much fluoride in tablet form at ages 6 to 12 months and at ages 3 to 6 years than Swedish children. Moreover, there were methodological differences in assessment: in Sweden only incisors were recorded, whereas in the USA group the recordings were occasionally based on erupted premolars, which tend to be more severely affected than incisors.

There is no reason to suppose that fluoride available from food, including fluoridated salt and beverages, and from toothpaste has a different effect on maturing enamel than fluoride from water and tablets, although no investigations of this relationship have been available to the Panel.

Also apparent from the studies is the fact that there is no real threshold value for a fluoride intake which is not associated with the occurrence of dental fluorosis in the population.

In summary persons living permanently in communities with water fluoride concentrations of about 1 mg/L had in 10% to 12% mild forms of enamel fluorosis. The fluoride intake of children in these communities was calculated to be 0.02 to 0.1 mg/kg/day. At a fluoride intake from water of 0.08-0.1 mg/kg/day moderate (or worse) fluorosis was recorded in less than 5% of children (Dean, 1942). Very mild and mild forms of dental fluorosis occurred in 48% of children with a calculated fluoride intake from water of 0.043 mg/kg/day (Fejerskov *et al.*, 1996a).

3.2.2.1.3 Bone mineral density and fractures

All studies on the relationship between fluoride in drinking water and bone density or risk of bone fracture suffer from imprecise exposure assessment.

Four studies included in a meta-analysis of 18 ecological, cross-sectional and cohort studies on water fluoridation/natural fluoride content of water (up to 4-5 mg/L) and bone fractures reported a significant increase in bone mass with increasing fluoride intake in lumbar spine, a positive change in the femoral neck which was not significant and a negative change for the distal radius, which also was not significant. In this meta-analysis no effect on fracture incidence could be demonstrated (RR=1.02, 95% CI = 0.06-1.09) (Jones *et al.*, 1999).

Kröger *et al.* (1994) investigated 3222 perimenopausal women for bone density of the spine and found it to be 1% higher in 969 women who had lived for more than ten years in an area with fluoridated water (1.0-1.2 mg/L) than in 2253 women who had used drinking water with less than 0.3 mg fluoride /L, while there was no difference for the bone mineral density of the femoral neck. There was also no difference in self-reported incidence of fractures.

Seven thousand one hundred twenty nine postmenopausal women were investigated for bone density by Phipps *et al.* (2000). Women who had lived for more than 20 years in an area with fluoridated water showed 2% higher density in the lumbar spine and femur than women living in an unfluoridated community, but their radius bone density was lower. In this study the risk of incident fracture of the hip and spine was significantly lower among those exposed to fluoridated drinking water than in those not exposed. While there was no difference in risk for fracture of the humerus, the risk for fractures of the wrist was increased for those exposed to fluoridated water.

Karagas *et al.* (1996) found no significant difference in risk for hip and ankle fracture in men and women between 65 and 90 years living either in an area with fluoridated water (≥0.7 mg fluoride/L) or in a non-fluoridated area (≤0.3 mg fluoride/L). In men the relative rates of fractures of the proximal humerus and distal forearm were significantly increased (by 23% and 16%, respectively), in the fluoridated area however.

A comparison between a community with drinking water containing 4 mg fluoride/L with two control communities with 1 mg fluoride/L in the water showed that the relative risk of hip, wrist or vertebral fracture was 2.2 (95% CI=1.07-4.69) in women 55-80 years of age. The fluoride intake from beverages only in the high-fluoride community was estimated to be 72 µg/kg body weight/day (Sowers *et al.*, 1986).

In a retrospective cohort study involving 144,627 elderly persons who had lived at least 13 years in villages outside the public Finnish water system with fluoride concentrations in well water ranging from less than 0.05 mg/L up to 2.4 mg/L no associations between hip fractures in men or women of all ages and water fluoride content was found. However, in women between the age of 50 and 65 years at the start of the follow-up the relative risk for hip fracture increased with increasing well water fluoride concentrations in comparison with a fluoride concentration of ≤0.1 mg fluoride/L. This relationship was significant for fluoride concentrations of >1.5 mg/L (RR 2.09; 95% CI=1.16-3.76; $p<0.05$) (Kurttio *et al.*, 1999).

When fluoride levels in toenails (<2.0, 2-3.35, 3.36-5.5 and >5.5 mg fluoride/kg) collected between 1982 and 1984 were used as markers for chronic fluoride exposure in a case-control study involving 62,641 healthy nurses (53 cases of hip fracture, 188 cases of forearm fracture, 241 matched controls in 1988) a non-significant increase of the risk for forearm fracture (adjusted odds ratio 1.5; 95% CI=0.9-2.7) and a non-significant decreased risk for hip fracture (odds ratio 0.5; 95% CI=0.2-1.5) were calculated for the three highest quartiles of fluoride (Feskanich *et al.*, 1998).

Risk factors for fractures were determined in a 5-year prospective follow-up study in 3216 men and women above the age of 65 years and related to fluoride exposure from drinking water supply during ten years. For hip fractures a higher risk could be determined with drinking water fluoride levels of 0.11-0.25 mg/L (odds ratio 3.2) and >0.25 mg/L (odds ratio 2.4) in comparison with fluoride concentrations below 0.11 mg/L. However, no increased risk

was estimated for exposure to water with >0.7 or >1.0 mg fluoride/L (odds ratio 0.77; 95% CI=0.37-1.62; odds ratio 0.89; 95% CI=0.21-3.72, respectively) (Jacqmin-Gadda *et al.*, 1998).

No relationship between naturally occurring fluoride in drinking water on fractures of the hip could be demonstrated in a population-based case-control study in the United Kingdom. The contribution of drinking water to total fluoride intake in that study was small and probably less than one-third (Hillier *et al.*, 2000).

Li *et al.* (2001) studied the relationship between hip fracture and other fractures and exposure to fluoride from drinking water in 8266 Chinese men and women from six villages with different fluoride content in water (0.25-0.34, 0.58-0.73, 1.00-1.06, 1.45-2.19, 2.62-3.56, 4.32-7.97 mg/L). Fluoride intake from drinking water was estimated to be the main source of fluoride intake and to be on average 0.73, 1.62, 3.37, 6.54, 7.85 and 14.1 mg/day. The subjects of the study had lived in the same village for more than 25 years and were more than 50 years old when studied. The odds ratios (OR) for all fractures for the different fluoride exposure levels were:

Intake (mg/day)	**OR**	**p Value (relative to the intake of 3.37 mg/day)**
0.73	1.50	0.01
1.62	1.25	0.17
3.37	1.0	-
6.54	1.17	0.33
7.85	1.18	0.35
14.1	1.47	0.01

The difference was significant (p=0.01) for fluoride exposure at the lowest and at the highest level (0.73 and 14.13 mg/day) compared with a fluoride exposure of 3.37 mg/day in the village with a fluoride content of the water of 1.00-1.06 mg/L. For fractures of the hip the increase in prevalence with increasing fluoride exposure was significant only for the highest exposure group (14.13 mg/day; OR 3.26; p=0.02) compared to exposure to water of 1.00-1.06 mg fluoride/L. Contrary to fractures of all bones no increase in hip fracture incidence was seen with low-fluoride exposure (<1 mg fluoride /L). This study indicates a bimodal effect of fluoride exposure with an increase in the risk of fractures at all locations both with fluoride intakes lower and higher than about 3.5 mg/day, whereas the risk of fractures of the hip only increased with increasing fluoride exposure.

3.2.2.1.4 Carcinogenicity

In a series of epidemiological studies, both geographic and temporal associations between fluoride in drinking water and risk of cancer mortality were reported (Yiamouyiannis and Burk, 1977). These reports were extensively reviewed both by IARC (1982) and Knox (1985) and criticised because of methodological flaws in adjusting for differences in the age, race and sex of the compared populations.

A number of ecological studies in various countries did not find a consistent relationship between incidence of and mortality from all types of cancer and the consumption of fluoride-containing drinking water (Freni and Gaylor, 1992; Mahoney *et al.*, 1991; Yang *et al.*, 2000).

Lynch (1984) analysed the relationship between cancer incidence and fluoride in drinking water (both natural and added) in 158 municipalities with a total population of 1,414,878 in

1970. A total of 66,572 cancer cases (bladder, female breast, colon, lung, prostate, rectum and other sites combined) were evaluated for fluoride content in drinking water and duration of exposure in univariate and multivariate cancer-site, sex-specific statistical tests, which included eight sociodemographic variables. The results failed to support a fluoride-cancer association.

A comparison of the annual incidence rates of osteosarcoma for 1970-1988 in Edmonton (Province of Alberta, Canada), where water was first fluoridated in 1967, with rates in Calgary, where fluoridation was started in 1989 showed incidence rates of 0.27 and 0.29 per 100,000 inhabitants in Edmonton and Calgary, based on 26 and 29 cases, respectively, that is no link between fluoridation of water and osteosarcoma (Hrudey *et al.*, 1990).

In an update of an earlier analysis of cancer mortality by county in the United States related to drinking water fluoridation, 2,208,000 deaths by cancer in the Caucasian population were analysed, with special emphasis on cancers of bones and joints. The risk of death from cancers of bones and joints after 20-35 years of water fluoridation in both male and females was the same as in the years immediately preceding fluoridation (Hoover *et al.*, 1991).

An ecological study in areas of New Jersey observed a higher rate of osteosarcoma in fluoridated communities in 1979-1987 than in non-fluoridated communities with a risk ratio of 3.4 among males under 20 years of age in fluoridated communities. The analysis was based on 12 and eight cases in the fluoridated and non-fluoridated area, respectively (Cohn *et al.*, 1992). In a case-control study from New York State the self-reported lifetime intake of fluoride from drinking water and dental care products from 130 patients below the age of 24 years diagnosed to have osteosarcoma between 1978 and 1988 was compared to the lifetime intake of matched controls. Whereas no significant trend for risk was observed on a group basis, there was a decrease in the odds ratios for osteosarcoma with increasing exposure estimates for males, which was statistically significant (p=0.02) (Gelberg *et al.*, 1995). Consumption of fluoridated drinking water (>0.7 mg fluoride/L) between 1979 and 1989 was not found to be associated with an increased risk for osteosarcoma (odds ratio 1.0; 95% CI=0.6-1.5) in a case-control study in Wisconsin, USA (Moss *et al.*, 1995).

Another hospital-based case-control study, on the contrary, with 22 cases of osteosarcoma and 22 matched controls found that the odds ratio of disease for drinking fluoridated drinking water (>0.7 mg/L) during childhood (birth to 15 years) and during lifetime was 0.33 (95%CI 0.04, 2.50) (McGuire *et al.*, 1991).

From the available data no increased risk of developing cancer at the observed fluoride dose levels can be deduced.

3.2.2.1.5 Genotoxic effects

The frequency of SCEs was studied in peripheral lymphocytes obtained from about 700 Chinese adults who had resided for more than 35 years in the same area and consumed drinking water with fluoride concentrations from 0.11-5.03 mg/L. Half of the study population had inadequate nutritional intakes. The fluoride intake from food and water was calculated to range from 20 to 280 μg/kg body weight/day (1.2-15.3 mg/day). The fluoride concentration in plasma in the area with 5.03 mg fluoride/L was 5.56 μmol/L (106 μg/L). Plasma levels of fluoride were higher in persons with inadequate nutrition, and SCE frequencies were higher in such subjects from areas with low fluoride content in water (0.1

and 0.2 mg/L), but there were no significant differences between all the other groups and no differences in micronuclei in blood lymphocytes were observed (Li *et al.*, 1995b; Liang *et al.*, 1997).

One study investigated adult persons (>50 years) who had resided for more than thirty years in three communities with drinking water fluoride concentrations of 0.2 (n=66), 1.0 (n=63) and 4.0 mg/L (n=70) and who provided samples of the water which had been their main source, urine and blood samples. Mean plasma and urine fluoride concentrations reflected the fluoride content of the water (plasma: 1.1, 1.8 and 4.0 μmol/L; urine: 0.7, 1.1 and 2.8 mg/L). Peripheral blood lymphocytes showed an increased frequency of SCEs in the samples from the 4 mg/L-community (mean 5.9% compared to 5.2% (1 mg fluoride/L) and 5.5% (0.2 mg fluoride/L water) ($p<0.001$). Women showed a significantly higher overall frequency of SCE than males in all three communities ($p<0.05$). However, when 58 residents from the community with a fluoride content of 4 mg/L water were split in those (n=30) who drank this water and those (n=28) who used instead water from wells with a mean fluoride content of 0.3 mg/L, there was no difference in the frequency of SCE in their lymphocytes (Jackson *et al.*, 1997).

Increases in the frequency of SCEs and micronuclei in peripheral lymphoblasts have been reported in patients with skeletal fluorosis or residents from fluorosis-endemic areas in comparison to residents from non-fluorosis areas in various countries (China, India). SCE frequency was significantly higher in peripheral blood lymphocytes from 14 inhabitants of a village with fluoride in drinking water of 1.6-2.9 mg/L than in lymphocytes from 14 residents of a village with low-fluoride drinking water (0.6-0.8). However, this was not the case with 28 residents of two other high-fluoride villages. Chromosomal aberrations occurred in higher frequency in blood from all 42 residents of the villages with high-fluoride drinking water (Joseph and Gadhia, 2000). SCE frequency in peripheral blood lymphocytes was significantly increased in 53 patients with skeletal fluorosis aged 16-59 years from a district with drinking water containing 4-15 mg fluoride/L, compared to healthy residents of the same region and to subjects drinking water with a fluoride concentration of less than 1 mg/L. The rate of micronuclei in fluorosis patients was 2 to 3 times that of control subjects and intermediate in healthy fluoride exposed subjects (Wu and Wu, 1995). However, too little details on other life circumstances are given in these studies.

There were no effects on chromosomal aberrations or micronuclei in lymphocytes in seven female osteoporosis patients randomised to treatment with sodium fluoride or monofluorophosphate (fluoride dose 29 mg/day, range 22.6-33.9 mg/day) for an average of 29 (14-49) months when compared to seven matched placebo controls. Serum fluoride concentrations were 0.1-0.2 mg fluoride/L (van Asten *et al.*, 1998).

Rapaport (1956) reported an increased frequency of trisomy 21 with increasing fluoride content of drinking water based on information gathered in 1950-1956 on 687 cases admitted to institutions in four American states (Wisconsin, North and South Dakota, Illinois). These findings could not be confirmed in investigations in English cities (Berry, 1958), in Massachusetts (Needleman *et al.*, 1974) and in Sweden (Berglund *et al.*, 1980). In the last named study the incidence of trisomy 21 was related to the mean fluoride content in the water of the area where the mother lived and to the age of the mother. No influence of fluoride on the incidence of trisomy 21 was seen.

Genotoxic effects associated with a high exposure to fluoride have been observed, predominantly in persons with clinically manifest symptoms of fluoride toxicity (skeletal fluorosis). The data are insufficient for a dose-response assessment.

3.2.2.1.6 Reproductive effects

Chronic occupational exposure to fluoride compounds has been reported to have negative effects on sex hormone levels and menstrual cycle and to increase spontaneous abortion especially in persons with skeletal fluorosis. However, because of exposure to multiple substances, these reports are not conclusive (NRC, 1993).

In India, where fluorosis is endemic in areas with drinking water naturally containing up to 38.5 mg fluoride/L infertility in married men was reported (Neelam *et al.*, 1987 cited in Susheela and Kumar, 1991). In an ecological study the total fertility rate of women 10-49 years of age in the period 1970-1988 of states of the USA with at least one community water system providing drinking water with ≥ 3 mg fluoride/L was found to be negatively associated with fluoride content. Because this study used population means and not data on individual women, it remains unresolved if fluoride from drinking water is of influence on human fertility (Freni, 1994).

3.2.2.1.7 Other effects

Nephrotoxic effects of fluoride have not been reported in subjects with skeletal fluorosis due to high fluoride contents in drinking water and in subjects with osteoporosis on long-term treatment with fluoride (section 3.2.2.2).

Fluoride was reported to affect human thyroid function. Increases in serum thyroxine levels without significant changes in T_3 or thyroid stimulating hormone levels were observed in residents of regions in India with high levels of fluoride in the drinking water (up to 6.5 mg/L). Nonetheless fluoride is not considered to be an endocrine disruptor (ATSDR, 2001). In a review of the literature on fluorine and thyroid gland function the authors come to the conclusion that the increase of the metabolic rate observed in men suffering from symptomatic industrial fluorosis was not due to fluoride-induced hyperthyroidism and that the literature on a relationship between fluoride exposure and endemic human goitre neglected to take into account a concomitant iodine deficiency (Bürgi *et al.*, 1984). In two studies performed in two regions in China the intelligence of children was measured and related to the fluoride content of drinking water. 907 children (age 8-13 years) from four areas with different degrees of dental fluorosis prevalence were investigated. Urinary fluoride concentration in these children was 1.02-2.69 mg/L and it was highest in areas with a high incidence of fluorosis. In the non-fluorosis area the mean IQ was 89.9, it was 80.3 in the high-fluorosis area (<0.01). The percentages of children with low or borderline IQs were higher in areas with medium and severe fluorosis and no children with IQs >120 were found in these areas (Li *et al.*, 1995a).

The second study in 118 children aged 10-12 years, who were randomly selected from two villages which differed in the fluoride content of the drinking water (3.15 mg/L versus 0.37 mg/L) and the prevalence of dental fluorosis (86% and 14%, respectively) found an average IQ of 103 in the village with low-fluoride drinking water and of 92 in the other village (Lu *et al.*, 2000). The significance of these studies is doubtful due to missing data on other factors of relevance.

In a study with 197 children aged 7-11 years, who demonstrated dental fluorosis in 69%, no association between dental fluorosis and behaviour could be demonstrated (Morgan *et al.*, 1998).

3.2.2.2 Interventions, clinical studies

Fluoride compounds, mostly sodium fluoride or monofluorophosphate, alone or in combination with calcium and vitamin D, have been used in the prevention and treatment of age-dependent osteoporosis in doses ranging from 4.5 to 57 mg fluoride/day, because fluoride is known to elevate the trabecular volume by increasing both the number of osteoblasts and the formation period of the bone remodelling process. It interacts with bone cell mitogens and increases tyrosine protein phosphorylation. It selectively stimulates the carrier-mediated sodium-dependent transport of anorganic phosphate across the membrane of osteoblast-like cells and the stimulatory effect of insulin and insulin-like growth factor -1 (IGF-1) on phosphate transport in a dose-dependent fashion (Bonjour *et al.*, 1993). Although fluoride increases bone mineral density (BMD), there is a corresponding decrease in elasticity and strength of bone tissue (Aaron and de Kanis, 1991).

The evaluation of the effectiveness of fluoride for prevention and treatment is outside the task of this panel, but well-conducted and documented therapeutic trials can help in identifying fluoride doses that lead to adverse effects, although it must be borne in mind that the study subjects are mostly elderly (>50 years), predominantly female and were selected because of already existing changes in bone mass or density, with or without a history of vertebral fractures.

In a meta-analysis of eleven therapeutic studies involving 1429 postmenopausal women (age 50-86 years), with a duration of 2-4 years in ten studies and of 3 months in one study, an analysis of side-effects was included (Haguenauer *et al.*, 2000). All trials were randomised and included control groups which received calcium and/or vitamin D in the same dosage as the fluoride intervention group. The increase in lumbar spine BMD was found to be higher in the fluoride group than in the control group. The relative risk (RR) for new vertebral fractures was not significant at two years or at four years. The RR for new non-vertebral fractures was not significant at two years, but was increased at four years in the fluoride treated group (1.2; 95% CI 1.36-2.50) especially if high doses were used. The RR for gastrointestinal side effects was not significant at two years, but was increased at four years (2.18; 95% CI 1.69-4.57), especially if fluoride was used in high doses and in a readily available form. High fluoride doses had no effect on risk of vertebral fractures, but increased the risk of non-vertebral fractures and of gastrointestinal side effects. Table 5 lists these eleven studies and the observed adverse effects in relation to the fluoride dosis.

3.2.2.2.1 Skeletal effects

No differences in the occurrence of adverse skeletal effects (vertebral and non-vertebral fractures and lower-limb pain presumably caused by microfractures) were found in those studies where the fluoride dosis was 4.5 to 26 mg/day (up to 0.4 mg/kg/day) (Hansson and Roos, 1987; Christiansen *et al.*, 1980; Grove and Halver, 1981; Gambacciani *et al.*, 1995; Sebert *et al.*, 1995). In two studies (Reginster *et al.*, 1998; Pak *et al.*, 1995) there was on the contrary a significantly reduced occurrence of vertebral fractures in the fluoride group compared to the placebo group. Meunier *et al.* (1998) reported a significantly higher

incidence of lower-limb pain in the group receiving fluoride 20-26 mg/day compared to the placebo group. Some women with lower-extremity pain were roentgenographed and incomplete fractures were identified in most of them at least two weeks after the onset of pain.

Whereas bone mineral density increased in one study in the lumbar spine (+35%) and the femoral neck (+10%) in the fluoride-treated group (0.56 mg fluoride/kg/day) as compared to the placebo group, there was a decrease in the radius (-4%). Vertebral fracture rate did not differ significantly over four years between the treatment and the placebo group. Non-vertebral fractures occurred in the fluoride group (72 fractures) in higher frequency than in the placebo group (24 fractures); there were 13 hip fractures in the fluoride group and four hip fractures in the placebo group. The odds ratio for non-vertebral fractures was 3.2 (95% CI 1.8-5.6) in the fluoride group compared to the placebo group (Riggs *et al.* 1990). Fifty of the 66 women in the fluoride group who completed the four-year trial were treated for an additional two years. Bone mineral density measured at the lumbar spine continued to increase linearly, whereas the rate of decrease in bone mineral density of the radius became less (minus 1.2%/year versus minus 2.2%/year in the four previous years). Vertebral fracture rate decreased somewhat in the additional two years, as did the non-vertebral fracture rate. However the non-vertebral fracture rate remained higher than in the placebo group during the first four years. From multivariate analysis it appeared that the vertebral fracture rate was moderately decreased by sodium fluoride therapy in women whose serum fluoride level and lumbar spine bone mineral density increased, provided that the increase in serum fluoride level did not exceed 8 μM (152 μg/L) and the increase in bone mineral density did not exceed 17% per year (Riggs *et al.*, 1994).

3.2.2.2.2 Gastrointestinal effects

Table 5 lists also the frequency of gastrointestinal side effects of fluoride treatment studies. No gastrointestinal effects or the same frequency of nausea and dyspepsia as in the placebo group were observed in postmenopausal women administered 4.5-22 mg fluoride/day (assumed to correspond to 0.13-0.37 mg fluoride/kg body weight/day) over 12 weeks and up to 3 years (Hansson and Roos, 1987; Christiansen *et al.*, 1980; Grove and Halver, 1981; Reginster *et al.*, 1998; Gambacciani *et al.*, 1995; Meunier et al, 1998; Pak *et al.*, 1995; Sebert *et al.*, 1995).

Nine of 61 postmenopausal women treated with on average 57 mg fluoride/day (as sodium fluoride) over four years complained of severe nausea, vomiting and peptic ulcer or blood loss anaemia. These symptoms did not occur in the control groups without treatment or with calcium and estrogens alone (Riggs *et al.*, 1982). Nineteen of 101 postmenopausal women treated with 34 mg fluoride/day during four years had severe gastrointestinal complaints which led to dose reduction, compared to seven of 101 in the placebo group. This is an odds ratio of 2.9 (95% CI 1.2-7.1). The risk for peptic ulceration and anaemia was similar in both groups (Riggs *et al.*, 1990). Gastrointestinal symptoms occurred significantly more often in 45 postmenopausal women treated with 34 mg fluoride plus 1500 calcium/day over four years than in 38 women receiving only calcium (16/46 versus 6/38) (Kleerekoper *et al.*, 1991).

Table 5. Eleven therapeutical randomised studies in old-age osteopenia/osteoporosis grouped according to fluoride dosis. Outcome with regard to vertebral and non-vertebral fractures and other side effects

Reference	Sample size (treatment/ placebo)	Age (years)	Duration (years)	Fluoride treatment: Fluoride compound	Fluoride treatment: Dose (mg/day)	Fluoride treatment: Dose (mg/kg/d)[3)]	Other substances (mg/day)	Control group	Vertebral fractures: fluoride	Vertebral fractures: control / placebo	Non-vertebral fractures: fluoride	Non-vertebral fractures: placebo	Lower Limb Pain: verum	Lower Limb Pain: placebo	Gastrointestinal symptoms: verum	Gastrointestinal symptoms: placebo
Hansson and Roos, 1987	50 (25/25)	66	3	NaF	4.5		calcium 1000	calcium 1000	2/25	1/25						
	50 (25/25)	65	3	NaF	13.6		calcium 1000	placebo		1/25					4/25	
Christiansen *et al.*, 1980	177 (29/121)	50	2	NaF	9		a) calcium 500	calcium 500	N.R.		N.R.		N.R.		N.R.	
	(27/121)	50	2	NaF	9		b) calcium 500 Vit. D 50 µg	calcium 500								
Grove & Halver, 1981	28 (14/14)	74	0.23	NaF	9		calcium 500 Vit. D 360 µg	-							2/12	2/10
Reginster[1)] *et al.*, 1998	164 (84/80)	64	4	NaMFP	20	(0.32)	calcium 1000	calcium 1000	2/84 2.4% (95% Cl, 0.3-8.3)	8/80 10% (95% Cl, 4.4-18.8%	15/84	13/80	N.R.			
									p=0.05		N.S.				N.S.	
Gambacciani *et al.*, 1995	60 (30/30)	52	2	GluMFP	20		calcium 600	calcium 500	N.R.		N.R.		N.R.		7/21	6/21
Meunier *et al.*, 1998									33%	25.4%	all 29/208 = 13.9%	17/146 = 11.6%	all 17.8	4.8	59.2%	59.4%
											p=0.53					
	219 (73/146)	66	2	NaF	22.6	0.37	calcium 1000 Vit. D 20 µg	calcium 1000 Vit. D 20 µg			11/73		19.2%			
	214 (68/146)	66	2	NaMFP	19.8	0.33					7/68		13.2%			
	213 (67/146)	66	2	NaMFP	26.4	0.44			p=0.15		13/67		20.9%		N.S.	
													p=0.001			

Reference	Sample size (treatment/ placebo)	Age (years)	Duration (years)	Fluoride treatment: Fluoride compound	Fluoride treatment: Dose (mg/day)	Fluoride treatment: Dose (mg/kg/d)[3]	Other substances (mg/day)	Control group	Vertebral fractures: fluoride	Vertebral fractures: control / placebo	Non-vertebral fractures: fluoride	Non-vertebral fractures: placebo	Lower Limb Pain: verum	Lower Limb Pain: placebo	Gastrointestinal symptoms: verum	Gastrointestinal symptoms: placebo
Pak[1] *et al.*, 1995	110 (54/56)	67	4 [5]	NaF [4]	22.6	0.37	calcium 800	calcium 800	7/48	22/51	2/48	4/51	11.1%	14.3%	9.3%	7.1%
	after one year								p=0.001							
	99 (48/51)								=14.6%	=43.1%	p>0.2		p=0.78		p=0.74	
Sebert[2] *et al.*, 1995	94 (35/41)	61	2	NaMFP	26.4	0.43	calcium 500	calcium 500	N.R.	N.R.	2/45	0/49	5/45	2/49	10/45	9/49
									p=0.84		p>0.2		p=0.3		p>0.2	
Kleerekoper *et al.*, 1991	84 (46/38)	67	4	NaF	34	0.52	calcium 1500	calcium 1500	961/1000 patient years	723/1000 patient years	13/46 =28%	7/38 [6] =18%	N.R.		16/46 =35%	6/38 =16%
									p=031		p=0.29				p=0.05	
Riggs *et al.*, 1990	202 (101/101)	68	4	NaF	34	0.56	calcium 1500	calcium 1500	47.0/100 person years	52.5/100 person years	23/100 person years	7/100 person years	54	24	17	7
									RR 0.85 (95% Cl, 0.6-1.2)		RR 3.2 (95% Cl, 1.8-5.6)		RR 3.0 (95% Cl, 1.9-4.8)		RR 2.9 (95% Cl, 1.2-7.1)	
									N.S.							
Riggs[7] *et al.*, 1994	151 (50/101)	66	2	NaF	31.5	0.52	calcium 1500	see Riggs *et al.*, 1990	45/100 person years (0-6 years)		21/100 person years (0-6 years)		N.R.		N.R.	
									32/100 person years (4-6 years)		13/100 person years (4-6 years)					

Reference	Sample size	Age	Duration	Fluoride treatment			Other substances	Control group	Vertebral fractures		Non-vertebral fractures		Lower Limb Pain		Gastrointestinal symptoms	
				Fluoride compound	Dose											
	(treatment/ placebo)	(years)	(years)		(mg/day)	(mg/kg/d)[3]	(mg/day)		fluoride	control / placebo	fluoride	placebo	verum	placebo	verum	placebo
Riggs[1] *et al.*, 1982	165 (33/104)	63	4	NaF	57		calcium 800-1500 Vit. D 10 µg or 360 µg	10 µg Vit. D and calcium 1500-2000 and/or estrogens 0.625-2.5 and/or 360 µg Vit. D	304/1000 patient years	834/1000 patient years (10 µg Vit. D only)	N.R.		14/61	0/104	10/61	0/104
	(28/104)	63	4	NaF	57		calcium 1000-5000 estrogens 0.625-2.5 Vit. D 10 µg or 360 µg		53/1000 patients years							
									p<0.000001							

N.S. not significantly different
N.R. not reported
NaF sodium fluoride
NaMFP sodium monofluorophosphate
GluMFP L-glutamine monofluorophosphate

1) 10-33% of patients continued hormone replacement therapy
2) study population includes three male patients;
18 patients were not included in assessment of bone mineral density
3) dosis divided by reported mean body weight
4) slow release preparation
5) four cycles of 12 months treatment plus 2 months treatment pause
6) excluding “incomplete fractures” identified by ^{99}Tc-bone scans which occurred significantly (p=0.02) more often in the lower extremities of patients on fluoride treatment
7) 50 patients under fluoride treatment in the study of Riggs *et al.*, 1990, continued treatment for another 2 years

4. DOSE-RESPONSE ASSESSMENT

4.1 Skeletal fluorosis, bone density, fractures

4.1.1 Bone density and bone strength

Bone density increases with increasing fluoride content of bone as a consequence of an increasing fluoride intake both in animals and in humans. This effect is observed predominantly in cancelleous bone. This increase in bone fluoride content is accompanied by an increase in bone strength up to a certain level, thereafter bone strength decreases. Turner *et al.* (1992; 1995) showed in rats drinking for 16 weeks water with 16 mg fluoride/L (corresponding to an estimated intake of 0.11 mg/kg body weight/day), that the fluoride content in bone was ≤ 1200 mg/kg and bone strength increased by 38%, whereas rats drinking water with fluoride contents between 50-128 mg/L (corresponding to 2.5-7.2 mg/kg/day accumulated 10,000 mg/kg fluoride in bone and strength decreased by 20%.

Trabecular bone compressive strength in autopsy samples from the iliac crest was significantly ($p<0.05$) higher in women from Kuopio with fluoridation of drinking water (0.97 mg/L) than in women from an area with low fluoride content in the water (0.02-0.32 mg/L), however, no significant difference was found in men (Alhava *et al.*, 1980). There are no reliable measures for bone strength in humans. The available data are of uncertain relevance with regard to the risk for bone fractures and insufficient for conclusions on effective fluoride doses.

4.1.2 Skeletal fluorosis

The asymptomatic stage of skeletal fluorosis is associated with fluoride contents in bone ash of 3500-5500 mg/kg. Clinical stages I and II plus III have been found to have fluoride contents of 6000-7000 and >7500 mg/kg bone ash, respectively (Hodge and Smith, 1977). There are no parallel data on the fluoride intake associated with these levels of fluoride in bone.

Fluoride content of the skeleton increases with increasing intake of fluoride via water. In areas with water fluoride contents of <0.3, 1.0 and 4 mg/L fluoride in bone ash was 140-790, 400-2300 and 6900 mg/kg, respectively (Alhava *et al.*, 1980; Bergmann, 1994; Zipkin *et al.*, 1958). From studies in China and India a correlation between the fluoride content in drinking water and skeletal fluorosis can be deduced. Prevalences of 4.4% at water fluoride levels of 1.4 mg/L and of 63% at water fluoride levels of 6 mg/L were observed in India. Crippling fluorosis was consistently found in villages with more than 3 mg fluoride/L. An estimated total fluoride intake of 20 mg/day was associated with a fluorosis prevalence of 34%, whereas no fluorosis was observed in areas with an estimated total fluoride intake of less than 10 mg/day. Skeletal fluorosis started to appear after 10 years of residence in a village with an estimated daily fluoride intake of 36-54 mg/day and concerned 100% of the population after 20 years. Precise intake estimates from regions with higher fluoride concentrations in drinking water are lacking. Fluoride intake from diet and water in adults was estimated to be 0.84-4.69 mg/day in Indian villages without endemic skeletal fluorosis and 3.4-27.1 mg/day in fluorosis-prone villages (IPCS, 2002).

Numerous epidemiological data support a linear relationship between fluoride intake and bone fluoride content and between bone fluoride content and both incidence and severity of skeletal

fluorosis In the few cases of clinical skeletal fluorosis in which the fluoride intake could be estimated it ranged from 15 to 20 mg/day and the period of exposure was over 20 years. A more precise threshold dose for fluoride causing skeletal fluorosis can not be defined.

The Panel decided not to chose the data on skeletal fluorosis in relation to the fluoride content of the drinking water as the critical endpoint for setting an UL because too many assumptions on the effective fluoride dose were necessary.

The Panel decided also not to use the data on the relationship between fluoride intake via drinking water and radiographic skeletal changes decribed in Section 3.2.2.1.1 for setting a UL because of insufficient exposure estimates and the lack of more recent radiographic investigations.

4.1.3 Fractures

4.1.3.1 Observational Data

Although an association of an increased risk for hip fractures in the elderly with the fluoride content in drinking water has been reported, the opposite has been found as well (Jacqmin-Gadda *et al.*, 1998; Kurttio *et al.*, 1999; Li *et al.*, 2001; Sowers *et al.*, 1986) or no association (Hillier *et al.*, 2000; Karagas *et al.*, 1996; Kröger *et al.*, 1994).

In one study from China a bimodal relationship between fluoride content in drinking water and fluoride intake per day and risk of overall fractures was apparent. Compared to an exposure of 3.4 mg fluoride/day there was a significantly increased risk for fractures at all sites (OR 1.47; p=0.01) and for hip fracture (OR 3.26; p=0.02) at an exposure of 14.1 mg fluoride/day. A fluoride exposure of about 6.5 mg/day was associated with a non-significant increase in the risk of hip fracture (OR 2.13; p=0.15) compared to an exposure of 3.4 mg fluoride/day. Compared to a fluoride exposure of 3.4 mg/day there was a significantly increased risk for fractures at all sites at an exposure of 0.7 mg/day (OR 1.5; p=0.01) (Li *et al.*, 2001).

In the retrospective cohort study in Finland which involved 144,627 persons an increasing risk for hip fracture in women between 50 and 65 years of age with increasing fluoride concentration in drinking water was found. This relationship was significant for concentrations of 0.5-1.0 and of >1.5 mg/L compared to less than 0.1 mg/L (Kurttio *et al.*, 1999).

The study by Li *et al.* (2001) is considered as evidence that an increased risk of bone fractures occurs at a total intake of 14 mg fluoride per day and that there are data (although statistically not significant) suggestive of an increased risk of adverse bone effects at total intakes above about 6.5 mg fluoride/day. The study of Kurttio *et al.* (1999) is considered as supportive (IPCS, 2002).

4.1.3.2 Therapeutic studies

From therapeutical studies with fluoride administration in postmenopausal women of 0.25-6 years duration and which employed fluoride doses between 0.13 and 1.1 mg/kg body weight per day either as sodium fluoride or monofluorophosphate it appears that side-effects in the form of lower limb pain occurred in a significantly higher frequency when fluoride doses of

more than 0.4 mg/kg body weight were administered compared with the placebo group. Lower limb pain was indicative of incomplete fractures of the bone (Kleerekoper *et al.*, 1991; Meunier *et al.*, 1998; Riggs *et al.*, 1982, 1990 and 1994).

In one study involving 101 subjects in the fluoride treatment group (0.56 mg fluoride/kg body weight/day) and 101 subjects in the control group, of which two thirds completed the four-year study period, there was a significant increase in the occurrence of non-vertebral fractures (72 versus 24), with an odds ratio of 3.2 (95 CI 1.8-5.6). Vertebral fracture rate increased by 11% for each 1 μM (19 μg/L) increase in serum fluoride over baseline and it decreased with increasing bone mineral density of the lumbar spine. However, if this increase in bone mineral density went beyond 1.2 g/cm^2 an increase in vertebral fracture rate was observed (Riggs *et al.*, 1990; 1994). Fifty women from the fluoride group continued treatment for an additional two years, but only nine of these with 34 mg fluoride/day corresponding to 0.56 mg/kg/day (as sodium fluoride). The fluoride dosis in the other 41 women had been reduced because of side effects or by the patients themselves; four women took less than 18 mg fluoride/day. The lumbar spine, femoral neck, and femoral trochanter bone mineral density continued to increase and the bone mineral density of the radius continued to decrease. The vertebral fracture rate decreased compared to the years 0-4. The non-vertebral fracture rate decreased also but was still 3 times higher after six years than in the control group (Riggs *et al.*, 1994).

The Panel considers the fluoride dose of 0.56 mg/kg body weight per day, rounded up to 0.6 mg/kg/day to include the usual dietary intake from food and water, as the dose associated with a significant increase in the occurrence of non-vertebral fractures.

4.2 Dental fluorosis

Enamel fluorosis is caused by fluoride ingestion during the preeruptive formation and maturation of enamel of teeth. Therefore, the sensitive period is before the age of eight years. There is a clear dose-response relationship with a prevalence of 48% of very mild and mild forms of dental fluorosis at fluoride intakes from water of 0.043 mg/kg/day (Fejerskov *et al.*, 1996a). Very mild forms of dental fluorosis are of aesthetic concern only. From the data of Dean (1942), it appears that in areas with a fluoride content of water of 1 mg/L 10-12% of the residents had mild forms of fluorosis (very mild plus mild). The fluoride intake of children in these communities was found to be 0.02-0.1 mg/kg body weight/day. In areas with a fluoride concentration in water of ≤ 0.3 mg/L the fluorosis prevalence was 1%, whereas it was 50% in areas with a fluoride concentration in water of 2 mg/L, and in these areas a few cases (<5%) of moderate fluorosis were observed. The fluoride intake by children in these communities was 0.08-0.12 mg/kg/day. A fluoride dose of 0.1 mg/kg body weight/day was, therefore, described as a “threshold” dose for the occurrence of less than 5% of moderate forms of dental fluorosis in a population for the ages from birth to eight years (Dean, 1942; Fejerskov *et al.*, 1996a).

The Panel concludes that an intake of 0.1 mg fluoride/kg body weight/day in children up to the age of eight years can be considered as the dose below which no significant occurrence of moderate forms of fluorosis in permanent teeth will occur.

4.3 Gastrointestinal effects

Gastrointestinal symptoms like nausea, vomiting, anorexia, diarrhoea occur with fluoride intakes that also result in skeletal effects, i.e., with doses above 0.5 mg/kg body weight/day

(Kleerekoper *et al.*, 1991; Riggs *et al.*, 1990). However, these effects are more unpredictable and presumably dependent on other dietary factors like fluid intake and type of diet.

Severe clinical symptoms were observed in 22% of children on acute single dose ingestion of sodium fluoride amounts of about one mg fluoride/kg body weight (Augenstein *et al.*, 1991).

CONCLUSIONS AND RECOMMENDATIONS

1. DERIVATION OF THE TOLERABLE UPPER INTAKE LEVEL (UL)

The Panel has identified different critical endpoints for the derivation of the UL of oral fluoride intake for the age from one to eight years (moderate dental fluorosis) and for all ages above eight years (bone fracture). Different ULs are set for these groups.

1.1 Children up to the age of eight years

The data support a continuous relationship between fluoride intake during the period from birth to eight years of age and both incidence and severity of dental fluorosis. The occurrence of moderate enamel fluorosis was less than <5% in populations at fluoride intakes of 0.1 mg/kg body weight/day. Mild fluorosis is generally considered to be acceptable on a population basis, in view of the concomitant beneficial effect of fluoride in the prevention of caries. No uncertainty factor is deemed necessary to derive an UL from this intake, because it is derived from population studies in the susceptible group. For children up to the age of eight years this intake level of 0.1 mg/kg body weight/day is proposed as the UL. Calculated on a body weight basis the following age-related ULs for daily fluoride intake are set:

Age (years)	Tolerable Upper Intake Level (UL) for fluoride (mg/day)
1-3 years	1.5
4-8 years	2.5

1.2 Children older than eight years and adults

Therapeutic studies with fluoride in postmenopausal osteoporosis suggest an increasing risk for skeletal fractures at or above fluoride intakes of 0.6 mg/kg body weight per day. The Panel decided to apply an uncertainty factor of 5 to the intake of 0.6 mg fluoride/kg body weight/day, because, although the adverse effects were detected in a sensitive group of elderly postmenopausal women, the study duration was relatively short and the studies were not designed to systematically define a LOAEL. The epidemiological data with an observed significantly increased risk for fractures at all sites associated with a long-term total daily intake of fluoride of 14 mg/day are considered as supportive evidence. An intake of 0.12 mg fluoride/kg body weight/day converts on a body weight basis (60 kg) into an UL of 7 mg/day for adults.

On a body weight basis the following ULs are proposed:

Age (years)	Tolerable Upper Intake Level (UL) for fluoride (mg/day)
9-14 years	5
≥15 years	7

1.2 Pregnancy and lactation

There are no data which support the setting of a specific UL. The UL of 7 mg/day applies.

2. RISK CHARACTERISATION

There is a narrow margin between recommended intakes for the prevention of dental caries and the ULs.

2.1 Infants and children up to 8 years

The Panel did not establish an UL for infants. The Panel notes, however, that the maximum level recommended by the SCF (2003) for fluoride of 0.6-0.7 mg/L (0.1 mg/100 kcal; 600-700 kcal/L) in infant formula and follow-on formula will result in fluoride intakes of infants during the first half of the first year of life (body weight 5 kg) of about 0.1 mg/kg body weight per day. The maximum recommended fluoride content of formula will be exceeded if water containing more than 0.7 mg/L is used for preparation of the formula.

Breast-fed infants have very low fluoride intakes from human milk (2-40 μg/day) and are not at risk of developing enamel fluorosis even when given fluoride supplements of 0.25 mg/day.

Children will have fluoride intakes from food and water well below the UL provided the fluoride content of their drinking water is not higher than 1.0 mg/L.

An increase in the prevalence of mild dental fluorosis observed in some countries has been attributed to the inappropriate use of dental care products, particularly of fluoridated toothpaste.

2.2 Children older than eight years and adults

The probability of exceeding the UL of 5/7 mg fluoride/day on a normal diet is generally estimated to be low. However, consumption of water with a high fluoride content e.g. more than 2-3 mg/L predisposes to exceeding the UL.

3. RECOMMENDATIONS FOR FURTHER WORK

More reliable data on total daily fluoride intake and the identification of the main sources of fluoride, particularly in young children, are needed. The incidence and severity of dental fluorosis should be monitored as an indicator of fluoride exposure during childhood.

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Annex 1. Development of deciduous teeth (Wei, 1974 cited in Bergmann, 1994)

Tooth	first formation hard substance (months of gestation)	mature enamel (months of life)	eruption (months of life)	root completed (years of life)
Mandibula				
Incisors				
central	4.5	2.5	6	1.5
lateral	4.5	3	7	1.5
Canine	5	9	16	3.25
first praemolar	5	5.5	12	2.25
second praemolar	6	10	20	3
Maxilla				
Incisors				
central	4	1.5	7.5	1.5
lateral	4.5	2.5	9	2
Canine	5	9	18	3.25
Praemolar first	5	6	14	2.5
Praemolar second	6	11	24	3

Development of permanent teeth (Wei, 1974 cited in Bergmann, 1994)

Tooth	formation of hard substance [age in months (m) or years (y)]	mature enamel (age in years)	eruption (age in years)	root completed (age in years)
Mandibula				
Incisors				
central	3-4 m	4-5 y	6-7 y	9 y
lateral	3-4 m	4-5 y	7-8 y	10 y
Canines	4-5 m	6-7 y	9-10 y	12-14 y
Praemolars				
first	1.75-2 y	5-6 y	10-12 y	12-13 y
second	2.25-2.5 y	6-7 y	11-12 y	13-14 y
Molars				
first	at birth	2.5-3 y	6-7 y	9-10 y
second	2.5-3 y	7-8 y	11-13 y	14-15 y
third	8-10 y	12-16 y	17-21 y	18-25
Maxilla				
Incisors				
central	3-4 m	4-5 y	7-8 y	10 y
lateral	10-12 m	4-5 y	8-9 y	11 y
Canines	4-5 m	6-7 y	11-12 y	13-15 y
Praemolars				
first	1.5-1.75 y	5-6 y	10-11 y	12-13 y
second	2-2.25 y	6-7 y	10-12 y	12-14 y
Molars				
first	at birth	2.5-3 y	7-8 y	9-10 y
second	2.5-3 y	7-8 y	12-13 y	14-16 y
third	7-9 y	12-16 y	17-21 y	18-25 y

Annex 2. Dental fluorosis

I. Grading of dental fluorosis ("Dean's fluorosis index") (Dean, 1934 and 1942)

Grade	Criteria
Normal (0)	The enamel presents the usual translucent semivitriform type of structure . The surface is smooth and glossy and usually of a pale creamy white colour. Included under this heading are all persons showing hypoplasia other than mottling of the enamel.
Questionable (0.5)	The enamel shows slight aberrations in the translucency of of normal enamel, ranging from a few white flecks to occasional white spots, 1 to 2 mm in diameter. It is recommended that this diagnosis is best made on a group basis comparing groups of children from different areas and with demonstrated use of a common water supply from birth.
Very mild (1)	Small opaque paper white areas are scattered irregularly or streaked over the tooth surface, principally on the labial and buccal surfaces and involving less than 25% of the surface of the affected teeth. Small pitted white areas are frequently found on the summit of cusps. No brown stains are present. Mottling of the enamel of deciduous teeth is invariably of the very mild type, while permanent teeth of the same individual may show severe mottling.
Mild (2)	The white opaque areas on the surfaces of the teeth involve at least half of the tooth surface. The surfaces of molars, bicuspids and cuspids subject to attrition show thin white layers worn off and the bluish shades of underlying normal enamel. Faint brown stains are sometimes apparent, generally on the upper incisors.
Moderate (3)	No change is observed in the form of the tooth, but generally all of the tooth surfaces are involved. Surfaces subject to attrition are definitely marked. Minute pitting is often present. Brown stain is frequently a disfiguring complication.
Severe (includes former grades moderately severe and severe) (4)	A greater depth of enamel is involved, with a smoky white appearance. Pitting is frequent, observed on all the tooth surfaces and is often confluent. The hypoplasia is so marked that the form of the teeth is at times affected. Stains are wide-spread and range from a chocolate brown to almost black in some cases. Teeth often present as corroded.

II. Tooth Surface Index of Fluorosis (TSIF) (Horowitz *et al.*, 1984)

Score	Criteria
0	Enamel shows no evidence of fluorosis.
1	Enamel shows definite evidence of fluorosis, namely areas with parchment-white colour, that total less than one third of the visible enamel surface. This category includes fluorosis confined only to incisal edges of anterior teeth and cusp tips of posterior teeth ("snowcapping").
2	Parchment-white fluorosis totals at least one-third of the visible surface, but less than two-thirds.
3	Parchment-white fluorosis totals at least two-thirds of the visible surface.
4	Enamel shows staining in conjunction with any of the preceding levels of fluorosis. Staining is defined as an area of definite discoloration that may range from light to very dark brown.
5	Discrete pitting of the enamel exists, unaccompanied by evidence of staining of intact enamel. A pit is defined as a definite physical defect in the enamel surface with a rough floor that is surrounded by a wall of intact enamel. The pitted area is usually stained or differs in color from the surrounding enamel.
6	Both discrete pitting and staining of the intact enamel exist.
7	Confluent pitting of the enamel surface exists. Large areas of enamel may be missing and the anatomy of the tooth may be altered. Dark-brown stain is usually present.

III. Thylstrup-Fejerskov (TF)-Score (Thylstrup and Fejerskov, 1978; Fejerskov *et al.*, 1996)

Score	Criteria
0.	Normal translucency of the glossy creamy-white enamel remains after wiping and drying of the surface.
1.	Thin white opaque lines are seen running across the tooth surface. Such lines are found on all parts of the surface. The lines correspond to the position of the perikymata. In some cases, a slight "snowcapping" of cusps/incisal edges may also be seen.
2.	The opaque white lines are more pronounced and frequently merge to form small cloudy areas scattered over the whole surface. "Snowcapping" of incisal edges and cusp tips is common.
3.	Merging of the white lines occurs, and cloudy areas of opacity occur spread over many parts of the surface. In between the cloudy areas, white lines can also be seen.
4.	The entire surface exhibits a marked opacity, or appears chalky white. Parts of the surface exposed to attrition or wear may appear to be less affected.
5.	The entire surface is opaque, and there are round pits (focal loss of outermost enamel) that are *less than* 2 mm in diameter.
6.	The small pits may frequently be seen merging in the opaque enamel to form bands that are less than 2 mm in vertical height. In this class are included also surfaces where the cuspal rim of facial enamel has been chipped off, and the vertical dimension of the resulting damage is *less than* 2 mm.
7.	There is a loss of the outermost enamel in irregular areas, and *less than* half the surface is so involved. The remaining intact enamel is opaque.
8.	The loss of the outermost enamel involves more than half the enamel. The remaining intact enamel is opaque.
9.	The loss of the major part of the outer enamel results in a change of the anatomic shape of the surface/tooth. A cervical rim of opaque enamel is often noted.