EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Scientific opinion on Dietary Reference Values for fluoride

EFSA publication; Tetens, Inge

Link to article, DOI: 10.2903/j.efsa.2013.3332

Publication date: 2013

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
SCIENTIFIC OPINION

Scientific Opinion on Dietary Reference Values for fluoride

EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA)

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies (NDA) derived Dietary Reference Values (DRVs) for fluoride, which are provided as Adequate Intake (AI) from all sources, including non-dietary sources. Fluoride is not an essential nutrient. Therefore, no Average Requirement for the performance of essential physiological functions can be defined. Nevertheless, the Panel considered that the setting of an AI is appropriate because of the beneficial effects of dietary fluoride on prevention of dental caries. The AI is based on epidemiological studies (performed before the 1970s) showing an inverse relationship between the fluoride concentration of water and caries prevalence. As the basis for defining the AI, estimates of mean fluoride intakes of children via diet and drinking water with fluoride concentrations at which the caries preventive effect approached its maximum whilst the risk of dental fluorosis approached its minimum were chosen. Except for one confirmatory longitudinal study in US children, more recent studies were not taken into account as they did not provide information on total dietary fluoride intake, were potentially confounded by the use of fluoride-containing dental hygiene products, and did not permit a conclusion to be drawn on a dose-response relationship between fluoride intake and caries risk. The AI of fluoride from all sources (including non-dietary sources) is 0.05 mg/kg body weight per day for both children and adults, including pregnant and lactating women. For pregnant and lactating women, the AI is based on the body weight before pregnancy and lactation. Reliable and representative data on the total fluoride intake of the European population are not available.

KEY WORDS

fluoride, caries, Adequate Intake, Dietary Reference Value

1 On request from the European Commission, Question No EFSA-Q-2011-01211, adopted on 11 July 2013.
2 Panel members: Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhaus-Erberthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen. Correspondence: nda@efsa.europa.eu
3 Acknowledgement: The Panel wishes to thank the members of the Working Group on Dietary Reference Values for Minerals: Carlo Agostoni, Sue Fairweather-Tait, Marianne Geleijnse, Michael Hambidge, Ambroise Martin, Androniki Naska, Hildegard Przyrembel, Alfonso Siani, and Hans Verhagen for the preparatory work on this scientific opinion and EFSA staff: Anja Brönstrup for the support provided to this scientific opinion.


Available online: www.efsa.europa.eu/efsajournal

© European Food Safety Authority, 2013
SUMMARY
Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on Dietary Reference Values (DRVs) for the European population, including fluoride.

Fluoride has no known essential function in human growth and development and no signs of fluoride deficiency have been identified. Though fluoride is not essential for tooth development, exposure to fluoride leads to incorporation into the hydroxyapatite of the developing tooth enamel and dentin. The resulting fluoroapatite is more resistant to acids than hydroxyapatite. Thus, teeth which contain fluoroapatite are less likely to develop caries. Apart from incorporation of fluoride into the dentin and enamel of teeth before eruption, dietary fluoride exerts an anticaries effect on erupted teeth through contact with enamel during consumption, excretion into saliva and uptake into biofilms on teeth. In addition, fluoride interferes with the metabolism of oral microbial cells, by directly inhibiting, for example, glycolytic enzymes and cell membrane-associated H⁺ ATPases in microbial cells after entry of hydrofluoric acid into their cytoplasm.

In bone, the partial substitution of fluoride for hydroxyl groups of apatite alters the mineral structure of the bone. Depending on the dose, fluoride can delay mineralisation. There is evidence from animal studies for a biphasic effect of fluoride on bone strength, with increases in both bone strength and bone fluoride content at moderately high fluoride intake, and a decrease with higher fluoride intake.

Major dietary fluoride sources are water and water-based beverages or foods reconstituted with fluoridated water, tea, marine fish, and fluoridated salt. Fluoride absorption occurs by passive diffusion in both the stomach (20-25 %) and the small intestine. On average 80-90 % of ingested fluoride is absorbed. In adults, up to 50 % of absorbed fluoride is associated with calcified tissues, mainly bone, a small amount reaches soft tissues, and the remainder is excreted, predominantly via the kidney and to a small extent via sweat and faeces.

The role of fluoride in the prevention of caries has been known for many years. In epidemiological studies performed before the 1970s, when fluoride in drinking water was practically the only relevant source of fluoride intake, it was shown that the prevalence of caries was negatively correlated with the fluoride concentration of water. The fluoride concentration at which the caries preventive effect approached its maximum was 1 mg/L, and at that level only 10 % of the population was affected by mild dental fluorosis. The average daily fluoride intake of a child in a community with this “optimal” drinking water fluoride concentration of 1 mg/L was determined as being approximately 0.05 mg fluoride/kg body weight per day from both water and diet.

Since then, many studies have reviewed the efficacy of fluoride in different forms (water, milk, salt, tablets/drops, chewing gum) in preventing dental caries. However, very few of these studies provide information on total dietary fluoride intake, and the outcome measure for caries may have been affected by additional uses of non-dietary fluoride. Therefore, they do not permit a conclusion to be drawn on a dose-response relationship between dietary fluoride intake and caries risk.

The available data on the relationship between fluoride intake or intake deduced from the fluoride content of toenails and bone health did not provide evidence for a beneficial effect of fluoride on bone health.

As fluoride is not an essential nutrient, no Average Requirement for the performance of essential physiological functions can be defined. Because of the beneficial effect of dietary fluoride on the prevention of caries, the Panel considered that the setting of an Adequate Intake (AI) is appropriate and that data on the dose-response relationship between caries incidence and consumption of drinking water with different fluoride concentrations are sufficient to set an AI of 0.05 mg/kg body weight per day. The AI covers fluoride intake from all sources, including non-dietary sources such as toothpaste and other dental hygiene products.
No data are available to define a dose-response relationship between fluoride intake and caries for adults. The Panel considered that the AI for children of 0.05 mg/kg body weight per day can also be applied to adults, including pregnant and lactating women. For pregnant and lactating women the AI is based on the body weight before pregnancy and lactation.

Reliable and representative data on the total fluoride intake of the European population are not available. The available data on fluoride intake are variable but generally at or below 0.05 mg/kg body weight per day.
TABLE OF CONTENTS

Abstract ................................................................................................................................. 1
Summary ................................................................................................................................. 2
Table of contents .................................................................................................................. 4
Background as provided by the European Commission ......................................................... 6
Terms of reference as provided by the European Commission ............................................ 6
Assessment ............................................................................................................................. 8
1. Introduction ...................................................................................................................... 8
2. Definition/category .......................................................................................................... 8
2.1. Chemistry ...................................................................................................................... 8
2.2. Functions of fluoride ................................................................................................... 8
2.2.1. Dental health and tooth development .................................................................... 8
2.2.2. Bone health ............................................................................................................ 9
2.2.3. Other functions ...................................................................................................... 10
2.2.4. Health consequences of deficiency and excess ...................................................... 10
2.2.4.1. Deficiency ......................................................................................................... 10
2.2.4.2. Excess .............................................................................................................. 10
2.3. Physiology and metabolism of fluoride ....................................................................... 11
2.3.1. Intestinal absorption ............................................................................................. 11
2.3.2. Transport in blood ................................................................................................. 12
2.3.3. Distribution to tissues ............................................................................................ 12
2.3.4. Accumulation in the body ....................................................................................... 13
2.3.5. Elimination ............................................................................................................ 13
2.3.5.1. Kidney ................................................................................................................ 13
2.3.5.2. Faeces ................................................................................................................ 13
2.3.5.3. Breast milk ....................................................................................................... 14
2.4. Biomarkers of fluoride intake ...................................................................................... 14
2.4.1. Plasma .................................................................................................................... 14
2.4.2. Urine ....................................................................................................................... 14
2.4.3. Saliva ....................................................................................................................... 15
2.4.4. Sweat and milk ....................................................................................................... 15
2.4.5. Bone and dentin ..................................................................................................... 15
2.4.6. Hair ........................................................................................................................ 15
2.4.7. Nails ....................................................................................................................... 15
2.4.8. Enamel .................................................................................................................... 16
2.5. Biomarkers of fluoride body burden ........................................................................... 16
2.5.1. Plasma .................................................................................................................... 16
2.5.2. Bone and dentin ..................................................................................................... 16
2.6. Conclusion on biomarkers of fluoride intake and body burden .................................. 17
2.7. Effects of genotypes .................................................................................................... 17
3. Dietary sources and intake data ....................................................................................... 18
3.1. Sources ....................................................................................................................... 18
3.1.1. Water ..................................................................................................................... 18
3.1.1.1. Fluoride intake from water ................................................................................ 18
3.1.2. Food ...................................................................................................................... 19
3.1.3. Infant and follow-on formula ................................................................................ 20
3.1.4. Fluoridated salt .................................................................................................... 20
3.1.5. Fluoride-containing dental products ..................................................................... 20
3.2. Intake .......................................................................................................................... 20
3.2.1. Infants .................................................................................................................... 20
3.2.2. Children ................................................................................................................ 21
3.2.3. Adults .................................................................................................................... 21
4. Overview of Dietary Reference Values and recommendations ...................................... 22
4.1. Adults .......................................................................................................................... 22
4.2. Infants and children ........................................................................................................... 23
5. Criteria (endpoints) on which to base Dietary Reference Values ........................................ 24
5.1. Biomarkers as endpoints .................................................................................................. 24
5.2. Health consequences ...................................................................................................... 24
  5.2.1. Dental health/caries ....................................................................................................... 24
    5.2.1.1. Fluoride in drinking water and dental health/caries .............................................. 25
    5.2.1.2. Total fluoride intake and dental health/caries ..................................................... 25
    5.2.1.3. Prenatal fluoride supplements and dental health/caries ....................................... 26
    5.2.1.4. Conclusions on fluoride intake and dental health/caries ..................................... 26
  5.2.2. Bone health ................................................................................................................ 27
6. Data on which to base Dietary Reference Values ................................................................. 27
6.1. Infants and children ......................................................................................................... 27
6.2. Adults ................................................................................................................................ 28
Conclusions ................................................................................................................................ 28
Recommendations for research/need for data ........................................................................ 28
References ................................................................................................................................. 29
Appendices ............................................................................................................................... 39
Appendix A. Fluoride concentration in breast milk from different regions of the world .......... 39
Appendix B. Biomarkers of fluoride intake and body burden ................................................. 40
Appendix C. Fluoride intake of children (1-6 years) from food, beverages and dentifrice ...... 42
Appendix D. Assessment of caries prevalence and severity ..................................................... 44
Abbreviations ............................................................................................................................ 45
BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and if necessary to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on the nutrient and energy intakes for the European Community. The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context, EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context, EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002, the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on population reference intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance, EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically, advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, trans fatty acids;
- Protein;
- Dietary fibre.

4 Scientific Committee for Food, Nutrient and energy intakes for the European Community, Reports of the Scientific Committee for Food 31\textsuperscript{st} series, Office for Official Publication of the European Communities, Luxembourg, 1993.
Following on from the first part of the task, EFSA is asked to advise on population reference intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, EFSA is asked to provide guidance on the translation of nutrient-based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).
ASSESSMENT

1. Introduction

In 1993, the Scientific Committee for Food (SCF) adopted an opinion on the nutrient and energy intakes for the European Community but was unable to define a specific physiological requirement of fluoride for human health. The SCF noted that epidemiological evidence pointed to an inverse relationship between dental caries and regular fluoride intake, and that fluoride had a beneficial effect on dental health. Fluoride deficiency had not been described, whilst chronic excessive fluoride intake, particularly in regions with fluoride concentrations in drinking water (far) in excess of 1 mg/L, was known to lead to dental fluorosis (disturbed maturation of tooth enamel of different grades of severity dependent on intake) and, in the case of chronic total fluoride intakes > 10-25 mg/day, to skeletal fluorosis (sclerotic calcification of bone, tendons, ligaments and interosseous membranes).

2. Definition/category

2.1. Chemistry

Fluorine is a gaseous halogen with an atomic mass of 18.998 Da. It is the most electronegative and reactive of all elements, and therefore it occurs naturally only in anionic form, i.e. as fluoride (F\(^-\)), after reaction with metallic elements or with hydrogen. Fluorides are ubiquitous in air, water and the lithosphere, where they are seventh 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 (Ca\(_{10}\)(PO\(_4\))\(_6\)X\(_2\), with X = F, Cl, OH) in mica, hornblende, or as pegmatites like topaz and tourmaline. Most of the fluoride anion is strongly bound to metal ions and is 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, and is generally low.

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 μg/m\(^3\). Hydrogen fluoride (HF), a highly corrosive gas or liquid at room temperature, is used extensively by industry. It readily dissolves in water to hydrofluoric acid (HF\(_{aq}\)), which is a weak acid with a pK\(_a\) of 3.4. HF\(_{aq}\) is rapidly converted to fluoride salts.

Analysis of fluorides in aqueous solutions is performed mainly by potentiometry using ion-selective electrodes, or by ion chromatography.

2.2. Functions of fluoride

Fluoride in the body is mainly associated with calcified tissue (bone and teeth). Fluoride has been known to be useful in the control of caries development for more than a hundred years (Sampaio and Levy, 2011). At the beginning of the 20th century, it was observed that a lower prevalence of caries was associated with (mild cases of) the brown stains on teeth (“mottled enamel”) that occurred in some regions of the USA, and that were positively related to the fluoride content of local drinking water (McKay, 1933; Dean, 1938).

2.2.1. Dental health and tooth development

Tooth development starts in the embryo from tooth buds, which consist of the enamel organ, the dental papilla and the dental follicle. The cells from the enamel organ transform into ameloblasts which produce enamel, the cells of the dental papilla develop into odontoblasts which form dentin and pulp cells. The dental follicle develops into cementoblasts, osteoblasts and fibroblasts, which are responsible for the cementum of a tooth, the alveolar bone around a tooth and the periodontal ligaments, respectively. Dentin formation precedes enamel formation. Ameloblasts secrete proteins as constituents of the enamel matrix, which is partially mineralised to form the first enamel around the third to fourth month of pregnancy. Enamel formation is followed by enamel maturation when ameloblasts remove transport proteins involved in amelogenesis out of the enamel. Fluoride uptake
from the circulation into enamel occurs only during tooth formation. It is incorporated into the hydroxyapatite of the developing tooth enamel and dentin. Fluoride is not essential for tooth development, whilst adequate intakes of nutrients, particularly nutrients such as calcium, phosphorus and vitamins A, D and C, are needed for healthy tooth development.

Fluorohydroxyapatite is more resistant to acids than hydroxyapatite. The critical pH at which dissolution of apatite begins to be higher than mineral deposition is 5.5 for hydroxy- and 4.5 for fluoroapatite. Teeth which contain fluoroapatite are less likely to develop caries because of greater resistance to ingested acids or to acids generated from ingested sugars by the oral bacteria (Beltran and Burt, 1988; Buzalaf et al., 2011). Not all apatite in enamel and dentin is fluoridated, though. Replacement of hydroxyl groups by fluoride in the surface of enamel was found to be 8% in areas with fluoridated drinking water and 3% in non-fluoridated areas, and fluoride concentration in surface enamel of about 3 000 ppm decreases to about some hundred ppm at a depth of more than 10-20 µm (Weatherell et al., 1977).

Mature dental enamel is an acellular tissue and consists mainly of minerals (85% by volume), particularly hydroxyapatite \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) in long crystals which combine to form enamel prisms. The space between these prisms is filled with water (12%) and organic material (3%). The hydroxyl-groups of hydroxyapatite can exchange with fluoride from the fluid surrounding the enamel prisms and the outer surface of the tooth to form fluoroapatite \( \text{Ca}_{10}(\text{PO}_4)_6(\text{F})_2 \). This incorporation of fluoride into the maturing enamel occurs already pre-eruptively (Buzalaf and Levy, 2011).

Dentin is a cellular tissue and contains about 47% minerals, 20% water and 33% organic components by volume, mostly collagen upon which the apatite crystals are deposited. These are smaller than in enamel, and therefore have a greater surface and make dentin more susceptible to cariogenic attacks.

Apart from some incorporation of fluoride into the forming enamel of teeth before eruption, dietary fluoride will exert an anticaries effect on erupted teeth through contact with enamel during ingestion, excretion into saliva, and uptake into biofilms on teeth (Buzalaf et al., 2011). Fluoride present at constant low concentrations (in the saliva or the biofilm on the tooth surface and in the intercrystalline fluid of the enamel) will adsorb to the crystal surfaces and protect these from dissolution even if the pH falls due to acid production by bacteria (Featherstone, 1999) and, more importantly, it will also form calcium fluoride with calcium from saliva and calcium released from the enamel surface. Calcium fluoride globules will precipitate both on the biofilm and porous enamel sites and add to the fluoride reservoir within the mouth. Whilst under pH-neutral conditions oral fluids are supersaturated with respect to both hydroxy- and fluorohydroxyapatite and there is a tendency for calculus formation and (re)mineralisation of demineralised areas, at a drop in the pH of saliva and the biofilm fluid due to bacterial production of acids from sugars or due to dietary acids, these fluids will be undersaturated with respect to hydroxyapatite, causing hydroxyapatite to dissolve from the enamel subsurface layers. Because the oral fluids are still supersaturated with respect to fluorohydroxyapatite, this will be deposited on the surface layers. With repeated cycles of de- and remineralisation, more fluorohydroxyapatite will be deposited in the surface layer at the expense of hydroxyapatite. This layer will protect the subsurface tooth minerals from further acid attacks but will also hamper the repair of such demineralisation lesions. As a result, enamel crystals may be different from their original state in being more resistant to acid and containing more fluoride after repeated cycles of dissolution and reprecipitation (White and Nancollas, 1990; Featherstone, 1999). Dentin demineralises faster and remineralises slower than enamel, and higher fluoride concentrations are needed to enhance remineralisation and decrease demineralisation than for enamel (Herkstroter et al., 1991).

2.2.2. Bone health

In bone, the partial substitution of fluoride for hydroxyl groups of 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 necessarily in increased mechanical strength (Chachra et al., 1999). Depending on the dose, fluoride can delay mineralisation. 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 and a decrease with higher fluoride intake. Fluoride acts on osteoblasts and osteoclasts both in vivo and in vitro. It has a mitogenic effect on osteoblastic precursors (Bonjour et al., 1993). Whilst at fluoride concentrations of 0.05 mMol osteoclast function was enhanced, it was inhibited at concentrations of 0.8-1.6 mMol in dentin osteoclasts of chicken embryos in vitro (Taylor et al., 1990).

Sodium fluoride intake can increase bone mass, but the newly formed bone may lack normal structure and strength. The effect is more apparent in trabecular bone where volume and thickness is increased but without a concomitant increase in trabecular connectivity resulting in reduced bone quality (Everett, 2011). However, Sowers et al. (1986; 2005) found no association between serum fluoride concentrations and bone mineral density (BMD) or osteoporotic fractures after adjustment for BMD among female residents aged 20-92 years living in communities with mean fluoride concentrations in drinking water of 1 mg/L or 4 mg/L.

2.2.3. Other functions

Fluoride has no known essential function in human growth and development. Fluoride interferes with the metabolism of oral microbial cells, including cariogenic streptococci, by directly inhibiting, for example, glycolytic enzymes, and by enhancing the permeability of microbial cell membranes due to the entry of hydrofluoric acid formed in the acidic milieu created by plaque bacteria from the fermentation of dietary carbohydrate. In the alkaline cytoplasm, hydrofluoric acid dissociates, resulting in acidification and inhibition of glycolytic activity and cell membrane-associated H⁺ ATPases. In vitro studies with Streptococcus mutans have also shown that glucan synthesis is inhibited, which could decrease their plaque-forming capacity (Hamilton, 1990; ten Cate and van Loveren, 1999; Marquis et al., 2003). These antimicrobial effects might contribute to the anti-caries effect of fluoride, but it must be kept in mind that they have mostly been observed in in vitro or ex vivo experiments at fluoride concentrations that are higher than the concentration needed to reduce the solubility of apatite (Van Loveren, 2001). The Panel notes that the clinical relevance of these findings should be interpreted with caution.

2.2.4. Health consequences of deficiency and excess

2.2.4.1. Deficiency

No signs of fluoride deficiency have been identified in humans. One cohort study on infants from an area with a low fluoride content of drinking water described a higher rate of length and body weight gain with a fluoride supplement (0.25 mg/day from birth) than without (Bergmann, 1994). The Panel considers that this observation does not provide sufficient evidence to prove a causal relationship between fluoride intake and growth.

A lack of fluoride intake during development will not alter tooth development but may result in increased susceptibility of enamel to acid attacks after eruption. However, caries is not a fluoride deficiency disease.

The Panel concludes that fluoride is not an essential nutrient.

2.2.4.2. Excess

Acute ingestion of a large fluoride dose can provoke gastric and kidney disturbances, and can be lethal (Whitford, 2011). Acute excess fluoride intake interferes with calcium metabolism and many enzyme activities, activating both proteolytic and glycolytic functions and cell respiration by inhibiting Na⁺/K⁺-ATPase, and can be fatal with doses of 5-10 g in adults and 500 mg in small children (Lech, 2011).
Dental fluorosis

The studies by Dean (1942) had already shown that a positive relationship existed between water fluoride concentration and prevalence of dental fluorosis.

Dental fluorosis is an undesirable side-effect of excessive fluoride intake during critical periods of amelogenesis of both primary and secondary teeth. The sensitive period ranges up to eight years of age with the exception of the third molars, in which maturation of enamel is not completed before age 12-16 years (EFSA, 2005). Dental fluorosis is characterised by increased porosity due to subsurface hypomineralisation with a loss of enamel translucency and increased opacity. There is a correlation between severity of dental fluorosis and fluoride intake on a population basis, but severity of dental fluorosis varies individually at the same level of intake. There are indications from animal studies that genetic factors (dental fluorosis severity) and environmental factors (fluoride concentration in tooth) have similar influence on tooth biomechanical properties, whereas tooth material properties (mineralisation) are only influenced by environmental fluoride (Vieira et al., 2005; Everett, 2011). In a WHO report it is stated that it may not be possible to achieve effective fluoride-based caries prevention without some degree of dental fluorosis, regardless of which methods are chosen to maintain a low level of fluoride in the mouth (Petersen, 2003). Very mild forms of dental fluorosis are of aesthetic concern only, whilst in severe cases the teeth are stained brown, show enamel defects, are pitted and fragile, and may be deformed or break.

Based on its effects on dental fluorosis, the Tolerable Upper Intake Level (UL) for fluoride for children up to the age of eight years was set by EFSA (2005) at 0.1 mg/kg body weight per day or 1.5 mg/day and 2.5 mg/day for children aged 1-3 and 4-8 years, respectively.

Skeletal fluorosis

Chronic high intake of fluoride increases the risk of bone fractures and of the development of skeletal fluorosis in adults. In its review of the Maximum Contaminant Level Goal of 4 mg/L for fluoride in drinking water established by the US Environmental Protection Agency (EPA) in 1986 and confirmed in 1993, the majority of the committee of the National Research Council concluded that lifetime exposure to fluoride at drinking-water concentrations of 4 mg/L or higher is likely to increase fracture rates in the population, compared with exposure to 1 mg/L (NRC, 2006). Skeletal fluorosis occurs after many years of excessive fluoride intake (10-20 mg/day). If it is due to dietary intake, it is mostly the consequence of living in regions with high fluoride concentrations in drinking water. It is practically unknown in Europe.

Based on data from observational and intervention studies with regard to fractures, the UL for older children and adults was set at 0.12 mg/kg body weight per day or 5 and 7 mg/day for children and adolescents aged 9-14 and 15 years and older, respectively (EFSA, 2005).

2.3. **Physiology and metabolism of fluoride**

Gastric absorption, distribution in the body and renal excretion are pH-dependent. When the pH falls below the pK\textsubscript{a} of 3.4, more than 50% of fluoride occurs as undissociated HF and less as ionic F\textsuperscript{-} (Whitford, 1996). Because lipid bilayer membranes are much more permeable to HF than to F\textsuperscript{-}, fluoride crosses cell membranes as HF following a pH gradient from the more acidic to the more alkaline compartment (Buzalaf and Whitford, 2011). Fluoride is not metabolised and is not a substrate for any enzyme.

2.3.1. **Intestinal absorption**

Readily water-soluble fluorides (sodium fluoride, sodium silicofluoride, fluorosilicic acid, sodium monofluorophosphate) are rapidly and almost completely absorbed, in contrast to the low-soluble fluoride compounds calcium fluoride, magnesium fluoride and aluminium fluoride. Sodium monofluorophosphate needs dephosphorylation before absorption in the lower intestine.
Fluoride absorption occurs by passive diffusion in both the stomach (20-25%) and the small intestine. Higher acidity of the stomach increases gastric absorption as undissociated HF. Fluoride not absorbed in the stomach will be absorbed in the proximal small intestine as ionic F (Buzalaf and Whitford, 2011). The bioavailability (absorption) of equal fluoride doses (~ 10 mg) of different fluoride salts can be decreased by 40% through differences in preparation, e.g. coating (van Asten et al., 1996).

The presence of magnesium, phosphorus and aluminium decreases the absorption of fluoride. An inhibitory effect of calcium on fluoride absorption was shown with calcium from food but not with calcium supplements (Trautner and Einwag, 1987; Setnikar and Maurer, 1990; Shulman and Vallejo, 1990; Cerklewski, 1997; Setnikar et al., 1998). Fluoride ingestion with rice, with or without calcium or together with (meat) meals, was associated with significantly delayed absorption and reduced peak plasma concentrations of fluoride whilst not affecting the total amount absorbed (Pak et al., 1990; Warneke and Setnikar, 1993; McIntyre et al., 2001). Fluoride absorption from milk, milk-based infant formula and other calcium-rich foods can be as low as 25% (Ekstrand and Ehrnebo, 1979).

Fluoride in water, either naturally present or added as sodium fluoride or fluoro-silicic acid, was absorbed proportionally to the concentration; the time to reach maximum plasma concentrations (0.7-0.9 hour) and the dose-related time-plasma concentration curves (area under the curve, AUC) were not significantly different, and were not dependent on water hardness and calcium content. There was, however, large within- and between-subject variation in plasma concentrations (Cmax and AUC) (Maguire et al., 2005; Villa et al., 2008; Whitford et al., 2008).

The Panel notes that fluoride absorption is influenced by many factors, and that there is variability in the absorption efficiency of fluoride from different foods, but that on average 80-90% of the ingested fluoride is absorbed.

2.3.2. Transport in blood

Peak plasma fluoride concentrations after ingestion of a single dose are reached within 20-60 minutes, independent of the dose and of the nature of the fluoride ingested (Whitford et al., 2008).

Decline of plasma concentrations thereafter is due to uptake into calcified tissues and excretion into the urine. Plasma fluoride concentrations return to baseline within 3-11 hours. Plasma fluoride occurs in both ionic and non-ionic forms. Ionic fluoride (inorganic or free fluoride) is ultrafiltrable, not bound to plasma proteins or other compounds, and reflects current fluoride intake. It is not homeostatically controlled. It is twice as high in plasma as in blood cells and can be measured by potentiometry with the fluoride-ion-specific electrode, or by ion chromatography. The non-ionic fluoride in plasma consisting mostly of fat-soluble fluorocompounds can be detected by the same methods only after ashing and does not significantly change with fluoride intake. Although usually higher than the ionic fluoride, its biological significance is unknown. Plasma fluoride is the compartment from which fluoride is distributed to hard and soft tissues and for elimination from the body (Buzalaf and Whitford, 2011).

2.3.3. Distribution to tissues

Absorbed fluoride is rapidly distributed by the circulation to the intracellular and extracellular fluid where a steady-state is established. Body fluid and soft tissue fluoride concentrations are not under homeostatic control (Ekstrand et al., 1977). Approximately 1% of the absorbed amount of fluoride is found in soft tissue. The ratio of fluoride in soft tissue to fluoride in plasma is between 0.4 and 0.9, as shown in rats (Whitford et al., 1979). 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), whilst the blood-brain barrier is virtually impermeable to fluoride (tissue/plasma ratio < 0.1). Altering the pH gradient by changes in the extracellular pH, for example by diet, drugs, level of physical activity, altitude of residence, or in the course of diseases can promote the net flux of fluoride into or out of cells. Acidotic states can lead to higher plasma fluoride concentrations by a reduction of the renal excretion of fluoride. About 40% of absorbed fluoride is retained in calcified tissues (bone and
teeth) of adults where it is tightly but not irreversibly bound (Buzalaf and Whitford, 2011). In children below the age of seven years, fluoride retention is higher, around 55% (Villa et al., 2010). Remobilisation from bone is by interstitial ion exchange or by remodeling and resorption of bone (Buzalaf and Whitford, 2011).

Circulating fluoride passes the placenta and reaches the fetus. The fluoride concentration in the placenta can be higher than in maternal blood, and was observed to vary widely between individuals, possibly due to methodological difficulties (Shen and Taves, 1974). The concentration of fluoride in cord blood is about 75% of the concentration in maternal blood. The use of fluoride supplements (1.5 mg/day) during pregnancy doubled fetal blood concentrations (Shen and Taves, 1974; Caldera et al., 1988).

2.3.4. Accumulation in the body

The total fluoride content of the human body amounts to 2-5 g and depends on age and exposure to fluoride. The skeleton of a newborn contains only about 5-50 mg of fluoride. Ninety-nine percent of the total fluoride content of the body is concentrated in calcified tissue, bone and teeth. Bone is 80% cortical (compact) and 20% trabecular (cancellous, spongy) bone. Fluoride uptake by bone is initially by ion exchange in the sheath of bone crystallites, followed by incorporation into the hydration shell and migration of fluoride into the crystalline structure during recrystallisation (WHO, 1994). Fluoride concentration in bone increases with age, with past chronic fluoride intake, with residence at high altitude and in acidotic states, more rapidly in women than in men, and it is higher in cancellous than in compact bone. Fluoride is only taken up in newly-formed bone and during remodelling of bone in growing children. In adults, fluoride incorporation follows bone resorption and remodeling.

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

A positive correlation between the fluoride content of drinking water and bone fluoride content was reported (Chachra et al., 2010).

2.3.5. Elimination

2.3.5.1. Kidney

Absorbed fluoride which is not deposited in calcified tissue is mainly excreted via the kidney (around 60% in adults, 45% in children) (Villa et al., 2010). The percentage of absorbed fluoride excreted via the kidney in infants and young children can be as low as 10-20% because of a higher capacity of bone to accumulate fluoride. 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). Ionic fluoride is filtered in the renal glomeruli and partially reabsorbed in the renal tubuli (10-90%), dependent on the pH of the tubular fluid. Dietary or other factors that change the acid-base balance of the body and decrease the pH value of the urine will reduce renal excretion of fluoride and lead to higher fluoride concentrations in the body. The renal clearance of fluoride is 30-50 mL/min in adults (Schiffl and Binswanger, 1982; van Asten et al., 1996).

Fluoride excretion decreases with impaired renal function (Schiffl and Binswanger, 1980; Spak et al., 1985; Torra et al., 1998) and with an age-related decrease of glomerular filtration (Jeandel et al., 1992).

2.3.5.2. Faeces

About 10-20% of the daily total fluoride intake is excreted via the faeces (see Section 2.3.1.).
2.3.5.3. Breast milk

In Appendix A, fluoride concentrations in breast milk from different countries are compiled. Fluoride concentrations vary from non-detectable to 100 µg/L with a trend for lower concentrations in regions with low fluoride concentrations in drinking water (≤ 0.3 mg/L), with the exception of a study reporting values of around 500 µg/L for both ionic and total fluoride (Pasternak et al., 1998). Fluoride concentrations in human milk are significantly lower than in plasma, but are correlated (Sener et al., 2007).

Ekstrand et al. (1981) showed that a single fluoride dose of 1.5 mg given to mothers did not increase the fluoride concentration of their milk, whilst a supplement of 11.3 mg fluoride as sodium fluoride resulted in a peak fluoride concentration in milk of 60 µg/L after two hours which returned to baseline within eight hours (Ekstrand et al., 1984). This rapid change in concentration after ingestion of high boluses of fluoride may - besides differences in methodology - be partly responsible for the observed variance of values when sampling and diet are not standardised.

From the available information, the Panel considers that breast milk is a minor route of fluoride loss (less than 1 % of fluoride intake).

2.4. Biomarkers of fluoride intake

Fluoride concentrations in plasma, bone (surface), dentin, nails, hair, saliva, milk, sweat, enamel and urine have been assessed for a relationship to fluoride intake. Total fluoride intake estimates include both dietary and non-dietary sources. Markers of contemporary intake are fluoride concentrations in blood, bone surface, saliva, milk, sweat and urine whilst fluoride concentrations in bone, teeth, nails and hair are markers of historic fluoride intake (Rugg-Gunn et al., 2011) (see also Appendix B).

2.4.1. Plasma

Plasma fluoride concentrations are dependent on the total fluoride dose ingested, dose frequency and the plasma half-life. When water was the predominant fluoride source, the plasma concentration reflected the fluoride content of drinking water (WHO, 1994). More recently, the plasma concentration has been shown to be associated with total fluoride intake, and with fluoride dentifrice use, but not with dietary fluoride intake, including fluoride from water (Cardoso et al., 2006). Because of the rapid absorption of fluoride with peak plasma concentrations reached after about 20-60 minutes and return to baseline within 3-6 hours, it is advisable to measure fasting values, but as yet there are insufficient data across age groups to define normal plasma concentrations and conclude from plasma concentrations on individual fluoride intake (Rugg-Gunn et al., 2011).

2.4.2. Urine

Fluoride excretion in the urine is a biomarker of contemporary fluoride intake provided assumptions about percentage gastrointestinal absorption, faecal excretion, retention in calcified tissue and renal excretion are correct, which may be the case on a population basis but not for individuals. Data of simultaneous measurements of 24-hour total fluoride intake and urinary excretion from studies in young children (n = 212, 0.15-7 years of age) and in adults (n = 269, 18-75 years of age) were recently analysed (Villa et al., 2010). Linear relationships were found for both children and adults between daily fluoride intake and daily fluoride excretion in urine, but the intercepts and the slopes for both age groups were significantly different and reflected the greater percentage retention of total fluoride intake in children compared to adults, without any influence of sex. Ranges of fluoride excretion associated with ranges of total daily fluoride intake have been defined for specific age groups in specific conditions, for example different fluoride concentrations in drinking water (Villa et al., 2010). However, the width of the 95 % confidence interval (10-15 %) of the linear relationship indicates that fluoride excretion in urine is suitable to predict fluoride intake for groups only, but not for individuals (Rugg-Gunn et al., 2011).
2.4.3. Saliva

Fluoride concentrations in ductal and glandular saliva closely follow the plasma concentration, but at a lower level (about two-thirds of the plasma concentration (Ekstrand et al., 1977; Whitford et al., 1999a). In 20 healthy adults ingesting no fluoride or 1 mg fluoride/day via milk, salt or tablets for 30 days, fluoride concentration in saliva increased about ten-fold with fluoridated milk and fluoride tablets and about six- to seven-fold with fluoridated salt. Saliva flow and pH did not change (Toth et al., 2005).

Kaiser et al. (2006) investigated the changes in salivary fluoride content in 15 healthy volunteers following the consumption of different meals prepared with 5 g of fluoridated salt and following rinsing with water (1 mg fluoride/L). Fluoride content rose significantly within five minutes from baseline (32-34 μg/L to 111-150 μg/L) and had almost returned to baseline at 60 minutes with all tested meals.

Because of the rapid changes in the fluoride concentration of saliva following fluoride intake (or use of dentifrice) only ductal saliva is a reliable marker of plasma fluoride concentration as an indirect indicator of fluoride intake; however, it is not easily obtained.

2.4.4. Sweat and milk

Fluoride concentrations in sweat are similar to those in plasma (1-3 μmol/L; 19-57 μg/L), but difficulties in standardised sample collection and lack of available data do not allow a conclusion to be drawn from fluoride concentrations in sweat regarding fluoride intake (Rugg-Gunn et al., 2011).

The available data on fluoride concentration in human milk (see Section 2.3.5.3.) do not permit a conclusion to be drawn on the dietary fluoride intake of lactating women (Rugg-Gunn et al., 2011).

2.4.5. Bone and dentin

Fluoride retention in bone (and dentin) is proportional to long-term fluoride intake and, moreover, dependent on the turnover rate of bone, on age, sex and the type of bone (Caraccio et al., 1983). Infants and young children will retain up to 75% of the absorbed fluoride dose in skeletal tissue. There is a steady-state relationship between fluoride in plasma and fluoride in the hydration shell of bone crystallites with a net transfer of fluoride to the bone surface with rising plasma fluoride concentrations. The fluoride content of surface bone, therefore, may reflect contemporary fluoride intake whilst fluoride in mature bone reflects chronic or historical fluoride intake (Pessan and Buzalaf, 2011; Rugg-Gunn et al., 2011).

2.4.6. Hair

The fluoride content in hair was found to reflect the fluoride content of the metabolic environment during formation of the hair, and to be highly correlated with fluoride content in drinking water (Schamschula et al., 1985) and also with dental fluorosis incidence in a study in 12 year-old children in communities with widely different water fluoride concentration (Mandinic et al., 2010). In this study, fluoride in hair was significantly correlated to dental fluorosis incidence (r = 0.62; p < 0.01) which occurred only in the region with the high-fluoride well water (11 mg/L) (r = 0.61; p < 0.01) (Mandinic et al., 2010). Practical and methodological problems detract from the usefulness of hair fluoride content for the estimation of fluoride intake of different populations (and the prediction of risk for fluorosis).

2.4.7. Nails

Like for hair, the concentration of fluoride in nails (50% higher in fingernails than in toenails) is proportional to the intake over longer periods of time, taking into account the nail growth rate (Schamschula et al., 1985; Czarnowski and Krechniak, 1990; Whitford et al., 1999b). An additional daily intake of 3.0 or 1.8 mg fluoride over 30 days in both men and women resulted three months later in an increase of the fluoride content of fingernails, and with some further delay also of toenails.
Dietary Reference Values for fluoride

Whitford et al., 1999b). Subjects living in areas with a high fluoride concentration in water (1.6-3.1 mg/L) had 1.8 and 2.9 times higher fluoride concentrations in fingernails than subjects from areas with intermediate (0.5-1.1 mg/L) or low (< 0.11 mg/L) fluoride concentration in water, respectively (Schamschula et al., 1985).

The Panel notes that higher fluoride intakes are reflected in the fluoride contents of nails, but that there are insufficient data for defining a dose-response relationship.

2.4.8. Enamel

In contrast to skeletal bone and dentin which accumulate fluoride throughout life and in proportion to the absorbed dose of fluoride, the fluoride concentration in enamel is indicative of the amount taken up during tooth formation, and only the surface layers of enamel of erupted teeth are affected by the fluoride concentrations in the mouth. Enamel maturation of deciduous teeth is completed between the age of 2-12 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 (EFSA, 2005). In areas with low fluoride concentrations in drinking water (≤ 0.1 mg/L) the fluoride concentration at an enamel depth of 2 μm averaged 1 700 mg/kg, and with fluoride concentrations in water of 1 mg/L it was 2 200-3 200 mg/kg. When water contained 5-7 mg/L of fluoride the concentration in enamel was 4 800 mg/kg. Such concentrations are usually accompanied by dental fluorosis (NRC, 1993).

Post-eruptive fluoride uptake of enamel depends on the fluoride concentration in saliva, food, dental plaque and dental products (WHO, 1994). The fluoride content in enamel biopsies from 137 children aged 14 years was higher with higher fluoride concentration in drinking water (0.09 versus 1.9 mg/L) and higher in superficial (0.44-0.48 μm) than in deeper (2.4-2.6 μm depth) enamel biopsies: 1 549 and 641 versus 3 790 and 2 110 mg/kg, respectively (Schamschula et al., 1985).

2.5. Biomarkers of fluoride body burden

The body burden of fluoride is reflected in blood, bone, teeth and urine concentrations of fluoride, whilst fluoride concentrations in saliva and sweat may be related to concentrations in blood (see also Appendix B).

2.5.1. Plasma

Plasma fluoride concentrations increase with age and with increasing fluoride content of bone, and as a consequence of renal insufficiency (Ekstrand and Whitford, 1988). Compared to normal subjects, serum fluoride concentrations were ten-fold higher in patients with both skeletal and dental fluorosis due to high fluoride concentrations in drinking water (> 8 mg/L) (Jha et al., 1982). There are insufficient data across age groups to define normal plasma concentrations and to conclude from plasma concentrations on individual fluoride body burden (Rugg-Gunn et al., 2011).

2.5.2. Bone and dentin

The non-exchangeable inner compartment of bone may be a suitable indicator of the total life-long body burden of fluoride (Pessan and Buzalaf, 2011; Rugg-Gunn et al., 2011).

Dentin, which like bone slowly increases in fluoride content throughout life and, unlike bone, does not undergo resorption, would be the most suitable indicator of the total fluoride body burden, and is easier to obtain than bone biopsies, for example by analysis of extracted teeth (Pessan and Buzalaf, 2011).

Both bone and dentin fluoride concentrations cannot be used to predict the total fluoride body burden of an individual but are suitable for comparisons of groups with different habitual intakes.
2.6. Conclusion on biomarkers of fluoride intake and body burden

The Panel considers that 24-hour urinary fluoride excretion can be used as a biomarker of contemporary fluoride intake for population groups. However, for different age groups, the relationship between intake and excretion varies with renal function and acid-base balance.

The Panel considers that various biomarkers may be suitable biomarkers of contemporary fluoride intake (enamel surface, bone surface) or the body burden of fluoride (dentin, bone), but that it is impractical to obtain samples for measurement. The Panel also considers that there are insufficient data for fluoride concentrations in plasma, (ductal) saliva, (toe)nail, hair and enamel surface to define a dose-response relationship and values associated with caries prevention.

The Panel considers that sweat and human milk are not suitable as markers of contemporary fluoride intake.

The Panel concludes that none of the listed biomarkers permit an estimation of the fluoride intake of individuals, and that none of them can be used for defining DRVs.

2.7. Effects of genotypes

From numerous animal studies, particularly in mice, it appears that the response to environmental fluoride of processes involved in tooth and bone formation and architecture is determined by the genetic background. The identification and characterisation of fluoride-responsive genetic variations (e.g. polymorphisms) may lead to a better understanding of the mechanisms by which fluoride affects mineralisation, and to the identification of human population groups at risk for either the beneficial or the adverse effects of fluoride (Everett, 2011).

Twin studies investigating the proportion of variation in susceptibility to caries due to genes support a role of genetics in tooth decay (Liu et al., 1998). There is evidence for a stronger genetic influence on primary teeth than on permanent teeth. Genes involved in saliva flow and composition, tooth morphology, taste preferences and enamel and dentin formation might determine the risk of contracting caries besides environmental parameters like age, oral hygiene, dietary fluoride levels, and ethnicity (Wang et al., 2012). The Iowa Fluoride Study showed an association of caries scores at the age of about five years and single-nucleotide polymorphisms (SNPs) in three genes (DSPP, coding for dentin sialophosphoprotein, AQP5, coding for aquaporin-5, and KLK4, coding for kallikrein 4). However, the observed associations were not related to fluoride exposure (Wang et al., 2012).

Huang et al. (2008) found that homozygosity for the P allele of the COL1A2 PvuII (coding for the pro-alpha2 (I) chain of collagen) was associated with an increased risk for dental fluorosis compared to children carrying the homozygous genotype pp from the same fluoride-rich area (OR 4.85, 95 % CI 1.22-19.32), but the risk was not elevated when the control population was recruited from low-fluoride areas.

Individuals with the homozygous P genotype of COL1A2 PvuII have been found to have a higher risk of fracture (Suuriniemi et al., 2003) and a lower BMD/bone mineral content (Lau et al., 2004) than those with the homozygous p genotype. However, no association of polymorphisms of genes involved in bone health and sensitivity to fluoride was found (Huang et al., 2008; Ba et al., 2009).

The Panel considers that the currently available data on genes related to saliva flow and composition, to enamel and dentin formation, and to collagen and bone formation are suggestive for genetically determined differences in susceptibility to both the beneficial and the adverse effects of fluoride on dental and bone health, but do not provide evidence for defining fluoride intakes for the prevention of caries or for maintaining bone health.
3. Dietary sources and intake data

3.1. Sources

Major fluoride food sources are water and water-based beverages or foods reconstituted with fluoridated water, for example soup or infant formulae, marine fish, fluoridated salt, and tea. Oral exposure to fluoride occurs through water, food (including fluoridated table salt available, for example, in Austria, Czech Republic, France, Germany, Greece, the Netherlands, Spain and Switzerland), fluoride supplements and cosmetic dental products.

Water fluoridation in Europe is done in Ireland (population coverage 74%) and selected regions of the UK (population coverage 9%), Spain (population coverage 3%) and Portugal (population coverage 1%) (Cheng et al., 2007; SCHER, 2010).

The most important fluoride salts for human use are sodium and potassium fluoride, which are easily soluble in water. They are permitted for addition to foods (e.g. salt)\(^5\) and for fluoridation of water. For use in food supplements, also calcium fluoride and sodium monofluorophosphate are permitted\(^6\).

For fluoridation of drinking water, silicofluorides (e.g. (hydro)fluorosilicic acid (H\(_2\)SiF\(_6\)), sodium silicofluoride, disodium hexafluorosilicate (Na\(_2\)SiF\(_6\)), hexafluorosilicate or hexafluorosilicic acid) are the most commonly used fluoridating agents.

3.1.1. Water

All waters contain fluorides. The concentration of fluoride in ground water in the EU is generally low, but there are large regional differences due to different geological conditions. Surface water usually has a lower fluoride concentration than ground water, most often below 0.5 mg/L, and sea water has a concentration between 1.2 and 1.5 mg/L. The concentration of fluoride naturally occurring in drinking water in EU Member States ranges from 0.1 to ca. 6.0 mg/L, and shows large variation between and within countries, e.g. Ireland < 0.01-5.8 mg/L, Finland 0.1-3.0 mg/L, and Germany 0.1-1.1 mg/L (SCHER, 2010). Mean and maximum concentrations of fluoride in tap water in Belgium differ substantially and amount to 0.08 mg/L and 1.24 mg/L, respectively, for the Walloon region, 0.14 mg/L and 1.39 mg/L, respectively, for the Flemish region, and 0.07 and 0.08 mg/L, respectively, for Brussels (Vandevijvere et al., 2009). Council Directive 98/83/EC\(^7\) on the quality of water for human consumption permits a maximum fluoride concentration of drinking water of 1.5 mg/L.

Bottled water is increasingly substituting tap drinking water. A large variation in the concentration of fluoride has been observed, reaching up to 8 mg/L (EFSA, 2005; SCHER, 2010). Natural mineral waters which contain more than 1 mg fluoride/L can be labelled as “contains fluoride”. According to Directive 2003/40/EC\(^8\), the fluoride content of natural mineral waters must not exceed 5 mg/L, and 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 seven years of age”, and shall indicate the actual fluoride content.

3.1.1.1. Fluoride intake from water

Conventional estimates are that about 75% of dietary fluoride comes from water and water-based beverages (USDA, online) that contain more than 0.3 mg/L of fluoride. About 63% of the population

---


on US public water systems are receiving water that is naturally high in fluoride or fluoridated. From US and Canadian studies, the total fluoride intake of adults in areas with different fluoride concentrations of drinking water was estimated to be 0.3-1 mg/day and 1.4-3.4 mg/day with water fluoride concentrations < 0.3 mg/L and 1.0 mg/L, respectively (IOM, 1997).

Vandevijvere et al. (2009) assessed fluoride intake through bottled and tap water consumption in the Belgian adult population, taking into account regional differences. Mean intake of fluoride through water consumption in Flanders was 1.4 ± 0.7 mg/day (97.5th percentile: 3.1 mg/day), while in the Walloon region it was on average 0.9 ± 0.6 mg/day (97.5th percentile: 2.4 mg/day).

Data on measured fluoride intake via water (both tap water and beverages) in Europe are not available but estimates have been made assuming different scenarios of water consumption based on the EFSA concise database, on the results of consumption surveys across Europe, and assuming different fluoride concentrations to illustrate the magnitude of the impact of the fluoride concentration in water on fluoride ingestion (SCHER, 2010) (see Table 1).

Table 1: Fluoride intakes of adolescents (> 15 years) and adults via water and water-based beverages in the EU (SCHER, 2010)

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>Water consumption (mL/day)</th>
<th>Fluoride concentration (mg/L)</th>
<th>Fluoride intake from water (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median water consumption in EU countries and mean fluoride concentration in water</td>
<td>1 321</td>
<td>0.1</td>
<td>0.13</td>
</tr>
<tr>
<td>Highest water consumption (97.5th percentile) and mandatory fluoride concentration of 0.8 mg/L in Ireland</td>
<td>3 773</td>
<td>0.8</td>
<td>3.02</td>
</tr>
<tr>
<td>Highest water consumption (97.5th percentile) and highest permitted fluoride concentration of 1.5 mg/L</td>
<td>3 773</td>
<td>1.5</td>
<td>5.66</td>
</tr>
<tr>
<td>Highest observed consumption (97.5th percentile) of tap water and fluoride concentration of 3 mg/L; worst case scenario</td>
<td>2 800</td>
<td>3.0</td>
<td>8.40</td>
</tr>
</tbody>
</table>

3.1.2. Food

Fluoride content in food is generally low (0.1-0.5 mg/kg) except when food is prepared with fluoridated water. An exception is tea which can contain considerable amounts of fluoride (170-400 mg/kg dry weight in black and green teas made from young leaves and two to four times as much in brick tea made from mature leaves; tea infusions contain 0.34-5.2 mg/L) (Schmidt and Funke, 1984; Wei et al., 1989; Chan and Koh, 1996), dependent on type of tea, brewing procedure and fluoride concentration 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 dairy products 0.05-0.15 mg/kg, bread, cereals and cereal meals 0.1-0.29 mg/kg, meat and meat products 0.15-0.29 mg/kg, eggs 0.18 mg/kg, and fish and fish sticks 0.48-1.91 mg/kg (Bergmann, 1994; EVM, 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. Dried herbs, which are eaten in small amounts only, contain up to 2.0 mg fluoride/kg.

The USDA National Fluoride Database of selected beverages and foods contains fluoride values for 400 foods across 23 food groups (mean ± SE, median, percentiles, ranges) (USDA, online). Except for foods processed with water, i.e. fluoridated water, these values can be expected to also apply to Europe, where most countries do not have a fluoridated water supply.
3.1.3. **Infant and follow-on formula**

Infant formula, with the exception of soy protein-based formula, has a low fluoride content when the powder is prepared with distilled water (0.01-0.05 mg/L). The use of naturally fluoride-containing or fluoridated drinking water will change the fluoride concentration of infant formula considerably as shown by model calculations (Buzalaf and Levy, 2011). Similar differences in the fluoride content of infant formulae prepared with low-fluoride (0.2 mg/L) and high-fluoride (1 mg/L) water, and in intakes from such formulae, were calculated by Fomon et al. (2000). In its report on the essential requirements of infant and follow-on formulae, the SCF recommended that the maximum fluoride content of infant and follow-on formulae should be 100 µg/100 kcal, whereas a minimum level was not defined (SCF, 2003)\(^9\).

3.1.4. **Fluoridated salt**

Another dietary source of fluoride is fluoridated salt, which contains 200-250 mg fluoride/kg of salt, depending on national regulations, mostly in the form of potassium fluoride. The use of fluoridated salt may be restricted to use at home, or it can be used in the preparation/production of meals and foods as well. The amount of fluoridated salt ingested per person per day is estimated to be 3 g in France, where 35 % of salt is fluoridated (Afssa, 2003), and 2 g in Germany, corresponding to an additional fluoride intake of 0.50-0.75 mg/day.

3.1.5. **Fluoride-containing dental products**

Dental products (toothpaste, rinses and gels) which contain fluoride but are not considered a dietary source can increase the total intake of fluoride considerably, especially when used inappropriately (Burt, 1992).

3.2. **Intake**

There is a lack of data on total fluoride intake from dietary and non-dietary sources based on analyses of individual actual diets. In most instances, food diaries or food frequency questionnaires are used to determine the habitual amounts of food or beverages consumed, and these amounts are combined with fluoride concentrations in food from food composition databases, from analysed fluoride concentrations in food items, or from duplicates of the food consumed. No such data are available from Europe.

3.2.1. **Infants**

Breast-fed infants have a low fluoride intake. An intake of 0.8 L of human milk by an infant weighing 5 kg corresponds to a fluoride intake of 1.6-8 µg/day or approximately 0.3-1.6 µg/kg body weight per day (Bergmann, 1994; Fomon et al., 2000). Ekstrand (1989) calculated the fluoride intake of young infants from human milk and from different formulae with measured fluoride concentrations, and also calculated how much the intake is influenced by the fluoride concentration of the water used for preparation or dilution: use of water with 1.0 mg fluoride/L compared to 0.15 mg/L increases the fluoride intake of the infant five-fold.

One non-European longitudinal observational cohort study, the Iowa Fluoride Study, initiated in 1991 to examine how fluoride exposures and ingestion beginning at birth relate to the occurrence of dental fluorosis and caries, provides fluoride intake data (total and from individual sources) from birth to 8.5 years of age. Recruitment was between 1992 and 1995 from eight different hospitals in Iowa. Parents of the 1,389 children participating in the study were asked to complete validated questionnaires at age 6 weeks and 3, 6, 9 and 12 months, every four months until three years, and every half year thereafter concerning the child’s ingestion of water, beverages and foods made with water, other foods and beverages, fluoride supplements and use of fluoride dentifrice during the preceding period, and other information like height and body weight. The reliability of the answers in

---

the questionnaires was assessed seven to ten days after they were returned, with percentage agreement for most questions > 90 %. Individual fluoride intake was calculated from the fluoride concentration in water used by each individual, while average product category fluoride concentrations were determined in the study or taken from the literature. The participants had dental examinations at 5, 9 and 13 years. At the age of 13-16 years 607 children remained in the study.

In the Iowa Fluoride Study, fluoride ingestion from water, dentifrice, supplements by infants and young children was assessed from zero to 36 months. Fluoride intake per day was highest from zero to three months: 0.075 mg/kg body weight. It was 0.06 mg/kg body weight at six and nine months, 0.035 mg/kg body weight at 12 and 16 months, and 0.043 mg/kg body weight from 20-36 months. For most children, water fluoride intake was the predominant source, especially up to age 12 months (Levy et al., 2001).

3.2.2. Children

In the Iowa Fluoride Study, the total fluoride intake of 785 children was assessed between 16 and 36 and 36 to 72 months of age. There was a steady decline of fluoride intake per kg body weight with age (Levy et al., 2003). These data and other data based predominantly on measured dietary fluoride intakes are given in Appendix C, including intake from fluoridated dentifrice use and its contribution to total daily fluoride intake. It appears that the main contribution to total daily fluoride intake comes from water and from the use of fluoridated toothpaste. In assessing the relationship between fluoride intake and tooth and bone health, this contribution, though not dietary, cannot be neglected.

3.2.3. Adults

The French Food Safety Agency estimated that the intake of fluoride through food (water, toothpaste and supplements excluded) is about 2 mg/day for adults (Afssa, 2003).

The average total dietary fluoride intake of the adult population in the UK, including tea but excluding drinking water, was estimated from the 1997 Total Diet Study to be 1.2 mg/day (EVM, 2001). Earlier, a fluoride intake of 1.78 mg/day (from both food and beverages) and of 0.4 mg/day from foods only for UK adults had been estimated from six-day dietary records and measured fluoride concentrations of 93 separate food items (Taves, 1983). In Sweden, the fluoride intake of adults from food and beverages in areas with low fluoride concentrations in drinking water (< 0.4 mg/L) was 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).

The dietary fluoride intake (solids and beverages) of German children and adults was estimated from measured fluoride concentrations in food and beverages, and from consumption data, to be 0.191 mg and 0.379 mg/day in adolescents aged 12-14.9 years and in adults, respectively. This intake was modified considerably by the fluoride concentration of drinking water (more than doubled with a fluoride content of 1 mg/L compared to 0.3 mg/L) and also by the use of fluoridated salt (0.25 mg fluoride per gram of salt consumed), whilst the contribution through fluoridated dental products was not taken into account (Bergmann, 1994).

The estimated fluoride intake via food, supplements and toothpaste of the US population is shown in Table 2.
The skeletal fluorosis and has not been shown to be associated with adverse effects including moderate dental fluorosis. Estimated intakes in children in areas with water fluoridation (0.7-1.1 mg/L) between 1943 and 1988 were close to 0.05 mg/kg body weight per day. Average dietary fluoride intakes of adults ranged from 0.02-0.05 mg/kg body weight per day, but 0.05 mg fluoride/kg body weight per day was chosen as the AI for all ages above six months. The reference weights for adults were calculated from the body mass index (BMI) and median heights of young adults (19-30 years) in NHANES III 1988-1994. Based on a reference weight of 76 kg, the AI for males was set at 3.8 mg/day and rounded to 4 mg/day. For females it was set at 3.1 mg/day and rounded to 3 mg/day based on a reference weight of 61 kg.

The German-speaking countries (D-A-CH, 2013) accepted the value of 0.05 mg/kg body weight per day as adequate total fluoride intake for caries protection. Reference weights were calculated for a BMI of 22 kg/m² (women) and 24 kg/m² (men) based on German average height values. Depending on the fluoride content of drinking water, the intake of fluoridated table salt and/or fluoride supplements was recommended.

The UK COMA (DH, 1991) concluded that no physiological requirement for fluoride was apparent and therefore no Recommended Nutrient Intake (RNI) for fluoride was set. A safe intake was set at 0.05 mg/kg body weight per day because this exposure was considered below the dose associated with skeletal fluorosis and has not been shown to be associated with adverse effects.

The World Health Organization (WHO/FAO, 2004), the Nordic countries (NNR, 2004), the Scientific Committee for Food (SCF, 1993) and the Netherlands Food and Nutrition Council (1992) did not derive DRVs for fluoride for adults.

### Table 2: Estimated average chronic inorganic fluoride intake from non-water sources of the US population (NRC, 2006)

<table>
<thead>
<tr>
<th>Age</th>
<th>Fluoride intake (µg/kg body weight per day) from</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Food (a)</td>
<td>Toothpaste (b)</td>
</tr>
<tr>
<td>All infants (&lt; 1 year)</td>
<td>9.6</td>
<td>0</td>
</tr>
<tr>
<td>Breast-fed</td>
<td>4.6</td>
<td>0</td>
</tr>
<tr>
<td>Non-breast-fed</td>
<td>11.4</td>
<td>0</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 years</td>
<td>21</td>
<td>11.5</td>
</tr>
<tr>
<td>3-5 years</td>
<td>18.1</td>
<td>11.4</td>
</tr>
<tr>
<td>6-12 years</td>
<td>12.3</td>
<td>7.5</td>
</tr>
<tr>
<td>Adolescents 13-19 years</td>
<td>9.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-49 years</td>
<td>11.4</td>
<td>1.4</td>
</tr>
<tr>
<td>≥ 50 years</td>
<td>10.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Females (d) 13-49 years</td>
<td>10.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>

(a): Corrected for the contribution from powdered or dried tea at 987.72 ppm instead of 5 ppm used in the analysis by EPA (2004).

(b): Based on Levy et al. (1995), assuming two brushings per day with fluoride toothpaste (1 000 ppm F) and moderate rinsing. The estimated exposures are: 0 mg/day for infants; 0.15 mg/day for children aged 1-2 years; 0.25 mg/day for children aged 3-5 years; 0.3 mg/day for children aged 6-12 years; 0.2 mg/day for adolescents aged 13-19 years; 0.1 mg/day for all adults and females aged 13-49 years. The calculated exposure in µg/kg body weight per day is based on the body weights from EPA (2004).

(c): Based on American Dental Association (ADA) (online) schedule. The estimated exposures are: 0.25 mg/day for infants and children aged 1-2 years; 0.5 mg/day for children aged 3-5 years, and 1 mg/day for children aged 6-12 years and adolescents aged 13-19 years.

(d): Women of childbearing age.

### 4. Overview of Dietary Reference Values and recommendations

#### 4.1. Adults

The US Institute of Medicine (1997) concluded that in the absence of data to determine an Estimated Average Requirement (EAR) for fluoride, an Adequate Intake (AI) could be derived based on estimated intakes that have been shown to maximally reduce the occurrence of caries in the population without causing adverse effects including moderate dental fluorosis. Estimated intakes in children in areas with water fluoridation (0.7-1.1 mg/L) between 1943 and 1988 were close to 0.05 mg/kg body weight per day. Average dietary fluoride intakes of adults ranged from 0.02-0.05 mg/kg body weight per day, but 0.05 mg fluoride/kg body weight per day was chosen as the AI for all ages above six months. The reference weights for adults were calculated from the body mass index (BMI) and median heights of young adults (19-30 years) in NHANES III 1988-1994. Based on a reference weight of 76 kg, the AI for males was set at 3.8 mg/day and rounded to 4 mg/day. For females it was set at 3.1 mg/day and rounded to 3 mg/day based on a reference weight of 61 kg.

The German-speaking countries (D-A-CH, 2013) accepted the value of 0.05 mg/kg body weight per day as adequate total fluoride intake for caries protection. Reference weights were calculated for a BMI of 22 kg/m² (women) and 24 kg/m² (men) based on German average height values. Depending on the fluoride content of drinking water, the intake of fluoridated table salt and/or fluoride supplements was recommended.

The UK COMA (DH, 1991) concluded that no physiological requirement for fluoride was apparent and therefore no Recommended Nutrient Intake (RNI) for fluoride was set. A safe intake was set at 0.05 mg/kg body weight per day because this exposure was considered below the dose associated with skeletal fluorosis and has not been shown to be associated with adverse effects.

The World Health Organization (WHO/FAO, 2004), the Nordic countries (NNR, 2004), the Scientific Committee for Food (SCF, 1993) and the Netherlands Food and Nutrition Council (1992) did not derive DRVs for fluoride for adults.
## Table 3: Overview of Dietary Reference Values (DRVs) for fluoride for adults

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 19</td>
<td>3.8 (d)</td>
<td>2.5</td>
<td>4</td>
<td>0.05</td>
</tr>
<tr>
<td>≥ 19</td>
<td>2</td>
<td>4</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

(a): Guiding values for total intake; in case of a fluoride content of drinking water ≤ 0.7 mg/L, various measures of additional fluoride intake are listed (fluoride supplements, fluoridated table salt). The recommended dose of fluoride supplements depends on the fluoride content of drinking water (< 0.3 mg/L vs. 0.3-0.7 mg/L).

(b): Adequate Intake.

(c): Safe intake (mg/kg body weight per day).

(d): Including pregnant and lactating women.

### 4.2. Infants and children

For infants and children from six months onwards, the IOM (1997) set an AI of 0.05 mg fluoride/kg body weight per day, considering fluoride intake from all sources. The reference weights considered were adapted from NHANES III 1988-1994 and, from age four years onwards, were calculated from BMI and median heights observed for children aged 4-8 and 9-13 years, and for adolescents aged 14-18 years.

For infants and children, the German-speaking countries (D-A-CH, 2013) chose the AI of 0.05 mg/kg body weight per day for caries protection, and combined it with reference body weights based on median values for US infants and children. The intake of fluoride supplements and fluoridated table salt was recommended depending on the fluoride content of drinking water, unless the fluoride content of drinking water is > 0.7 mg/L. It was noted, though, that the fluoride intake from table salt would be very low for infants and young children due to a low salt intake.

The UK COMA (DH, 1991) derived a safe fluoride intake for children up to six years of age of 0.12 mg/kg body weight per day, based on the observation that fluoride intakes up to this level are found in areas with fluoridated water and are not associated with cosmetically significant dental mottling. For children over six years, a safe intake was set at 0.05 mg/kg body weight per day because this exposure was considered below the dose associated with skeletal fluorosis.

The World Health Organization (WHO/FAO, 2004), the Nordic countries (NNR, 2004), the Scientific Committee for Food (SCF, 1993) and the Netherlands Food and Nutrition Council (1992) did not derive DRVs for fluoride for infants and children.
Table 4: Overview of Dietary Reference Values (DRVs) for fluoride for infants and children

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DRV (mg/day)</td>
<td>4-&lt;12</td>
<td>6-12</td>
<td>6-12</td>
<td>6-12</td>
</tr>
<tr>
<td>DRV (mg/day)</td>
<td>0.5</td>
<td>0.2 (c)</td>
<td>0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1-&lt;4</td>
<td>1-3</td>
<td>1-3</td>
<td>1-6</td>
</tr>
<tr>
<td>DRV (mg/day)</td>
<td>0.7</td>
<td>0.5</td>
<td>0.7</td>
<td>0.12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4-&lt;10</td>
<td>4-6</td>
<td>4-8</td>
<td>6-18</td>
</tr>
<tr>
<td>DRV (mg/day)</td>
<td>1.1</td>
<td>0.8</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>Age (years)</td>
<td>10-&lt;13</td>
<td>10-12</td>
<td>9-13</td>
<td>-</td>
</tr>
<tr>
<td>DRV (mg/day)</td>
<td>2.0</td>
<td>1.5</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>13-&lt;19</td>
<td>13-19</td>
<td>14-18</td>
<td>-</td>
</tr>
<tr>
<td>DRV (mg/day)</td>
<td>3.2 (males)</td>
<td>2.0</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.9 (females)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a): Guiding values for total intake; in case of a fluoride content of drinking water ≤ 0.7 mg/L, various measures of fluoride intake are listed (fluoride supplements, fluoridated table salt). The recommended dose of fluoride supplements depends on the fluoride content of drinking water (<0.3 mg/L vs. 0.3-0.7 mg/L) and age;
(b): Adequate Intake, as reported on page 507;
(c): Adequate Intake;
(d): Safe intake (mg/kg body weight per day);
(e): Adequate Intake, as reported on page 172.

5. Criteria (endpoints) on which to base Dietary Reference Values

5.1. Biomarkers as endpoints

The Panel considers that presently none of the available biomarkers are suitable for use in setting a DRV for fluoride. This is due to insufficient data to define a dose-response relationship and values associated with caries prevention, and due to the impracticality of obtaining samples for the measurement of potentially suitable biomarkers (see Section 2.6.).

5.2. Health consequences

5.2.1. Dental health/caries

Caries is a major oral health problem in most industrialised countries, affecting 60-90% of schoolchildren and the vast majority of adults (Petersen, 2003).

Caries or dental decay is a disease of the hard tissues of the teeth that is caused by the action of microorganisms in dental plaque on fermentable carbohydrates. Caries is the result of repeated cycles of de- and remineralisation of the tooth surface, when the balance is on the side of demineralisation. Fluoride can contribute to the prevention of caries (Buzalaf et al., 2011), but caries is not a fluoride deficiency disease. Caries development is multifactorial (dietary sugars, extent and frequency, microbial population and composition of plaque, genetics and the oral environment).

Caries can be arrested or reversed provided it has not yet resulted in cavitation, i.e. loss of enamel substance. The process leading to caries is the same in deciduous (primary) and permanent (secondary) teeth, but due to anatomical differences, different surfaces and different types of teeth are affected in the two dentitions. Because of thinner enamel and dentin layers in primary teeth, a higher rate of progression and earlier involvement of the dental pulpa in primary teeth may occur. Generally, approximal surfaces are affected more than occlusal surfaces in primary teeth. Predominant caries of the labial surfaces of the upper anterior teeth in young children is also termed “early childhood caries”. Appendix D explains how caries is documented with respect to extent and intensity in deciduous and permanent teeth.
5.2.1.1. Fluoride in drinking water and dental health/caries

In the 1930s it was noted that in communities with water fluoride concentrations of 0.7-1.2 mg/L the caries prevalence was 40-60 % lower than in communities with low water fluoride concentrations and it was concluded that fluoride has a beneficial effect in increasing the resistance to caries in children (Dean et al., 1942) and at all ages (Russell and Elvove, 1951).

Based on epidemiological studies it was shown that the prevalence of caries was negatively correlated with the fluoride concentration of water, whilst dental fluorosis was positively correlated with the fluoride concentration (Dean and Elvove, 1936). The water fluoride concentration at which the caries preventive effect approached its maximum was 1 mg/L, and at that level only 10 % of the population was affected by mild fluorosis (according to Dean’s fluorosis index, see EFSA (2005)). The water fluoride concentration at which fluorosis becomes apparent in the population (2 mg/L) corresponds to a daily intake of 0.1 mg fluoride/kg body weight per day up to the age of 12 years. McClure determined that the average daily fluoride intake of a child in a community with a drinking water fluoride concentration of 1 mg/L would be approximately 0.05 mg fluoride/kg body weight per day from both water and diet (McClure, 1943). Both the concentration of 1 mg fluoride/L in drinking water and the fluoride intake of 0.05 mg/kg body weight per day were termed “optimal” in reducing caries prevalence and keeping dental fluorosis prevalence and severity in the population low. The “optimal” water fluoride concentration to reduce caries incidence and estimated fluoride intakes in the US population in both areas with and without water fluoridation (McClure, 1943; Singer and Ophaug, 1979; Ophaug et al., 1980b, 1980a; Dabeka et al., 1982; Ophaug et al., 1985; Featherstone and Shields, 1988) were the basis for setting the adequate fluoride intake of infants and children at 0.05 mg/kg body weight per day (Burt, 1992).

The efficacy of water fluoridation in preventing caries has been confirmed in a number of predominantly observational studies, either cross-sectional or pro- and retrospective cohort studies. In a systematic review which included 26 studies (of moderate quality and moderate risk of bias) reported in 73 publications between 1951 and 2000 on the effects of water fluoridation versus no fluoridation, a mean difference in the percentage of caries-free children of 15.4 % (95 % CI 10.8, 20.1; p < 0.001) was calculated as well as a mean difference in change in dmft/DMFT score (see Appendix D) of 2.3 (95 % CI 1.8, 2.8; p < 0.001), which after adjustment for baseline dmft/DMFT, setting, validity score and age in a multivariate regression model was 2.61 (95 % CI 2.31, 2.91) (McDonagh et al., 2000). A preventive effect of water fluoridation on caries development in children (difference in percentage of caries-free subjects and of dmft or DMFT scores) was also shown in another extensive systematic review (NHMRC, 2007). In a meta-analysis based on studies in adults only, the prevented fraction of caries through water fluoridation was calculated to be 27 % (95 % CI 19 %, 34 %) (Griffin et al., 2007).

A pre-eruptive effect of fluoride through increasing fluoridation of the developing enamel is supported by some evidence (Groeneveld et al., 1990; Murray, 1993), but is difficult to differentiate from the more important cariostatic effect of fluoride on erupted teeth. The relative effects of pre- and post-eruptive exposure to fluoride from water on caries experience of first permanent molars was assessed in Australian children aged 6-15 years. Pre-eruption exposure reduced caries of different locations significantly, whilst post-eruptive exposure alone was not effective. The maximum caries-preventive effect was achieved by combined pre- and post-eruptive exposure to fluoridated water (Singh et al., 2003; Singh and Spencer, 2004; Singh et al., 2007).

5.2.1.2. Total fluoride intake and dental health/caries

A dose-response assessment has been attempted in 601 children from the Iowa Fluoride Study using total daily intake data (i.e. food, water, and dental hygiene products, see also Section 3.2.1.) from birth to nine years in combination with dental examination for caries at age five and nine years, and for dental fluorosis at age nine years. 153 children had neither fluorosis at age nine years nor caries at ages five and nine years; 202 children had no fluorosis at age nine years, but caries at either age five or nine years; 96 had fluorosis at age nine years but no caries at ages five and nine years; 150 had both...
fluorosis at age nine years and caries at one or both dental examinations. The estimated mean daily fluoride intake in children with neither fluorosis nor caries was at or below 0.05 mg/kg body weight per day at all time points until the age of four years, and declined thereafter. There was considerable individual variation of fluoride intake in this group of children with values as low as 0.01 and as high as 0.2 mg/kg body weight per day at single time points. Children with fluorosis alone had significantly higher mean fluoride intakes than those without fluorosis and caries, whilst the intake of children with caries only mirrored the intake of children without caries and fluorosis but was slightly lower (Warren et al., 2009).

The efficacy of fluoride in different forms (water, milk, salt, tablets/drops, chewing gum) has been assessed in systematic reviews (Yeung et al., 2005; Griffin et al., 2007; NHMRC, 2007; Ismail and Hasson, 2008; Espelid, 2009; Tubert-Jeannin et al., 2011). A controlled trial on the efficacy of fluoridated sugar (Mulyani and McIntyre, 2002) is also available.

The consumption of fluoride supplements (tablets, drops/lozenges, up to 2 mg fluoride/day) by children reduced in the majority of systematically reviewed studies the caries increment in permanent teeth (by about 25 %) (Espelid, 2009), and in one randomised controlled trial (RCT) on children with cleft lip and/or palate by 50-70 % (Lin and Tsai, 2000), whilst the effect on deciduous teeth was inconsistent or questionable. Systematic reviews of studies on the effect of fluoridated milk (Yeung et al., 2005; NHMRC, 2007; Espelid, 2009) and of fluoridated salt (NHMRC, 2007; Espelid, 2009) provided no evidence for a beneficial effect on caries in children. A single RCT assessed the effect of fluoridated sugar on the development of caries in children and found it to be positive compared to non-fluoridated sugar (Mulyani and McIntyre, 2002). In older adults (n = 160, 58-84 years of age), an RCT of 15 months’ duration assessed the effect of fluoride applied in milk on dental root caries. The numbers of root caries index reversals (i.e. higher numbers of inactive caries lesions and lower numbers of more severe active lesions than at baseline) were significantly (p < 0.05) higher in the fluoride intervention groups than in the placebo group. In the intervention groups, but not in the placebo group, electric resistance measurements at the carious lesions increased (p < 0.05), indicating that remineralisation had occurred (Petersson et al., 2011).

5.2.1.3. Prenatal fluoride supplements and dental health/caries

Leverett et al. (1997) investigated the effect of daily prenatal fluoride supplements (1 mg fluoride) compared to placebo in an RCT on 798 children from a community with a low fluoride content in drinking water (< 0.3 mg/L) on caries incidence up to five years of age, and found no positive effect on caries. In a follow-up study, the fluoride content of enamel and of dentin of shedded primary teeth of 185 subjects was measured. Fluoride concentrations were higher in surface enamel (average 3 400-3 800 µg/cm³) than in tooth body enamel (about 1 350 µg/cm³) and still lower in dentin (380 µg/cm³), but there was no difference between teeth from children whose mothers had received fluoride supplements during pregnancy and teeth from children whose mothers had received placebo (Sa Roriz Fonteles et al., 2005). Fluoride supplements of 0.5 mg/day were given to all children until the second birthday as drops, and thereafter for another year as tablets (Leverett et al., 1997).

5.2.1.4. Conclusions on fluoride intake and dental health/caries

The Panel notes that very few of the many reviewed studies provide information on the total dietary fluoride intake besides stating the fluoride content of water or the amount of the interventional fluoride doses, and notes that the outcome measure for caries may have been affected by additional uses of non-dietary fluoride. Whilst fluoride in drinking water was practically the only source of fluoride intake around 40 years ago and total dietary fluoride intake could be assumed to be reliably estimated from drinking water consumption and could be used to estimate a dose-response relationship, this is no longer the case. Therefore, all studies after the 1970s and reviews of the effect of fluoride intake via diets, supplements or water on caries are potentially confounded by the use of fluoride-containing dental hygiene products, and do not permit a conclusion to be drawn on a dose-response relationship between dietary fluoride intake and caries risk. The Panel also notes the methodological difficulties in the measurement of fluoride concentrations in food and beverages and the wide variation of such
concentrations, which enhance the difficulties in obtaining representative intake data to enable a dose-response assessment between total fluoride intake and caries. Moreover, the majority of studies have not systematically addressed other factors which influence caries development (e.g. diet, dental hygiene, environment, and genetic disposition), thereby making studies incomparable and not suitable for defining DRVs for fluoride.

5.2.2. Bone health

Fluoride accretion in bone increases bone density by stimulating the formation of new bone (Everett, 2011), but excessive long-term intake reduces bone strength and increases risk of fracture and skeletal fluorosis (stiffness of joints, skeletal deformities).

One systematic review evaluated six studies investigating the relationship between fluoride intake from water, milk and salt with added fluoride and bone health. There was no eligible study on fluoridated milk and salt. Three systematic reviews and three cross-sectional studies on fluoridated water were eligible, including the systematic review by McDonagh et al. (2000). Overall, there was little evidence for a beneficial relationship between fluoride intake and bone health (NHMRC, 2007).

In a nested case-control study involving 62,641 healthy nurses, fluoride concentrations 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 of chronic fluoride intake, and the association with fracture incidence was assessed (53 cases of hip fracture, 188 cases of forearm fracture, 241 matched controls in 1988). Comparing women in the three highest quartiles of toenail fluoride to those in the lowest quartile resulted in an adjusted odds ratio of 1.5 (95% CI 0.9, 2.7) for forearm fracture and of 0.5 (95% CI 0.2, 1.5) for hip fracture (Feskaniich et al., 1998). The results of this study do not permit a conclusion to be drawn on the effects of fluoride on bone health and fracture risk.

The Iowa Fluoride Study includes the Iowa Bone Development Study. This project involves the same children as the Iowa Fluoride Study and looks at dietary, genetic and physical activity factors, and how these affect bone growth. Parents were asked to complete questionnaires about the amount of physical activity their children had, and the children's diets were analysed for calcium, vitamin D, phosphorus and fluoride. The mean fluoride intake estimated by AUC was 0.68 mg (SD 0.27) per day from birth to 11 years when bone examinations (BMD, bone mineral content by whole body and lumbar spine DXA scans) were performed in 481 children. After adjustment for confounders, no girls’ or boys’ bone outcomes were statistically significantly related to any of the fluoride intake measures (Levy et al., 2009). The Panel concludes that this longitudinal prospective observational study does not provide evidence for a relationship between fluoride intake (total and from different sources) and bone mineral status at the age of 11 years, and that the duration of follow-up may have been too short for an assessment of other parameters of bone health.

From the available data, no beneficial effect of fluoride on bone health can be deduced.

6. Data on which to base Dietary Reference Values

The Panel concludes that fluoride is not an essential nutrient. Therefore, no Average Requirement (AR) for the performance of essential physiological functions can be defined. Because of the beneficial effect of dietary fluoride on prevention and severity of caries, the Panel considers that the setting of an AI is appropriate.

6.1. Infants and children

The Panel considers that data on the dose-response relationship between caries incidence and consumption of drinking water with different fluoride concentrations which were confirmed by more recent data on total fluoride intake from a study in the US are sufficient to set an AI of 0.05 mg/kg body weight per day. The AI covers fluoride intake from all sources, including non-dietary sources.
6.2. Adults

The Panel considers that no data are available to define a dose-response relationship between fluoride intake and caries for adults. Reliable and representative data on the total fluoride intake of the European population are not available. The available data on fluoride intake are variable and generally at or below 0.05 mg/kg body weight per day. The Panel considers that the AI for children of 0.05 mg/kg body weight per day can also be applied to adults, including pregnant and lactating women.

CONCLUSIONS

The Panel concludes that the AI of fluoride from all sources for both children and adults can be set at 0.05 mg/kg body weight per day. Table 5 lists the AI for age groups of children and adults calculated with the relevant reference body weights and rounded, where necessary. For pregnant and lactating women the AI is based on the body weight before pregnancy and lactation, because there is no evidence that a fluoride intake above the AI for non-pregnant women has a beneficial effect on the dental health of the child, and because the low fluoride content of breast milk does not increase significantly with higher fluoride intakes.

Table 5: Summary of Adequate Intake for fluoride for infants, children and adults

<table>
<thead>
<tr>
<th>Age</th>
<th>Reference weight (kg)</th>
<th>Adequate Intake from all sources (mg/day)</th>
<th>Reference weight (kg)</th>
<th>Adequate Intake from all sources (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-11 months</td>
<td>8.9 (a)</td>
<td>0.4</td>
<td>8.2 (a)</td>
<td>0.4</td>
</tr>
<tr>
<td>1-3 years</td>
<td>12.2 (b)</td>
<td>0.6</td>
<td>11.5 (b)</td>
<td>0.6</td>
</tr>
<tr>
<td>4-6 years</td>
<td>19.2 (c)</td>
<td>1.0</td>
<td>18.7 (c)</td>
<td>0.9</td>
</tr>
<tr>
<td>7-10 years</td>
<td>29.0 (d)</td>
<td>1.5</td>
<td>28.4 (d)</td>
<td>1.4</td>
</tr>
<tr>
<td>11-14 years</td>
<td>44.0 (e)</td>
<td>2.2</td>
<td>45.1 (e)</td>
<td>2.3</td>
</tr>
<tr>
<td>15-17 years</td>
<td>64.1 (f)</td>
<td>3.2</td>
<td>56.4 (f)</td>
<td>2.8</td>
</tr>
<tr>
<td>≥ 18 years</td>
<td>68.1 (g)</td>
<td>3.4</td>
<td>58.5 (g)</td>
<td>2.9</td>
</tr>
</tbody>
</table>

(a): Median body weight-for-age of male or female infants, respectively, aged 9 months (WHO Multicentre Growth Reference Study Group, 2006).
(b): Median body weight-for-age of boys and girls, respectively, aged 24 months (WHO Multicentre Growth Reference Study Group, 2006).
(c): Median body weight of boys and girls, respectively, aged 5 years (van Buuren et al., 2012).
(d): Median body weight of boys and girls, respectively, aged 5 years (van Buuren et al., 2012).
(e): Median body weight of boys and girls, respectively, aged 12.5 years (van Buuren et al., 2012).
(f): Median body weight of boys and girls, respectively, aged 16 years (van Buuren et al., 2012).
(g): Median body weight of 18 to 79-year-old men and women, respectively, based on measured body heights of 16 500 men and 19 969 women in 13 EU Member States and assuming a BMI of 22 kg/m² (see Appendix 11 in EFSA NDA Panel (2013)).

RECOMMENDATIONS FOR RESEARCH/NEED FOR DATA

The Panel recommends systematically producing and collecting analytical data on the fluoride content of foods, beverages and water for human consumption in EU Member States, and on their variability by standardised methodology, to enable better assessments of total fluoride intake and of fluoride intake from different sources, and to determine the major contributors to dietary fluoride intake.

The Panel recommends pursuing the validation of biomarkers of actual and chronic fluoride intake. 24-hour urinary fluoride excretion appears to be the most promising for contemporary intake, and the influence of different sources of fluoride on excretion should be measured.
REFERENCES


Dabeka RW, Karpinski KF, McKenzie AD and Bajdik CD, 1986. Survey of lead, cadmium and fluoride in human milk and correlation of levels with environmental and food factors. Food and Chemical Toxicology, 24, 913-921.


SCHER (Scientific Committee on Health and Environmental Risks of the European Commission), 2010. SCHER pre-consultation opinion on critical review of any new evidence on the hazard profile, health effects, and human exposure to fluoride and the fluoridating agents of drinking water. 18 May 2010. 55 pp.


van Buuren S, Schönbeck Y and van Dommelen P, 2012. Collection, collation and analysis of data in relation to reference heights and reference weights for female and male children and adolescents (0-18 years) in the EU, as well as in relation to the age of onset of puberty and the age at which different stages of puberty are reached in adolescents in the EU. Project developed on the procurement project CT/EFSA/NDA/2010/01. 59 pp.


Viswanathan G, Gopalakrishnan S and Siva Ilango S, 2010. Assessment of water contribution on total fluoride intake of various age groups of people in fluoride endemic and non-endemic areas of Dindigul District, Tamil Nadu, South India. Water research, 44, 6186-6200.


## APPENDIX A. FLUORIDE CONCENTRATION IN BREAST MILK FROM DIFFERENT REGIONS OF THE WORLD

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of samples</th>
<th>Maternal fluoride intake (mg/day)</th>
<th>Stage of lactation (months)</th>
<th>Fluoride concentration (µg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Several</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>2-95</td>
<td>IPCS (2002)</td>
</tr>
<tr>
<td>Finland</td>
<td>24</td>
<td>1.0 mg/L</td>
<td>1-3</td>
<td>Median 5, range 3-6 (ionic); median 7.0, range 4.3-14.0 (total)</td>
<td>Esala et al. (1982)</td>
</tr>
<tr>
<td>Canada</td>
<td>210 (total)</td>
<td>1.0 mg/L</td>
<td>Not reported</td>
<td>Mean 7.1, median &lt; 4 (range &lt; 2 - 97)</td>
<td>(Dabeka et al., 1986)</td>
</tr>
<tr>
<td>Sweden</td>
<td>143</td>
<td>1.0 mg/L</td>
<td>Colostrum</td>
<td>5.3 ± 0.4 (mean ± SEM)</td>
<td>Spak et al. (1983)</td>
</tr>
<tr>
<td>Kenya</td>
<td>27</td>
<td>Mean 22.1, range 9.5-37.2 (a), water fluoride 9 mg/L</td>
<td>Mean 10.2, range 0.5-44</td>
<td>Mean 33, range 11-73</td>
<td>Opinya et al. (1991)</td>
</tr>
<tr>
<td>Guatemala</td>
<td>29</td>
<td>Not reported</td>
<td>3</td>
<td>Mean &lt; 0.5 (all median ± SD)</td>
<td>Parr et al. (1991)</td>
</tr>
<tr>
<td>Hungary</td>
<td>63</td>
<td></td>
<td></td>
<td>13.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Nigeria</td>
<td>10</td>
<td></td>
<td></td>
<td>24.7 ± 9.7</td>
<td></td>
</tr>
<tr>
<td>Philippines</td>
<td>32</td>
<td></td>
<td></td>
<td>118 ± 13</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>10</td>
<td></td>
<td></td>
<td>17 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Zaire</td>
<td>61</td>
<td></td>
<td></td>
<td>6.8 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>444</td>
<td>Not reported</td>
<td>1-6</td>
<td>Mean 3-4 at every month, range below detection limit to 25</td>
<td>Bergmann (1994)</td>
</tr>
<tr>
<td>Poland</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>513 ± 55 total (mean ± SD)</td>
<td>Pasternak et al. (1998)</td>
</tr>
<tr>
<td>Turkey</td>
<td>57</td>
<td>Not reported</td>
<td>First week</td>
<td>Mean 19 ± 4, range 5-25</td>
<td>Koparal et al. (2000)</td>
</tr>
<tr>
<td>Thailand</td>
<td>65</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Mean ± SD: 17 ± 20</td>
<td>Chuchkaiwong et al. (2000)</td>
</tr>
<tr>
<td>India</td>
<td>20</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Mean 80 ± 132, range 50-100</td>
<td>Rahul et al. (2003)</td>
</tr>
<tr>
<td>Egypt</td>
<td>60</td>
<td>Not reported</td>
<td>Not reported</td>
<td>4.6 ± 2.5 (mean ± SD); median 3.2; range 1.9-11.4</td>
<td>Hosssny et al. (2003)</td>
</tr>
<tr>
<td>Turkey</td>
<td>125</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Mean ± SD: 40 ± 10</td>
<td>Sener et al. (2007)</td>
</tr>
<tr>
<td>India</td>
<td>15</td>
<td>Mean (range):-colostrum</td>
<td>&lt; 1 month</td>
<td>40 ± 10</td>
<td>Viswanathan et al. (2010)</td>
</tr>
</tbody>
</table>

(a): Measured in duplicate samples of food and beverages consumed in 24 hours.
(b): Bound fluoride was 4% of total fluoride.
(c): Average dietary intake estimated from household survey per age groups multiplied by measured fluoride concentrations in water; 1) area with about 1 mg/L; 2) area with 1-2 mg/L; 3) area with > 2 mg/L water.
## APPENDIX B. BIOMARKERS OF FLUORIDE INTAKE AND BODY BURDEN

<table>
<thead>
<tr>
<th>Concentration in</th>
<th>“Normal” range</th>
<th>Reflects</th>
<th>Influencing factors</th>
<th>References</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Baseline after overnight fasting: 9.3-24 µg/L; 0.5-1.3 μmol/L</td>
<td>Actual fluoride intake; interstitial and intracellular F⁻</td>
<td>Site of collection, age, acid-base balance, altitude, haematocrit, genetic background</td>
<td>Whitford (1996); Rugg-Gunn et al. (2011)</td>
<td>Suitable for prediction of fluoride intake of groups, not of individuals</td>
</tr>
<tr>
<td>Sweat</td>
<td>Baseline similar to plasma; 19-57 µg/L; 1-3 μmol/L</td>
<td>Plasma fluoride, actual fluoride intake</td>
<td></td>
<td>Whitford (1996)</td>
<td></td>
</tr>
<tr>
<td>Saliva, ductal</td>
<td>Not established; ratio ductal submandibular or parotid saliva to plasma 0.61-0.88 and 0.32-0.55, respectively</td>
<td>Plasma fluoride, actual fluoride intake</td>
<td>Not influenced by saliva flow stimulation</td>
<td>Ekstrand (1977); Oliveby et al. (1989a, 1989b, 1989c); Whitford (1996); Whitford et al. (1999a)</td>
<td>Whole saliva not suitable. Ductal saliva potentially suitable to predict fluoride intake of groups but difficult to obtain</td>
</tr>
<tr>
<td>Urine, 24-hour</td>
<td>Observed ranges of excretion per age groups and under defined conditions of intake</td>
<td>Actual fluoride intake</td>
<td>Acid-base balance, urinary pH, renal function, age</td>
<td>Villa et al. (2010); Rugg-Gunn et al. (2011)</td>
<td>Suitable for prediction of total daily fluoride intake of groups, not of individuals</td>
</tr>
<tr>
<td>Milk</td>
<td>Total fluoride in fluoridated areas 52 µg/L or 2.7 μmol/L; in non-fluoridated areas 46 µg/L or 2.4 μmol/L</td>
<td>Neither fluoride intake nor plasma fluoride</td>
<td></td>
<td>Dirks et al. (1974); Ekstrand et al. (1981); Koparal et al. (2000)</td>
<td>Not suitable as marker of fluoride intake</td>
</tr>
<tr>
<td>Nails</td>
<td>Not established</td>
<td>Recent; plasma fluoride concentration and average intake over protracted periods (≥ 3 months)</td>
<td>Water fluoride concentration, growth rate, age, sex, metabolic environment during formation; not influenced by renal function, urinary pH, urinary flow</td>
<td>Whitford et al. (1999b); Correa Rodrigues et al. (2004); Buzalaf et al. (2006)</td>
<td>Toenails more suitable than fingernails, concentration in fingernails &gt; toenails and rise earlier than in toenails (about 3.5 months following additional intake) Methodological problems and external contamination possible. Suitable for epidemiological subchronic exposure to fluoride; no predictor of dental fluorosis</td>
</tr>
<tr>
<td>Hair</td>
<td>Not established</td>
<td>Recent; plasma fluoride concentration and average intake over protracted periods</td>
<td>Metabolic environment during formation; water fluoride concentration</td>
<td>Schamschula et al. (1985)</td>
<td>Methodological problems and external contamination possible</td>
</tr>
<tr>
<td>Concentration</td>
<td>“Normal” range</td>
<td>Reflects</td>
<td>Influencing factors</td>
<td>References</td>
<td>Remarks</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------</td>
<td>----------</td>
<td>---------------------</td>
<td>------------</td>
<td>---------</td>
</tr>
<tr>
<td>Bone</td>
<td>Normal concentrations not established to indicate “desirable” levels of intake</td>
<td>Acute fluoride intake in exchangeable bone surface compartment; total life-long body burden of fluoride in non-exchangeable inner compartment</td>
<td>Age, sex, genetics, site (cancellous versus compact bone), historical fluoride intake, acid-base balance, altitude, bone remodeling rate, renal function</td>
<td>Chachra et al. (2010); Villa et al. (2010)</td>
<td>Not suitable because of invasive sample collection</td>
</tr>
<tr>
<td>Dentin</td>
<td>Normal values not established</td>
<td>Total body burden of fluoride</td>
<td>Age, historical fluoride intake, acid-base balance, altitude, renal function</td>
<td>Richards et al. (1992); Vieira et al. (2004)</td>
<td>Potentially suitable as indicator of total fluoride body burden in extracted teeth</td>
</tr>
<tr>
<td>Enamel</td>
<td>Not established</td>
<td>The biologically available fluoride at the time of tooth formation and post-eruptive fluoride uptake from saliva, food, dental plaque and dental products into the outer enamel layer after eruption</td>
<td>Habitual pre- and postnatal fluoride exposure</td>
<td>Schamschula et al. (1985); WHO (1994); Sa Roriz Fonteles et al. (2005)</td>
<td>Not suitable because of invasive sample collection and variations in sample preparation and analysis</td>
</tr>
</tbody>
</table>
### APPENDIX C. FLUORIDE INTAKE OF CHILDREN (1-6 YEARS) FROM FOOD, BEVERAGES AND DENTIFRICE

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of participants, fluoride content of drinking water</th>
<th>Total fluoride intake (μg/day)</th>
<th>Total fluoride intake (μg/kg body weight per day)</th>
<th>From food (μg/day)</th>
<th>From dentifrice (μg/day)</th>
<th>Method; remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-36 months</td>
<td>Iowa Fluoride Study, n = 630, water fluoridated; Fluorosis in permanent incisors at age nine years, n = 163; No fluorosis at age nine years, n = 367</td>
<td>705 (median)</td>
<td>196 (median)</td>
<td>600 (median); p &lt; 0.001</td>
<td>158 (median)</td>
<td>Diet diary at 1.5, 3, 6, 9, 12, 16, 20, 24, 28, 32, 36 months, then every six months. Analysis of drinking water and typical foods for fluoride; brand toothpaste, frequency of brushing. Fluoride intake during first 24 months via toothpaste and water was significantly related to fluorosis of maxillary incisors</td>
<td>Levy et al. (2006); Levy et al. (2010);</td>
</tr>
<tr>
<td>36-72 months</td>
<td>Iowa Fluoride Study, n = 785, water fluoridated</td>
<td>About 800</td>
<td>About 50</td>
<td>From food 10-15 %, from water 20-30 %, from other beverages 35 %</td>
<td>30 % of total</td>
<td>See above</td>
<td>Levy et al. (2003)</td>
</tr>
<tr>
<td>16-40 months</td>
<td>Fluoridated versus non-fluoridated area</td>
<td>Majority of fluoride intake from toothpaste</td>
<td>Duplicate diet and estimate of toothpaste left on brush after tooth brushing</td>
<td>Duplicate diet method and analysis of residual toothpaste</td>
<td>Guha-Chowdhury et al. (1996)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 years</td>
<td>n = 33, area with fluoridated water</td>
<td>Mean ± SD: 130 ± 87</td>
<td>Mean ± SD: 25 ± 13 μg/kg per day, 310 ± 160 μg/day</td>
<td>Mean ± SD: 106 ± 85 μg/kg per day, 1 340 ± 1 080 μg/day</td>
<td>Duplicate plate; dentifrice remaining on brush, fluoride electrode</td>
<td>de Almeida et al. (2007)</td>
<td></td>
</tr>
<tr>
<td>3-4 years</td>
<td>Fluoridated area, n = 32, compared to non-fluoridated area, n = 34</td>
<td>680 (mean) 490 (mean)</td>
<td>36 27</td>
<td>Mean ± SD: 360 ± 170 150 ± 60, from food and beverages</td>
<td>Duplicate diet method and analysis of residual toothpaste</td>
<td>Guha-Chowdhury et al. (1996)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Number of participants, fluoride content of drinking water</td>
<td>Total fluoride intake (μg/day)</td>
<td>Total fluoride intake (μg/kg body weight per day)</td>
<td>From food (μg/day)</td>
<td>From dentifrice (μg/day)</td>
<td>Method; remarks</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------------</td>
<td>------------------------------</td>
<td>-----------------------------------------------</td>
<td>-------------------</td>
<td>-------------------------</td>
<td>-----------------</td>
<td>-----------</td>
</tr>
<tr>
<td>3-6 years</td>
<td>Healthy, n = 11, fluoride in drinking water 0.25 mg/L</td>
<td>Mean ± SD: 931 ± 392</td>
<td>Mean ± SD: 53 ± 21</td>
<td>Mean ± SD: From food 203 ± 116, from dairy products 23 ± 22, from beverages 144 ± 126</td>
<td>Mean ± SD: 274 ± 176</td>
<td>Duplicate diet collection over two days, homogenised and analysed; analysis of toothpaste left on brush after brushing of teeth</td>
<td>Haftenberger et al. (2001)</td>
</tr>
</tbody>
</table>
APPENDIX D. ASSESSMENT OF CARIES PREVALENCE AND SEVERITY

For comparison of caries disease levels in populations, and for the control of the effectiveness of interventions, standardised methods of assessment are needed (WHO, 1997; Fisher et al., 2012). Caries status can be given as:

- **Dmft index**: the number of obviously decayed, missing or filled teeth in the deciduous dentition; if a missing tooth has been extracted, the “m” may be changed for an “e”; the maximum score is 20;

- **DMFT index**: the number of decayed, missing or filled teeth in the permanent dentition; the maximum score is 28, or 32 if the 3rd molars are included;

- **Dmfs index**: the number of decayed, missing or filled surfaces in the deciduous dentition; the maximum score is 88 for 20 teeth;

- **DMFS index**: the number of decayed, missing or filled surfaces in the permanent dentition; the maximum score for 28 teeth is 128.

The “d” and the “D” may, in addition, be graded into three steps: 1 signifying visible change without cavitation; 2 some cavitation; 3 cavitation reaching into the dentin.

In addition, for epidemiological research and the assessment of effects of interventions in longitudinal studies the following parameters are of interest:

- the percentage of caries-free subjects in a population;

- the prevented fraction (PF), e.g. as D(M)FS, which is the mean caries increment in the control group minus the increment in the intervention group divided by the increment in the control group;

- the absolute caries reductions (or increments) per year;

- the proportion of children developing new caries;

- the number of children needed to treat (NNT) to prevent one carious tooth/surface. These can be calculated by combining the overall prevented fraction with an estimate of the caries increment in the control groups of the individual studies.

Data from “clinical and radiological examinations combined” are preferable over data from “clinical” assessment only (Marinho et al., 2003).
<table>
<thead>
<tr>
<th>ABBREVIATIONS</th>
<th>MEANING</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA</td>
<td>American Dental Association</td>
</tr>
<tr>
<td>Afsssa</td>
<td>Agence française de sécurité sanitaire des aliments</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate Intake</td>
</tr>
<tr>
<td>AQP</td>
<td>Aquaporin</td>
</tr>
<tr>
<td>AR</td>
<td>Average Requirement</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>maximum concentration</td>
</tr>
<tr>
<td>COL1A2</td>
<td>collagen, type I, alpha 2</td>
</tr>
<tr>
<td>COMA</td>
<td>Committee on Medical Aspects of Food Policy</td>
</tr>
<tr>
<td>D-A-CH</td>
<td>Deutschland- Austria- Confderatio Helvetica</td>
</tr>
<tr>
<td>dmft/DMFT</td>
<td>decayed, missing or filled teeth, see Appendix D</td>
</tr>
<tr>
<td>DoH</td>
<td>Department of Health</td>
</tr>
<tr>
<td>DRV</td>
<td>Dietary Reference Value</td>
</tr>
<tr>
<td>DSPP</td>
<td>dentin sialophosphoprotein</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated average requirement</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>EPA</td>
<td>US Environmental Protection Agency</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>EVM</td>
<td>Expert Group on Vitamins and Minerals</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>HF</td>
<td>Hydrogen fluoride</td>
</tr>
</tbody>
</table>
IOM  US Institute of Medicine of the National Academy of Sciences
KLK  Kallikrein
NHANES  National Health and Nutrition Examination Survey
NHMRC  National Health and Medical Research Council
NNR  Nordic Nutrition Recommendations
NRC  National Research Council
OR  Odds ratio
RCT  Randomised controlled trial
RNI  Recommended Nutrient Intake
SCF  Scientific Committee on Food
SCHER  Scientific Committee on Health and Environmental Risks of the European Commission
SD  Standard deviation
SE  Standard error
SEM  Standard error of the mean
SNP  Single nucleotide polymorphism
UK  United Kingdom
UL  Tolerable Upper Intake Level
US  United States
USDA  United States Department of Agriculture
WHO  World Health Organization