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SCIENTIFIC OPINION

Updated consumer risk assessment of fluoride in food and drinking water including the contribution from other sources of oral exposure

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

This updated risk assessment evaluated evidence on potential adverse health effects of fluoride related to all sources of oral exposure as mandated by the European Commission. Fluoride benefit assessment was not included. Effects on the central nervous system, thyroid and bone were prioritised. Evidence from human studies indicates that total fluoride intake is associated with adverse effects on the developing brain at drinking water concentrations > 1.5 mg/L. The evidence of such associations below 1.5 mg/L was not sufficiently consistent to draw conclusions for risk assessment. Using drinking water concentration of 1.5 mg/L as a reference point, a safe level of intake including all sources of oral exposure of 3.3 mg/day was established for pregnant women to protect the fetus. This safe level of intake was extended to apply to other adults and children > 8 years. It is considered protective also against possible adverse effects on thyroid function and bone mineralisation, for which associations have been observed at water concentrations > 1.5 mg/L. Dental fluorosis was considered the most sensitive endpoint for children ≤ 8 years. Tolerable upper intake levels (UL) of 1.0, 1.6 and 2.0 mg/day were established for infants, toddlers and children 4–8 years, respectively. These ULs are considered protective against other possible adverse effects of fluoride, including neurodevelopmental outcomes. Aggregate exposure included intake of fluoride from food, drinking water, discretionary salt and (ingested) dental care products. Aggregate exposure based on the mean concentration of fluoride in EU drinking water (submitted data) was below the above health-based guidance values (HBGVs) for all age groups. Aggregate exposure exceeds the HBGVs at the 95th percentile of intake in the scenario of the P95 concentration of fluoride in EU drinking water, for all age groups except adolescents. The risk assessment suggests that the current legal limit for drinking water (1.5 mg/L) in the EU is not sufficiently protective.

KEYWORDS

bone, CNS, dental health, developmental neurotoxicity (DNT), exposure assessment, fluoridation, fluoride, fluorosis, neurodevelopment, neurotoxicity, thyroid

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SUMMARY

In the context of recent studies suggesting a relationship between intake of fluoride from drinking water with less than 1.5 mg fluoride/L and neurotoxicity in children, EFSA was mandated by the European Commission to carry out a consumer risk assessment for fluoride in food and drinking water, taking into account all relevant hazard information and all sources of oral exposure. The Scientific Committee considers that dental care products and fortified foods are major relevant sources and they were included in the assessment.

Chemistry

The scope of this update includes elemental or ionic fluoride as well as compounds that can release either of these fluoride forms. It does not cover fluorinated compounds where fluoride is covalently bound or those with a toxicological profile not attributed to fluoride. To ensure accurate fluoride quantitation, correct sample preparation and measurement interpretation are critical to avoid matrix effects or interferences and ensure correct recovery.

Previous assessments by EFSA and other agencies

Fluoride benefits and risks have been previously assessed by EFSA and other agencies. Although the beneficial properties of fluoride are out of scope of this mandate, the Scientific Committee noted that benefits for prevention of dental caries have previously been acknowledged by establishment of an adequate intake by the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA Panel) in 2013. In 2005, the NDA Panel also established tolerable upper intake levels (ULs)[1] of 1.5 and 2.5 mg/day for children of 1–3 and 4–8 years, respectively, based on less than 5% prevalence of moderate to severe dental fluorosis. For children and adults aged 9–14 and ≥ 15 years, ULs of 5 and 7 mg/day were established, respectively, based on increased risk for bone fractures observed in randomised controlled trials among postmenopausal women. Among more recent assessments, the U.S. National Toxicology Program concluded, in their assessment finalised in 2024, that fluoride exposure corresponding to water concentrations of ≥ 1.5 mg/L were associated with adverse neurodevelopmental outcomes in children. Health Canada concluded in their 2023 assessment that '*there is not a sufficient basis at this time to recommend a specific point of departure and health-based value for neurocognitive effects*'. In their assessment Health Canada identified water concentration of 1.56 mg F/L '*as the point of departure for deriving the health-based value*', based on dental fluorosis. However, Health Canada has not yet established a 'health-based value'.

Prioritisation of health effects for systematic literature review

Among all endpoints reported in the literature to be associated with fluoride exposure, those related to the central nervous system (CNS), thyroid and bone were prioritised by the Scientific Committee for a systematic literature review and comprehensive risk assessment. Studies in humans and experimental animals were assessed as separate lines of evidence through the weight of evidence approach. Other lines of evidence included information on fluoride kinetics and relevant in vitro evidence. New evidence on well-established adverse effects of fluoride, such as skeletal and dental fluorosis, was not subject to systematic literature review for several reasons. First, the direct link (or causality) between fluoride and these outcomes is well established. Second, in line with the EFSA NDA Panel assessment of 2005, it was concluded that skeletal fluorosis likely occurs at higher exposures compared to exposures resulting in bone fractures for which the UL for adults was established in 2005. Third, the UL for children was already based on dental fluorosis and screening of the new literature did not suggest that new evidence would challenge studies published prior to 2003.

Association of fluoride intake with central nervous system (CNS) development

The available prospective studies examining associations with CNS development were judged to be relevant and reliable. These studies primarily examined associations between exposure to fluoride during pregnancy in relation to later assessment of IQ in the offspring. Those studies were conducted in populations exposed to relatively low concentration of fluoride in drinking water (< 1.5 mg/L). Although associations with lower IQ scores were observed in some studies, the overall evidence from these studies was judged to be inconclusive. To provide more clarity around this conclusion, further studies addressing neurodevelopmental outcomes in children exposed pre- and/or postnatally to low levels of fluoride (< 1.5 mg/L in drinking water) are recommended.

The available cross-sectional studies were judged to be of moderate relevance and reliability, given that exposure to fluoride from drinking water in these studies is expected to have been ongoing, thereby preceding the outcome. The cross-sectional studies were most often conducted in populations exposed to relatively high fluoride concentrations in drinking water (> 1.5 mg/L). A large majority of those studies reported associations between fluoride concentrations in drinking water and lower IQ in children. The relative consistency of these findings observed across different populations increases the confidence in their findings. It was therefore concluded that exposure to fluoride in drinking water higher than 1.5 mg/L during

[1]The UL is the maximum level of total chronic daily intake of a nutrient (from all dietary sources) which is not expected to pose a risk of adverse health effects to humans (EFSA NDA Panel 2024). The UL is also defined as a level of intake above which the risk of adverse effects begins to increase.

fetal development and/or postnatally is associated with lower IQ scores in children. The cross-sectional studies did not provide further clarity on possible associations with lower IQ at concentrations of fluoride in drinking water < 1.5 mg/L.

In the human studies, the mean water fluoride concentrations correlated with mean urinary and/or serum fluoride concentrations across studies. Although concentrations of fluoride in urine and serum can be considered an indirect marker for total fluoride intake, the total intake of fluoride received by the study participants is uncertain. For the purpose of this assessment, total intake was estimated using default factors and (conservative) assumptions (see below and Section 3.8.2 in the opinion).

Studies in experimental animals provide evidence that adverse effects of fluoride on neurobehavioural indicators begin to occur at dose levels above 2.1 mg/kg bw per day, with adverse effects at organ level (brain) reported at doses of 3.5 mg/kg bw per day and higher. However, the mechanistic data and the non-specific effects reported at molecular or cellular level do not provide reliable additional support for effects of fluoride on the brain or a mode of action relevant to effects in the CNS. As concentration of fluoride in control feed was rarely reported, the total fluoride intake of the experimental animals in the in vivo studies is uncertain and may be overestimated with the use of default feed concentrations. Fluoride accesses the brain during development and possibly in adulthood, but the fraction of the dose reaching the brain could not be estimated. Therefore, it was not possible to estimate the target tissue concentrations that are associated with the effects reported in vivo.

Overall, for neurodevelopment and neurotoxicity, the Scientific Committee concluded that there is reasonable confidence in the evidence from both human and animal studies suggesting that an association with neurodevelopmental outcomes may occur at relatively high fluoride exposures, i.e. above 1.5 mg/L in drinking water. The conclusion is primarily based on the evidence from human studies with supporting evidence from animal studies. The evidence for associations at lower concentrations is inconclusive.

Association of fluoride intake with thyroid function

Human cross-sectional studies suggest that living in areas with relatively high fluoride concentrations in drinking water (> 1.5 mg/L) is associated with increased serum TSH. The biological relevance of the TSH increases reported in these studies is subject to some uncertainty due to the modest effect sizes and inconsistent associations with concentrations of thyroid hormones (T3 and T4). Results from few, mostly Tier 3 (high risk of bias), animal studies and the available in vitro studies provide insufficient evidence to support adverse effects of fluoride on thyroid function or a mode of action on the thyroid.

Association of fluoride intake with bone health

New evidence on the relationship between fluoride exposure and bone health from human studies suggests that changes in bone mineral density and increased risk of fractures may occur below the UL of 7 mg/day (at ~ 3 mg/day) established by the EFSA NDA Panel (2005). However, the new evidence was not sufficiently robust to identify a more precise estimate of oral intake at which changes in bone mineral density become adverse, or to provide a more precise estimate of intake at which fracture risk might start to increase. The new evidence does not suggest that adverse associations between fluoride and measures of bone health occur at fluoride concentrations < 1.5 mg/L in drinking water. Relevant and reliable evidence from animal studies consistently shows effects of fluoride on bone starting from about 6 mg/kg bw per day. Consistent with the previous EFSA conclusion, an association of fluoride exposure with bone cancer risk is not supported by new studies published since 2005.

Synthesis of the evidence and selection of critical endpoints

The weight of evidence in this Opinion indicates that adverse effects on neurodevelopment are observed at intakes below the UL of 7 mg/day established by the EFSA NDA Panel in 2005, and there are some indications that this also applies to thyroid and bone health. The fetus of exposed pregnant women is most sensitive to the potential adverse effects of fluoride on neurodevelopment due to the vulnerability of the developing human brain. Since development continues postnatally, fluoride effects on the CNS may also be relevant for the developing offspring from exposure occurring after birth. However, there was insufficient evidence to directly assess this relationship. Hence, effects on the developing CNS were selected as the critical endpoint applicable to pregnant women and the developing offspring. Based on the weight of evidence, adverse effects on thyroid function or bone health are unlikely to occur at fluoride intakes below those identified as critical for neurodevelopment.

For toddlers 1–3 years and children 4–8 years, the available evidence on dental fluorosis is still considered robust and relevant. This critical endpoint is also relevant for infants < 1 year, as mineralisation of permanent teeth begins after birth. Based on the weight of evidence, adverse effects on the CNS, thyroid function or bone health are unlikely to occur in children at exposures to fluoride below those associated with dental fluorosis. Therefore, the Scientific Committee considered dental fluorosis also to be a relevant and reliable critical endpoint infants, toddlers and children up to 8 years.

Derivation of reference point

The Scientific Committee concluded, based on a number of studies in children, that lower IQ scores in children are consistently reported at drinking water fluoride levels above 1.5 mg/L. There were not sufficient data for dose–response modelling of this endpoint. Hence, it is important to note that the concentration in drinking water where adverse effects begin to occur

is subject to uncertainty. Adverse associations have been reported at lower concentrations in some prospective studies, but the evidence is not conclusive. As a result, it was judged appropriate to use the value of 1.5 mg/L drinking water as a reference point (RP) for neurodevelopmental effects, supported by the overall evidence on the prioritised endpoints from human and animal studies.

Benchmark dose modelling was possible for drinking water concentration data (benchmark concentration, BMC) and data on dental fluorosis from the Dean, 1942 study and was based on two criteria: the severity grade (mild, moderate, severe) and the prevalence reflected in the BMR. Moderate to severe dental fluorosis representing onset of adversity and a benchmark response (BMR) of 1% resulted in a lower bound of the BMC (BMCL) of 1.7 mg/L in drinking water. This was not considered sufficiently protective since more recent evidence indicates that moderate fluorosis may occur at lower concentrations of fluoride in drinking water (considering total fluoride intake has changed since the 1942 study). The Scientific Committee selected a more conservative approach based on the combined prevalence from mild to severe dental fluorosis, with a BMR of 5%, that resulted in a BMCL of 1.4 mg/L in drinking water, where the probability for cases of moderate severity in the population is minimal. A conservative approach is justified due to the uncertainty about the total intake in the population of the pivotal study, as well as the cumulative and non-reversible nature of the adverse effect. The Scientific Committee considers that the BMCL of 1.4 mg/L in drinking water is an appropriate RP from which to establish ULs that are protective against dental fluorosis in infants (< 1 year), toddlers (1–3 years) and children (4–8 years). This reference point is slightly lower, and therefore more protective, than the value of 1.5 mg/L that is used as reference point for possible effects on neurodevelopment.

Establishing health-based guidance values[2]

The Scientific Committee notes that the effects reported in the literature are not the result only of fluoride present in drinking water, but of the total fluoride intake of the participants in the human studies and the experimental animals. Therefore, the health-based guidance value (HBGV) must represent the total intake to which drinking water is but one contributor. The estimated total intake of study participants included contributions from food and dental care products. The RPs for both dental fluorosis and for effects in the CNS are obtained from human studies. Hence, no interspecies uncertainty factor is needed. The Scientific Committee concluded that an uncertainty factor to account for interindividual variability is not needed because the evidence for both endpoints is obtained from diverse, relevant and sensitive populations.

Because the RP for effects on the CNS is derived based on evaluation of the overall evidence and is not derived numerically though dose–response characterisation, a UL could not be established. Instead, the Scientific Committee established a 'safe level of intake'[3] of 3.3 mg/day for pregnant women based on CNS effects in the offspring following exposure to fluoride from maternal intake. Considering that other evidence indicates that associations with changes in thyroid function and bone mineralisation in adults also occur at drinking water fluoride concentrations > 1.5 mg/L, the safe level of intake established for pregnant women is considered protective against other potential adverse effects and is applicable to other adults and children > 8 years.

The Scientific Committee established ULs of 1.0, 1.6 and 2.0 mg/day for infants < 1 year, toddlers 1–3 years and children 4–8 years, respectively, based on dental fluorosis. Although effects on the CNS are also in principle applicable to infants, toddlers and children, the Scientific Committee concluded that the reference point for dental fluorosis is more robust (obtained from dose–response data) and an UL based on dental fluorosis should also be protective against CNS and other potential adverse effects of fluoride.

An uncertainty analysis of the HBGVs was performed based on expert knowledge elicitation. Assessment of overall uncertainties was based on the contribution of all lines of evidence. The results of the uncertainty analysis are presented in detail in Section 5.1 of the opinion. The established HBGVs represent the best outcome of the assessment (protective values of highest confidence) based on the weight of the available evidence. The credible intervals of the HBGVs represent the uncertainty around the HBGV contributed by different lines of evidence of lower confidence and the information (or lack information) they provided.

Dietary and non-dietary exposure assessment

Dietary and non-dietary exposure assessment was conducted using EFSA databases and methods for age groups older than 12 weeks. Data relevant to exposure through dental care products and fluoridated salt were obtained from the literature and previous EFSA assessments. Aggregated exposure was assessed by age group based on fluoride from major sources, including food, drinking water, fluoridated discretionary salt (not accounted for in food) and dental care products (assuming 100% of product is ingested by children ≤ 8 years and 10% is ingested by adults and children > 8 years). Intake from drinking water was based on data submitted to EFSA of current drinking water fluoride concentrations in European countries representing naturally occurring fluoride in drinking water or water fluoridation. Based on drinking water concentration data submitted to EFSA, > 86% of samples contain < 0.3 mg/L fluoride and > 97% of samples contain

[2]The term HBGV is used in this opinion when referring to both safe level of intake and UL established for fluoride.

[3]A safe level of intake is the only type of HBGV proposed when the RP is not derived from dose–response data and, therefore, a UL cannot be established. It is generally defined as '*the maximum amount that can be confidently concluded poses no risk of adverse effects in the population*' (EFSA NDA Panel 2024). In the case of fluoride, the evidence below the safe level of intake does not allow conclusion on safety, but it is nonetheless considered inconsistent and insufficient to result in a lower HBGV.

< 0.7 mg/L fluoride. Intakes based on these data are presented as the 'basic scenario', when using the mean concentration of fluoride in water estimated from the submitted data and 'water P95 scenario', when using the P95 concentration of fluoride in water estimated from the submitted data. Intake from drinking water was also assessed according to scenarios assuming that all drinking water consumed contained fluoride up to the current legal limits of 1.5 mg/L (tap or bottled water, 'legal limit 1' scenario) or 5 mg/L (bottled water, 'legal limit 2' scenario) in the European Union. The drinking water scenarios referring to legal limits of water fluoridation indicate the highest potential intakes and overestimate actual intakes.

The contribution of the mean dietary exposure (food and drinking water) to the mean total aggregated oral exposure to fluoride ranged from 19% in children to 66% in adults. Main contributing food categories (> 10% to the total exposure in the basic scenario) included 'Grains and grain-based products', 'Milk and dairy products', 'Tea beverages' and 'Drinking water' for all age groups. 'Food products for young population' was also a main contributor for infants. Under the 'basic scenario' of water fluoride, contributions to the mean total aggregated oral exposure of fluoride from all sources ranged as follows: mean exposure from discretionary salt (not accounted for within the food categories) from 15% in 'other children' (4–8 years) to 33% in adults (this source is not applicable to infants), and mean exposure from ingested dental care products from 15% in adults to 75% in infants and toddlers. In the 'water P95 scenario' and two water fluoridation scenarios up to current legal limits ('legal limit 1' and 'legal limit 2' scenarios), the contribution of dietary sources (food and drinking water) increased, reaching up to 74%, 84% and 89%, respectively, and the contribution of the other sources decreased respectively.

An uncertainty analysis of exposure by age group was performed based on expert knowledge elicitation. Uncertainties in exposure assessment were related to analytical methods used for food composition data, amount of fluoridated salt consumed, amount of toothpaste used and fraction of ingested product. The impact of each source of uncertainty was estimated separately for each age group, resulting in 90% certainty ranges for the respective aggregated exposures and for each source of exposure (drinking water, food (excluding drinking water), fluoridated salt and dental care products). The results of the uncertainty analysis are presented in detail in Section 5.2 of the opinion. Considering that sources of fluoride exposure vary among EU Members States and additional sources of fluoride intake may be applicable in some Member States, the related sources of uncertainty and their respective ranges can be used to support risk management decisions under different exposure scenarios.

Risk characterisation

The estimated mean and P95 intakes based on the 'basic scenario' are below the HBGVs for all age groups, except for the top of the P95 intake exceeding the ULs of toddlers (slightly) and children 4–8 years. Exceedances of the HBGVs for infants (slightly), toddlers, children and adults are noted for aggregate oral exposures at the P95 intake level of the 'water P95 scenario'. Intakes at the mean and P95 ranges of 'legal limit 1' scenario exceed the HBGVs for all populations groups. Intakes at the mean and P95 of 'legal limit 2' scenario exceed the HBGV for adults and children > 8 years (legal limit 2 scenario is not applicable to younger age groups).

An uncertainty analysis of the risk characterisation was performed based on expert knowledge elicitation. The results of the uncertainty analysis are presented in detail in Section 5.3 of the opinion. The analysis provides the likelihood of no concern or of likely exceedance of the established HBGVs to support risk management decisions under different exposure scenarios and level of certainty.

Conclusions

The Scientific Committee concluded that the mean and P95 intakes of fluoride based on typical drinking water concentrations in Europe (basic water scenario) do not exceed the established HBGVs, except for the top of the P95 intake that exceeds the UL for toddlers (slightly) and children 4–8 years. The ULs are exceeded for infants (slightly), toddlers and children 4–8 years at the highest drinking water concentrations in Europe (P95 water scenario). The exceedance of UL in these age groups suggests that mild forms of dental fluorosis may occur. For children 4–8 years, dental fluorosis may occur in the molar teeth which develop during this period. A conservative assumption of 100% ingestion of fluoridated dental care products is included.

For adults the safe level of intake is exceeded at levels of exposure associated with high contributions from all the following sources combined in descending order: drinking water, food, fluoridated discretionary salt and dental care products. Such exceedances in the subgroup of pregnant women would indicate increased risk of adverse effects on fetal neurodevelopment.

Because the RP derived for CNS effects (1.5 mg fluoride/L) and the RP for dental fluorosis (1.4 mg/L) are respectively equal or nearly equal to fluoride concentration corresponding to the current legal limit for drinking water, aggregate intake under the 'legal limit 1' scenario will lead to exceedances of the HBGVs. As a result, the current legal limit for fluoride in drinking water is not considered sufficiently protective.

The Scientific Committee concluded that the risk for adverse effects in the CNS from fluoride exposure is related to ingested fluoride. It does not apply to exposure through topical applications of fluoridated dental care products (recommended use), as long as the products are not ingested.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background

In 2013, EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) adopted a Scientific Opinion on dietary reference values for fluoride (EFSA NDA Panel, 2013). It concluded that 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 Adequate Intake (AI) is appropriate because of the beneficial effects of dietary fluoride on prevention of dental caries. The AI is based on epidemiological studies showing an inverse relationship between the fluoride concentration of water and caries prevalence. 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.

In Directive (EU) 2020/2184[4] on the quality of water intended for human consumption a Limit Value of 1.5 mg/L was established for fluoride in drinking water. In Directive 2003/40/EC[5] on natural mineral waters a labelling requirement was established that natural mineral waters with a fluoride concentration exceeding 1.5 mg/L shall bear on the label the words 'contains more than 1.5 mg/L of fluoride: not suitable for regular consumption by infants and children under 7 years of age'. Both limits were based on the 1998 Opinion of the Scientific Committee for Food (SCF, 1998) that especially as concerns the occurrence of dental fluorosis, there is no reason to deviate from the level of 1.5 mg/L, which is also in line with WHO Guidance (WHO, 2004).

Although drinking water and beverages are the main contributors to the consumer exposure to fluoride, studies also identified concentrations of fluoride in tea, fish, vegetables, cereals and cereal-based products, food for infants and young children and various other foods. In addition, non-dietary sources, such as fluoride-containing dental hygiene products also contribute to the overall exposure to fluoride.

Recently studies have become available suggesting a relation between neurotoxicity in children and intake of fluoride which might cause effects due to exposure to concentrations below 1.5 mg/L in drinking water.

1.1.2 | Terms of Reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002 the Commission asks EFSA for an updated consumer risk assessment for fluoride in food and drinking water, taking into account:

- available information on the occurrence of fluoride in food and drinking water;
- an exposure assessment considering the levels of fluoride in food and drinking water and the contribution from other known sources of exposure;
- available scientific information on the hazards of fluoride.

1.2 | Interpretation of the Terms of Reference

In this Opinion, consumer risk from exposure to fluoride through food and drinking water and contribution from other sources is re-evaluated. The Scientific Committee noted that for this mandate only elemental fluoride and fluoride ion will be assessed and that every compound that can release elemental or ionic fluoride will be considered. This re-evaluation is based on existing assessments (see Section 1.4) and additional relevant literature on the hazard properties of fluoride. Although fluoride is not an essential trace element, it has been recognised as beneficial for its prevention of dental caries and therefore adequate intakes (AI) for fluoride were established by the EFSA NDA Panel (2013). This risk assessment aims to interpret the evidence on potential adverse effects of fluoride within the context of its benefits, even though re-evaluation of the beneficial effects is out of scope of this mandate. In that context the principles described in the EFSA Statement that proposed a harmonised approach for establishing health-based guidance values (HBGVs) for compounds used in regulated products that are also nutrients (EFSA Scientific Committee, 2021) are considered while recognising that fluoride is not an essential nutrient. In addition to the beneficial properties of fluoride, assessment of risks from occupational exposure to fluoride and exposure to fluoride from medicines are out of the scope of this opinion.

The request to consider the contribution from other sources requires a comprehensive exposure assessment in which the relative contribution of food and water is assessed along with other sources contributing to oral exposure. Regarding these other sources, the SC considers that dental care products are the major sources that are relevant and these will be included in the assessment. Non-oral sources of exposure to fluoride are out of the scope of this opinion.

[4]Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption. OJ L 435, 23.12.2020, p. 1–62.

[5]Commission Directive 2003/40/EC of 16 May 2003 establishing the list, concentration limits and labelling requirements for the constituents of natural mineral waters and the conditions for using ozone-enriched air for the treatment of natural mineral waters and spring waters. OJ L 126, 22.5.2003, p. 34.

The following sources are considered to be relevant to the occurrence of fluoride in food and drinking water (tap, well, bottled): natural occurrence (including occurrence as a contaminant), use of relevant fluoride compounds in plant protection products (PPPs), food supplements, fortified foods (e.g. fluoridated salt), flavouring substances and additives, and presence of relevant fluoride compounds as impurities in any of these applications. In addition, fluoride compounds relevant to drinking water fluoridation and fluoride compounds in materials in contact with drinking water from abstraction to tap are relevant to the overall exposure to fluoride.

1.3 | Consultations

In line with its policy on openness and transparency, EFSA consulted EU Member States and interested parties through an online public consultation held between 11 December 2024 and 9 February 2025. The comments received were considered by the working group and incorporated into the current Opinion, where appropriate, before adoption of the Opinion by the EFSA Scientific Committee. The technical report of the outcome of the public consultation (EFSA-Q-2021-00358) is published separately as Annex A to this opinion.

1.4 | Additional information

1.4.1 | Legislation

A Limit Value of 1.5 mg/L has been established for fluoride in drinking water in Directive (EU) 2020/2184. Natural mineral waters which contain more than 1 mg fluoride/L are authorised to be labelled as 'contains fluoride' by Directive 2009/54/EC.[6] According to Directive 2003/40/EC, the fluoride content of natural mineral waters must not exceed 5 mg fluoride/L, and mineral waters exceeding 1.5 mg fluoride/L shall bear on the label the words 'contains more than 1.5 mg fluoride/L: not suitable for regular consumption by infants and children under seven years of age', and shall indicate the actual fluoride content.

Maximum residue levels (MRLs) for fluoride ion in food of plants and animal origin have been established under Regulation (EC) No 396/2005[7] on pesticide residues. These MRLs have been legally implemented by Commission Regulation (EU) 2022/1321[8] and are based on the uses of sulfuryl fluoride as fumigant on emptied cereal mills, empty grain storage rooms, tree nuts, raisins and cocoa beans assessed by EFSA (2021). They are also based on the background levels reported in a Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) for fruits, vegetables, dried herbs, tea and animal commodities (EFSA NDA Panel, 2013).

A Scientific Opinion by the Panel on food additives, flavourings, processing aids and materials in contact with food (AFC Panel) published in 2005, listed the substance silicic acid, magnesium-sodium-fluoride salt (CAS number: 037296-97-2) used in food contact materials in list 3 with the restriction of 0.15 mg fluoride/kg food.

1.4.2 | Previous assessments establishing health-based guidance values for fluoride

In 2005, the NDA Panel established a tolerable upper intake level (UL) of fluoride of 0.1 mg/kg bw per day in children aged 1–8 years. This was based on the prevalence of moderate to severe dental fluorosis of less than 5% observed in children ingesting 0.08–0.12 mg/kg bw per day of fluoride (EFSA NDA Panel, 2005). This UL is equivalent to 1.5 and 2.5 mg/day in children aged 1–3 years and 4–8 years, respectively. The Panel established a UL of 0.12 mg/kg bw per day for ages 9 years and over, on the basis of evidence of increased risk for bone fractures obtained from postmenopausal women receiving 0.6 mg/kg bw per day of fluoride therapeutically, with application of an uncertainty factor (UF) of 5. This is equivalent to a UL of 5 mg/day in children aged 9–14 years and 7 mg/day for children ≥ 15 years and for adults (including pregnant and lactating women). No UL for infants was established. The Panel noted that a maximum fluoride level of 0.6–0.7 mg/L in infant formula and follow-on formula was recommended by the Scientific Committee on Food, equivalent to an intake of about 0.1 mg/kg bw per day in infants during the first 6 months of life (body weight 5 kg) (SCF, 2003).

In the 2013 Scientific Opinion on Dietary Reference Values (DRV) for fluoride, the NDA Panel established an adequate intake (AI) of fluoride from all sources (including non-dietary sources such as toothpaste and other dental hygiene products) of 0.05 mg/kg bw per day for infants from 6 months onwards, children and adults, including pregnant and lactating women considering its beneficial effect on the prevention of dental caries (EFSA NDA Panel, 2013; Table 1). This was based

[6]Directive 2009/54/EC of the European Parliament and of the Council of 18 June 2009 on the exploitation and marketing of natural mineral waters (Recast). OJ L 164, 26.6.2009, p. 45–58.

[7]Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1–16.

[8]Commission Regulation (EU) 2022/1321 of 25 July 2022 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for fluoride ion, oxyfluorfen, pyroxsulam, quinmerac and sulfuryl fluoride in or on certain products. OJ L 200, 29.7.2022, p. 1–24.

on a fluoride concentration of 1 mg/L in drinking water at which the caries preventive effect was considered to approach its maximum; at that level only 10% of the population was found to be affected by mild dental fluorosis.

TABLE 1 DRVs for fluoride from NDA Panel (2005, 2013).

	Age group	mg/day	mg/kg bw per day	Basis
Adequate Intake (AI)	All	0.05		Adequate Intake from all sources (including non-dietary sources); intake associated with prevention of dental caries
Tolerable upper intake level (UL)	Infants (< 1 years)	Not established [0.6–0.7 mg/L infant and follow-on formula]		No specific UL established; maximum fluoride content recommended by SCF in infant formula and follow-on formula
	1–3 years	1.5	0.1	RP = 0.1 mg/kg bw per day as dose associated with occurrence of less than 5% of moderate to severe dental fluorosis; no UF applied
	4–8 years	2.5	0.1	
	9–14 years	5	0.12	RP = 0.6 mg/kg bw per day as dose associated with an increased risk of skeletal fractures in postmenopausal women, with UF = 5
	≥ 15 years	7	0.12	

Abbreviations: RP, reference point; UF, uncertainty factor.

In 2008, the US Environmental Protection Agency (EPA) carried out a quantitative dose–response assessment providing an oral reference dose of 0.08 mg/kg bw per day based on the critical health effect of pitting of the enamel in severe dental fluorosis (U.S. EPA, 2010). Confidence in the reference dose was considered to be medium.

1.4.3 | Previous assessments without health-based guidance values

In the framework of the Pesticide Peer Review (2010) under Directive 91/414/EEC,[9] a draft assessment report on sulfuryl fluoride was prepared by the United Kingdom as rapporteur Member State, in which an acceptable daily intake (ADI) for that substance was established (EFSA, 2010a). EFSA concluded that no scientifically based conclusion could be drawn on the ADI for fluoride (EFSA, 2010a).

In a 2004 Scientific Opinion related to fluorine as undesirable substance in animal feed, the Panel on Contaminants in the Food Chain (CONTAM) concluded that the fluoride concentrations in foods from animal origin contribute only marginally to human exposure (EFSA CONTAM Panel, 2004).

In a 2005 Scientific Opinion, the CONTAM Panel assessed fluoride concentration limits in natural mineral waters relative to the 2005 NDA-established UL for adults (7 mg/day). The Panel noted that with a consumption of 1 L mineral water/day, the UL would be exceeded at the maximum reported fluoride concentration (8 mg/L) but not if the maximum limit for fluoride concentration in mineral water was reduced to 1 mg/L. At a maximum limit of 5 mg/L, exposure would exceed the UL in the population below 15 years but not above 15 years. The UL for a child of 1–3 years of age (1.5 mg/day) would be reached with consumption of 200 mL of mineral water containing 8 mg/L fluoride, excluding exposure from other sources (EFSA CONTAM Panel, 2005).

The EC Scientific Committee on Health and Environmental Risks (SCHER) assessed the hazardous and beneficial health effects of fluoride in 2011 in the context of drinking water fluoridation (SCHER, 2011). The opinion concluded that a threshold for dental fluorosis in children could not be determined and the margin between the protection against caries and dental fluorosis is narrow. It was noted that endemic skeletal fluorosis has not been reported in the EU general population. The committee concluded that there was not sufficient evidence to support that fluoride intake from drinking water at the level occurring in the EU affects thyroid function, male and female reproductive capacity, children's neurodevelopment and IQ or is associated with osteosarcoma. The committee also concluded that water fluoridation and topical fluoride applications appear to prevent caries, primarily on permanent teeth and that topical application is a more efficient intervention.

The EC Scientific Committee on Cosmetic Products and Non-food Products Intended for Consumers assessed the safety of fluorine compounds in oral hygiene products for children under the age of 6 years (SCCNFP, 2003). The Opinion concluded that the maximum permitted concentration of 0.15% (1500 mg/kg) fluorine does not pose a safety concern when used by children under the age of 6 years. If toothpaste containing fluoride within the permitted concentrations is the sole source of exposure and if it is used as recommended, the risk that children below 6 years will develop dental fluorosis is considered minimal. The committee also concluded that there is strong evidence that toothpaste containing 0.15% (1500 ppm) is effective at preventing dental caries in all age groups and that the cariostatic effect decreases when the concentration is reduced until 1000 ppm, below which the cariostatic effect is not established.

In 2023, a Health Canada expert panel meeting on the health effects of fluoride in drinking water considered moderate dental fluorosis as the key health effect on which to base a human health risk assessment for fluoride in drinking water

[9]Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1–32.

(Health Canada, 2023). The expert panel was provided with a Health Canada-commissioned review on fluoride health effects in humans and animals with the task to provide expert opinion and recommendations to Health Canada. The expert panel agreed with the use of a 1% lower limit benchmark dose of 1.56 mg F/L as the point of departure (PoD) for deriving the health-based guidance value based on moderate dental fluorosis data from Dean (1942). The panel recommended 0–4 years as the period of greatest susceptibility. After 8 years of age, the panel concluded that there would be essentially no risk of dental fluorosis. No PoD could be identified for neurobehavioural effects reported in literature based on Health Canada-commissioned independent systematic review (unpublished). Due to the uncertainty about possible neurocognitive effects at low levels of exposure, the panel recommended the use of an UF for database deficiency for deriving the health-based guidance value but was unable to recommend a specific numeric UF, leaving this decision to Health Canada (Health Canada, 2023).

In 2024 the National Toxicology Program (NTP) published a systematic review of human, experimental animal and mechanistic studies to evaluate the evidence for an association between exposure to fluoride and neurodevelopment and cognition, in adults and children separately (NTP, 2024). The body of evidence from experimental animal and mechanistic studies was considered poor or inadequate to conclude on the association between fluoride exposure and human health effects. Limited evidence examining cognitive effects in adults were available while literature in children assessing IQ or other cognitive or developmental outcomes was more extensive. NTP concluded with moderate confidence that higher estimated fluoride exposures (e.g. drinking water concentrations that exceed 1.5 mg/L of fluoride) are consistently associated with lower IQ in children. NTP also concluded that uncertainty remains in findings at the lower fluoride exposure range (NTP, 2024). Before the finalisation of this opinion, the NTP meta-analysis of studies that assessed the potential association of fluoride exposure with cognitive development was also published (Taylor et al., 2025). This evidence synthesis effort (74 studies; 10 cohort studies, 64 cross-sectional studies) indicated inverse associations and a dose–response association between fluoride measurements in urine and drinking water and children's IQ. For the dose–response association and when fluoride exposure was estimated by drinking water alone at concentrations less than 1.5 mg/L, the available evidence was limited and characterised by uncertainty.

In 2024, New Zealand Office of Prime Minister's Chief Science Advisor (OPMCSA) updated the 2021 review on the risks and benefits of drinking water fluoridation (OPMCSA, 2024). The review upheld the previous conclusions that drinking water fluoridation in New Zealand is safe and provides social equity of protection against dental caries. The higher risk for dental fluorosis in areas with high naturally occurring fluoride in drinking water was acknowledged but it was noted that this is not the case in New Zealand. Studies reporting on neurodevelopmental outcomes published between 2014 and 2023 were not considered to provide evidence of sufficiently high quality to suggest a causal link between fluoride and adverse effects at the levels of drinking water fluoridation in New Zealand.

1.4.4 | Methods of fluoride analysis

Various methods have been developed for the detection and quantitation of fluoride in food and beverages, drinking water, dental care products and biological matrices. This section describes only the most relevant methods of analysis for fluoride.

In this Opinion, the term 'fluoride' refers to non-organic fluorine (i.e. non-covalently bound), i.e. both free fluoride ion and inorganic bound fluorine, including inorganic fluoride salts, fluoride complexed with metals (e.g. with aluminium in tea) or fluoride fractions in proteins or fractions incorporated in biological matrices (bone, teeth).

Methods quantifying total fluorine may overestimate the occurrence of and consequently exposure to fluoride ions. The potential overestimation of the exposure resulting from such methods is taken into account in the uncertainty assessment.

An overview of the methods of analysis of fluoride most frequently found in the literature is presented in Appendix A, while a brief description of sample treatment and analyses is given below.

Pre-treatment

Pre-treatment of the sample before analysis is a fundamental step of the analytical process for fluoride determination, independent of the applicable analytical methodology (ion selective electrode (ISE), ion chromatography (IC), etc.) because the presence/absence and also the type of pre-treatment may impact fluoride recovery and the result of the analysis.

For water and water-based beverages, such as tea infusions or biological matrices, such as urine, samples are generally treated with a buffer solution to dissociate metal-fluoride complexes prior to analysis (Abuhaloob et al., 2015; Belete et al., 2017; Fernandez-Macias et al., 2020; Fojo et al., 2013; Goschorska et al., 2016; Helte et al., 2021; Janiszewska & Balcerzak, 2013; Kassahun & Chandravanshi, 2019).

Fluoride from solid matrices such as food can be acid-extracted with nitric, sulfuric or perchloric acid combined with heating in a heating block or microwave oven, but there is a risk of volatilisation in acidic media (El-Said & El-Sikaily, 2013; Kazi et al., 2018; Lou et al., 2017; Rocha et al., 2013). Silicon-assisted diffusion methods, where the released HF is trapped into an alkaline trapping solution, are widely used to concentrate fluoride ions after acid extraction. This can also be applied to concentrate fluoride ions from liquid matrices. After trapping the fluoride, dissociation of metal-fluoride complexes in the extracts is mandatory prior to determination using a buffer solution (Abuhaloob et al., 2015; EURL-SRM, 2023; Martinez-Mier et al., 2011, 2017; Zohoori & Maguire, 2016).

To increase fluoride recovery, alkaline fusion can be applied to a wide range of food and aqueous matrices at temperatures up to 600°C (Dagnaw et al., 2017; Dessalegne & Zewge, 2013). This pre-treatment is used to assess the total fluoride content, although the carbon-fluoride bond may withstand temperatures above 900°C. Additional analytical challenges have been identified for some specific matrices. Dairy matrices may require additional sample treatment, as direct measurement and diffusion methods can miss protein-bound and lipid-bound fluoride. Spano et al. (2023) developed recently a method quantifying fluoride in the different fractions of milk: free inorganic, inorganic reversibly adsorbed by emulsions or suspensions in the milk matrix, protein-bound and lipid-bound fluoride. In foods where food additives rich in aluminium, magnesium or calcium are present (such as in salt), the concentrations of metal-fluoride complexes can be elevated. Acid treatment and pre-heating (boiling) before analysis releases fluoride from metal-fluoride complexes increasing fluoride recovery up to five-fold (Esquivel-Pena et al., 2016). Pre-heating in a microwave oven results in slightly higher recoveries compared to boiling due to reduced volatilisation of fluoride. Potential underestimation of the actual concentrations of fluoride in these matrices is considered in the uncertainty analysis.

Fluoride analysis

The potentiometric determination of fluoride by means of an ISE is a technique used in several standardised methods that are widely used for fluoride analysis (CEN, 2012; Chinese standard WS/T 89–2015, 2015; EURL-SRM, 2023; U.S. EPA, 2024). The technique allows the quantitation of fluoride ions in the solution in which the electrode is immersed. Correct sample conditioning is required to ensure a constant ionic strength and pH value as OH^- ions can interfere with fluoride ions. The sensitivity of ISE in solution is reported in the range of 0.1 mg/L or lower. ISE has been used for fluoride determination in a variety of matrices such as drinking water, foods, beverages, dental care products and human tissues (see Appendix A).

Ion chromatography (IC) combined with a conductivity detector is also used for the determination of fluoride in solution and the technique is used in US Environmental Protection Agency standards for the analysis of aqueous samples (U.S. EPA, 1993, 1997, 2024). Both the stationary phase and the eluent must be selected so that co-eluting ions are avoided. Some methodologies allow the simultaneous analysis of multiple ions by IC. Sensitivity in solution is reported in the range of 0.6 µg/L or lower. This method has been used for the determination of fluoride in water, water-based beverages (e.g. tea infusions), serum or urine (see Appendix A).

Fluoride can be quantified in solution with colorimetric methods. A colorimetric method using SPADNS reagent (sodium 2-(p-sulfophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonate) measures the loss of colour of the zirconyl-SPADNS dye following complexation of fluoride with zirconium (U.S. EPA, 2024). The sensitivity of the SPADNS method in water is 0.1 mg/L or lower. More colorimetric methods using zirconium alizarin red S or other agents are reported (see Appendix A).

Total fluorine analysis

Some methods of analysis quantify total fluorine in the samples, i.e. free fluoride ions plus fluorine covalently bound to organic compounds (e.g. PFAS). This is done by e.g. microwave-induced plasma optical emission spectroscopy (MIP-OES), inductively coupled plasma mass spectrometry (ICP-MS), plasma assisted reaction chemical ionisation mass spectrometry (PARCI–MS) or atomic absorption spectroscopy (AAS) applying thermal treatment at high temperatures (up to 8000°C) (see Appendix A).

In samples of water for human consumption, Directive (EU) 2020/2184[10] sets low regulatory limits for substances containing covalently bound fluorine, such as PFAS and pesticide residues.[11] In such cases the analytical result based on total fluorine methodologies does not deviate (from a statistical point of view) from the result of a method limited to the determination of ionic fluoride only and can, therefore, be considered acceptable.

In food samples, residues of fluorine-containing pesticides may occur in concentrations up to the maximum residue levels (MRLs) set in Regulation (EC) No 396/2005. Total fluorine analysis following certain pre-treatment conditions might, therefore, include contribution of covalently bonded fluoride released from these compounds. To estimate the impact from measuring covalently bonded fluorine from pesticide use, the Scientific Committee calculated the total fluorine concentration in main dietary sources of fluoride such as tea, coffee, herbal infusions, cereals and vegetables, assuming occurrence of approved fluorine-containing pesticides at the MRL.[12] The outcome of this exercise showed that total fluorine analysis would be expected to overestimate the analytical result for fluoride when residues of pesticides co-occur with free fluoride in the food matrix.

Summary of methods of analysis

ISE and IC are standardised analytical methods for the determination of fluoride ions once extracted in solution from a variety of matrices (see Appendix A). The conditions of sample pre-treatment determine whether total or free fluoride is extracted and measured in the analytical methods.

[10]Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption. OJ L 435, 23.12.2020, p. 1–62.

[11]Limit for total PFAS in water: 0.0005 mg/L; limit of total pesticide residues in water: 0.0005 mg/L; limit of fluoride in water: 1.5 mg/L and mean occurrence in non-fluoridated water is 0.2 mg/L (see Table 2 in Section 2.2.2).

[12]https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/mrls.

A possible over- or underestimation of the actual exposure to fluoride due to the method and/or sample pre-treatment is further considered in the uncertainty analysis.

2 | DATA AND METHODOLOGIES

2.1 | Problem formulation

2.1.1 | Overall aim of the risk assessment

The overall aim is to provide an updated risk assessment for fluoride relevant to exposure of the EU population to fluoride present in food and drinking water that also considers exposure from its use in dental care products and other major sources. The hazard assessment is based on previously assessed literature and new evidence relevant for fluoride hazard identification.

2.1.2 | Target populations

The target population of the risk assessment is the European population, including specific vulnerable groups (such as pregnant women, infants, children) and consumer subgroups with high fluoride exposure from sources covered in the exposure assessment. These include subgroups with particular dietary preferences (e.g. high and frequent salt consumers) and/or high and frequent exposure to tap water and natural mineral water in regions/countries with naturally high content level of fluoride due to regional/national geological conditions.

2.1.3 | Fluoride compounds

The focus of the assessment is fluoride ion[13] in food, drinking water and non-dietary sources relevant to oral exposure. Fluorine compounds present in or added to food, drinking water and dental care products relevant to this assessment include those that release fluoride. Fluorinated compounds that do not release fluoride or with toxicological properties not attributed to fluoride are out of scope for this mandate (e.g. polyfluorinated compounds, sulfuryl fluoride). Cosmetic products or products used under medical prescription containing fluoride, other than dental care products are also excluded in the scope of the mandate.

2.2 | Data

2.2.1 | Hazard identification and characterisation

Data relevant to the hazard identification in both humans and laboratory animals were identified through a comprehensive literature search for relevant toxicity studies published after the last safety assessment (EFSA NDA Panel, 2005). Information on absorption, distribution, elimination and available physiologically based kinetic models from human and experimental animal kinetic studies has been collected.

Additional evidence relevant to fluoride toxicity and mode of action was sought with targeted literature searches for human, in vivo, in vitro and in silico studies.

2.2.2 | Dietary exposure assessment

2.2.2.1 | *Food consumption data*

Food consumption data from the EFSA Comprehensive European Food Consumption Database[14] (Comprehensive Database) were used for the dietary exposure assessment of fluoride. This database contains national data on food consumption at the individual level and is the most complete and detailed database currently available in the EU.

The food consumption data gathered in the Comprehensive Database were collected using dietary records covering 3–7 days per individual or repeated 24- or 48-h dietary recalls. Owing to the differences in the methods used for data collection, intake estimates are analysed per EU Member State.

Details on how the Comprehensive Database is used to assess the dietary exposure to food chemicals are published in a 2011 EFSA Guidance (EFSA, 2011). The latest version of the Comprehensive Database was updated in 2021 and contains

[13]Throughout the opinion, the term 'fluoride' refers to 'fluoride ion'.

[14]https://www.efsa.europa.eu/en/data-report/food-consumption-data.

results from 51 dietary surveys carried out in 24 Member States (MS) covering 97,154 individuals. Some surveys provide information on 'Pregnant women' ($n = 3$), 'Lactating women' ($n = 2$) and Vegetarians ($n = 1$). When two different dietary surveys are available for one country and age class, the most recent one is used in the dietary exposure assessment.

Since 2018, all consumption records in the Comprehensive Database have been coded according to the FoodEx2 classification system (EFSA, 2015). The FoodEx2 classification system consists of a large number of standardised basic food items aggregated into broader food categories in a hierarchical parent–child relationship. Additional descriptors, called facets, are used to provide additional information about the coded foods (e.g. information on food processing and packaging material).

For fluoride, a chronic dietary exposure assessment is relevant in the context of the Terms of Reference. For such an assessment, surveys in which food consumption data were collected during only 1 day are not considered appropriate, as described in 2011 EFSA Guidance (EFSA, 2011). Exclusion of these surveys resulted in a total of 41 dietary surveys carried out in 22 MSs covering 83,540 individuals. Table 2 provides an overview of the population groups and countries included in the dietary exposure assessment of fluoride.

According to the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age, exposure assessment for these infants should be carried out separately from older infants, following the procedure described in the guidance (EFSA Scientific Committee, 2017a, 2017b, 2017c). Based on this guidance, infants below 16 weeks of age should be excluded from the dietary exposure estimation of the infants age group. However, for the exposure assessment of fluoride, due to uncertainty in the reported individual ages of infants in the Comprehensive Database, the cut-off age was set at 12 weeks based on the existing individual age range of this group in this database. As a result, food consumption data of infants over 12 weeks, i.e. infants between 12 and 16 weeks of age, were included in the exposure assessment. Since the number of children within this age range in the database is limited, it is not expected that this will have affected the exposure estimate of fluoride for infants of 16 weeks up to 12 months of age.

TABLE 2 Population groups and countries included in the chronic dietary exposure assessment.

Population group	Age range	Countries with food consumption surveys covering more than 1 day
Infants	> 12 weeks to < 12 months old	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	≥ 12 months to < 36 months old	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, the Netherlands, Portugal, Slovenia, Spain
Other children	≥ 36 months to < 10 years old	Austria, Belgium, Bulgaria, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, the Netherlands, Portugal, Spain, Sweden
Adolescents	≥ 10 years to < 18 years old	Austria, Belgium, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	≥ 18 years to < 65 years old	Austria, Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Elderly	≥ 65 years to < 75 years old	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Very elderly	≥ 75 years old	Austria, Belgium, Denmark, France, Germany, Hungary, Ireland, Italy, Latvia, the Netherlands, Portugal, Romania, Sweden

In addition, Table C.1 in Annex C provides details on the dietary surveys included in the dietary exposure assessment.

2.2.2.2 | *Consumption data on discretionary fluoridated salt*

Dietary surveys are not considered a reliable source of information for the consumption of discretionary salt (fluoridated or not) that consumers add to their food (e.g. during preparation or while eating) as the use of this salt is poorly reported in surveys and subject to high uncertainty. For this reason, discretionary salt consumption (fluoridated or not) was estimated or extracted from the literature.

A scientific opinion from the EFSA ANS Panel (Scientific Opinion on the re-evaluation of sodium ferrocyanide (E 535), potassium ferrocyanide (E 536) and calcium ferrocyanide (E 538) as food additives (EFSA ANS Panel, 2018) reported EU estimates of the total salt intake data from urinary excretion of the sodium ranging in average from 8 g/day for children and adolescents population groups (6–18 years old) to 10 g/day for adults and elderly population groups (> 18 years old). The P75 was reported and/or estimated by the ANS Panel (based on the available data for the ratio P75/average of 1.23 for children and adolescents and of 1.63 for adults and elderly population groups), respectively at 10 and 15 g/day.

The European Commission's survey conducted in 2012 on the implementation of the EU salt reduction framework reported the daily consumption of salt intake among adults of 18 MSs (European Commission, 2012). Total daily consumption of salt was reported in average among adults from 5 to 13.3 g/day in males and from 5 to 11.3 g/day in females (EC, 22 Jan 2021, defining

dietary salt and sodium – overview of salt intake in adults across European countries[15]). The Scientific Committee notes that this daily salt consumption is in the same order of magnitude of the data published by the ANS Panel.

Discretionary salt (fluoridated or not) added during cooking and at the table comprises about 10%–15% of total salt intake (Sanchez-Castillo et al., 1987). The percentage of salt intake attributed to discretionary salt (table and cooking salt, fluoridated or not) is quite variable as illustrated by studies conducted in the UK (Farrimond et al., 1995; Henderson, 2003), Denmark (Andersen et al., 2009), Italy (Leclercq & Ferro-Luzzi, 1991) and worldwide (Bhat et al., 2020), and varies from 10% in the study in Denmark, more than one third of the total salt intake in the study in Italy to more than 50% in Romania (Bhat et al., 2020).

Assuming a percentage consumption of up to 15% of discretionary salt (fluoridated or not) over total salt consumption as reported in EFSA, 2006, and using the average and P75 total daily consumption of salt from the EFSA ANS Panel described above, the mean consumption of discretionary salt (fluoridated or not) would be 1.2 g/day for children and adolescents population groups (6–18 years old) and 1.5 g/day for adults and elderly population groups (> 18 years old) while the estimated P75 amount would be 1.5 g/day and 2.25 g/day, respectively. The Scientific Committee notes the substantial uncertainty associated with this consumption estimate.

2.2.2.3 | *Concentration data of fluoride in food and drinking water*

It was assumed that the fluoride concentration measured in food and drinking water is the concentration of the fluoride ion that results from the release of fluoride ions from all contributing sources and as measured by appropriate analytical methods (see Section 1.4.4). A list of fluoridated materials which are used in water transport pipes and which come in contact with drinking water was provided by ECHA. Contribution of any potential release of fluoride ions from such materials is captured in the concentration data of drinking water. Two types of concentration databases were available: the database of occurrence data submitted to EFSA and the EFSA food composition database. When a sufficient number of samples is available from the occurrence database, this data source is considered more reliable as detailed information and the analytical methods are available for each analysed sample while the EFSA food composition database only provides average values with no information at sample level. These two concentration databases are described below. It was deemed not necessary to perform a systematic literature search for fluoride occurrence data in food and water as the EFSA occurrence and composition databases provided sufficient data for all food categories.

2.2.2.4 | *Occurrence data submitted to EFSA*

Following a mandate from the European Commission to EFSA, a call for annual collection of chemical contaminant occurrence data in food was issued by the former EFSA Dietary and Chemical Monitoring Unit (now iDATA Unit) in December 2010. Since then, data have been submitted every year with a closing date on 1 October of each year. These data submissions include concentrations of fluoride in food and drinking water.

The data submitted to EFSA follow the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010c) and the EFSA Guidance on Standard Sample Description 2 (EFSA, 2013).

For the dietary exposure assessment of fluoride, analytical results available up to July 2022 on fluoride in food and drinking water were extracted from the EFSA occurrence database. In total, 3259 analytical results were available, which were provided by national authorities of eight MS between 2014 and 2021 for six food categories at the FoodEx2 level 1 classification and drinking water. The number of results reported per year and per country are shown in Table C.2 in Annex C.

Most of the analytical results were available for drinking water ($n = 3222$). For five of the six food categories fewer than six samples were available and thus data from the EFSA occurrence database were not included in the assessment, and data on these food categories from the EFSA composition database were used instead (see next section).

Twenty left-censored samples were available for infant formula, but the data provider was not able to provide clarifications on the form in which the samples were analysed (powder or ready-to-drink). Ten of these analytical results were referring to sulfuryl fluoride. Based on this, analytical results on infant formula from the EFSA occurrence database were not included in the assessment while data on milk powder and soy protein derived from the EFSA composition database were used instead (see next section). Only one sample was available for 'Herbal infusions (beverages) specific for infants and young children, liquid' thus this category was not included in the assessment.

In conclusion, only data for drinking water from the EFSA occurrence database were included in the assessment. Raw data were validated following the standard procedure described in EFSA 2022 and corrections were applied on identified mistakes. The raw data including information on actions taken concerning data validation are available at the EFSA Knowledge Junction community on Zenodo.[16]

Results referring to suspect sampling ($n = 154$) were excluded. One sample referring to well water with a concentration higher than the legal limit of 1.5 mg/kg was also excluded. After validation, a total of 3067 analytical results were used to

[15] https://knowledge4policy.ec.europa.eu/health-promotion-knowledge-gateway/defining-dietary-salt-sodium-overview-adults-4a_en.
[16] 10.5281/zenodo.15698508.

calculate fluoride concentrations in water for the dietary exposure assessment. Among these, > 86% of drinking water samples had fluoride concentration < 0.3 mg/L and > 97% of drinking water samples had < 0.7 mg/L fluoride.

Averages for specific water categories calculated on less than six samples, were not retained. These analytical results were included in the calculation of averages for categories at higher levels of the FoodEx2 classification if at least six samples were available.

Concentrations for samples of water identified as being fluoridated (samples with facet FORT = FLUOR or bottled water samples with a fluoride concentration between 1.5 and 5 mg/L, $n = 21$) were not used in calculating other water concentration averages and only linked to consumption events of fortified water.

For 792 out of 3067 available results (26%), there was no information about the analytical method. For 2275 samples, various methods for fluoride analysis in drinking water were reported by the national laboratories, in line with information from literature (see Section 1.4.4). The lack of information on the analytical method in 26% of samples and limitations regarding the sensitivity of some of the used methodologies were considered in the uncertainty analysis.

Left-censored data for fluoride in drinking water [results below limit of detection (LOD) or below the limit of quantification (LOQ)] were treated using the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (ICPS/WHO, 2019). This is the same method as indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b). The guidance suggests that the lower bound (LB) and upper bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). The LB was obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD (< LOD) or LOQ (< LOQ). The UB was obtained by assigning the numerical value of LOD to values reported as < LOD and LOQ to values reported as < LOQ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory. In addition, the middle bound (MB) was obtained by assigning the numerical value of LOD/2 to values reported as < LOD and LOQ/2 to values reported as < LOQ (maximum possible value), depending on whether LOD or LOQ was reported by the laboratory.

A mean LB, MB and UB occurrence value was then calculated at each level of the FoodEx2 classification.

Details on the occurrence data at the different FoodEx2 classification levels for drinking water are provided in Table C.3 in Annex C. These values of fluoride concentration in water (LB/MB/UB) were used in the dietary exposure assessment for the 'basic scenario'.

Additional values for the concentration of fluoride in water were used to assess exposure in potential 'water fluoridation' scenarios. Water fluoridation in the EU is only done in Ireland (population coverage 74%) and some regions of Spain (population coverage 3%) and Portugal (population coverage 1%) at concentrations levels ranging from 0.8 to 1.2 mg/L (Mullen, 2005; SCHER, 2011).

The first 'Water P95 scenario' makes use of the LB/MB/UB 95th percentile value of fluoride concentration in water as derived from the EFSA occurrence database. Because the LB P95 was equal to the UB P95, the P95 concentrations are shown as single values in Table 3.

Two additional scenarios were based on the maximum concentrations set by Directive 2020/2184/EC on the quality of water intended for human consumption and Directive 2003/40/EC on the constituents of natural mineral waters.

The value of 1500 µg/L was used for all types of drinking water in scenario 'Water legal limit 1', while in scenario 'Water legal limit 2', the value of 1500 µg/L was used for tap water and unspecified drinking water while the value of 5000 µg/L was used for bottled water. As according to Directive 2003/40/EC, 'water containing more than 1.5 mg fluoride/L is not suitable for regular consumption by infants and children under seven years of age', the 'Water legal limit 2' scenario was assessed only for older age groups.

The relevant water concentration values were used in each scenario to account for the effect of boiling and reconstitution of foods as described in next section.

The concentration of fluoride in water used in each of the scenarios are shown in Table 3. It is noted that LB, MB and UB differed only in the basic scenario.

TABLE 3 Fluoride water content in µg/L used in the relevant four dietary exposure assessment scenarios with lower bound (LB), middle bound (MB) and upper bound (UB) concentrations, as described in this section and in Section 2.3.7.

	Dietary exposure assessment scenarios					
	Basic					
Water typology	LB	MB	UB	Water P95	Legal limit 1	Legal limit 2
Unbottled water	143	176	209	700	1500	1500
Bottled water	199	219	238	740	1500	5000
Drinking water (unspecified)	185	208	231	700	1500	1500

2.2.2.5 | *Nutrient composition data submitted to EFSA*

Fluoride concentration data in food were derived from the EFSA Food Composition Database.[17] The data for fluoride was part of a deliverable of a procurement project to update the food composition database for the estimation of nutrient intake. This project was coordinated by the Institute of Food Research, Norwich, UK and the database was delivered in 2013 (Roe et al., 2013).[18] This database contains concentration data on fluoride in food from three European countries (Germany, Iceland and Slovenia).

An additional dataset of composition data for Denmark was downloaded from the Food Institute, Technical University of Denmark public food database[19] on March 2022.

Concentration data included in the national composition databases were derived from various sources such as scientific literature, analytical results, other foods considered to have analogous fluoride content or calculated from recipes. No information was available about the way left-censored data were treated and if included in the calculation of the mean values available in the nutrient composition database. The lack of detailed information represents a source of uncertainty affecting the exposure assessment and is documented in the uncertainty section.

Data reported with concentration equal to zero were not included in the analysis because it was unclear if they were below the limit of detection or not reported.

Three outlier values identified by expert judgement were excluded from the calculation of averages of the relevant food category (one value for wine and one value for fish from Denmark, one value for coffee with cream from Germany).

Values reported as 'Flowers used for herbal infusions' and 'Herbal infusion materials from leaves and herbs' by Germany were not attributed to these categories as they referred to tea ingredients as indicated in the free text field.

Composition data reported for different cooking methods were excluded from the average of the food category because the effect of cooking was taken into account assuming different scenarios for the fluoride concentration in water as described in Section 2.2.2.2 (Occurrence data submitted to EFSA).

For each food, the mean concentration of fluoride across all countries was calculated. These mean concentrations were then used to calculate a mean concentration at each level of the FoodEx2 classification (available in Table C.3 in Annex C).

For some food categories fluoride concentration was available for only one of the forms either 'as sold' (ex. powder) or 'as consumed' (ex. reconstituted). The concentration in the other form was calculated by applying standard dilution factors.

Based on the above the following calculations were made:

- For soups and porridges ready-to-eat the available fluoride concentration value in powder was divided by the dilution factor of 10 plus fluoride concentration in water in each of the 'water fluoridation' scenarios.
- For pasta, rice and other grains with the exception of corn, water concentration was added to the fluoride concentration in these foods to take into account the uptake of fluoride during cooking (boiling) and based on the evidence that 100% of the fluoride ion present in water used for cooking rice goes into the cooked products (Anasuya & Paranjape, 1996; Sawangjang & Takizawa, 2020). As a conservative approach in the absence of data for cooked pasta and other grains, it was assumed that the same processing factor applies as for cooked rice.
- For tea infusions, fluoride concentration was calculated from concentration in solid ingredients (powder and leaf) using a dilution factor of 75 plus fluoride concentration in water in each of the 'water fluoridation' scenarios.
- For coffee solid ingredients (powder, granules) a dilution factor of 18 was used.
- For coffee imitates beverages, fluoride concentration was calculated from solid ingredients concentration in coffee imitates divided by a dilution factor of 50 plus fluoride concentration in water in each of the four 'water fluoridation' scenarios.
- The concentration value for fluoridated salt (470 mg/kg) was not used as the value extracted from literature was considered more reliable (see Section 2.2.2.2 on fluoridated salt).
- A specific fluoride concentration for iced tea was not available but considering the importance of tea as contributor to fluoride exposure, the value available in literature for iced tea (1.1 mg/L, (Rodriguez et al., 2018)) was used in the assessment when eating events could be related to the consumption of iced tea (see Section 2.3.7).
- For infant and follow-on formulas, as no specific concentration data were available, the concentration in the powder form was assumed equal to the fluoride concentration in powder milk (for the milk-based formulas) and in soy proteins (for the soy-based formulas) (see Table C.5 of Annex C). The concentration in the ready-to-drink form for the basic scenario was calculated from the value in milk powder/soy protein divided by a dilution factor of 8 plus the relevant value for fluoride concentration in water in the basic scenario (Table 3). The concentrations in the water fluoridation scenarios were set to the legal limit provided by Delegated Regulation (EU) 2016/127; this is 700 μg/L for the ready-to-drink formula. It was noted that the fluoride concentration for the ready-to-drink infant formula of 156 μg/L calculated from the concentration of fluoride in milk powder before adding the contribution of fluoride from drinking water (185 μg/L, Table 3) is consistent with the fluoride concentration data reported in the Mintel database, where ready-to-drink

[17]For the dataset used, see Annex C, Table C.4.

[18]Finland, France, Germany, Italy, Sweden, The Netherlands and United Kingdom.

[19]http://frida.fooddata.dk, version 4, 2019.

products reporting fluoride content indicated a concentration between 100 and 200 μg/L (Mintel database).[20] Consistency was also observed between the fluoride concentration in infant formula ready-to-drink form used in the dietary exposure assessment in the basic scenario (LB = 342 μg/L) and the published data from Zohoori and Maguire (2016) and (FSAI, 2018). A fluoride concentration was reported ranging from 9 to 252 μg/L and from 81 to 438 μg/L respectively.

– For (non-tea) 'Herbal infusion materials from leaves and herbs' the value reported in EFSA NDA 2010 was used (2 mg/kg dry weight) and the concentration in the ready-to-drink was calculated using a dilution factor of 75.

Table 4 shows fluoride concentration ranges extracted from the EFSA food composition database used in the dietary exposure assessment for various food categories (μg/kg or μg/L) and Table 5 shows the LB, MB and UB concentration used for infant and follow-on formulas in the four scenarios (μg/kg or μg/L).

TABLE 4 Fluoride concentration ranges extracted from EFSA food composition database used in the dietary exposure assessment for various food categories (μg/kg or μg/L).

FOODEX2_L1_ID	N food categories	min_LB	median_LB	max_LB
Alcoholic beverages	63	10	130	500
Animal and vegetable fats and oils and primary derivatives thereof	25	100	125	300
Coffee, cocoa, tea and infusions	64	90	228	95,000
Composite dishes	161	30	350	2725
Eggs and egg products	19	580	984	5800
Fish, seafood, amphibians, reptiles and invertebrates*	203	0	1000	4800
Fruit and fruit products	216	20	135	680
Fruit and vegetable juices and nectars (including concentrates)	79	75	147	1180
Grains and grain-based products	299	20	565	1607
Legumes, nuts, oilseeds and spices	143	60	500	4000
Major isolated ingredients, additives, flavours, baking and processing aids	10	20	300	1100
Meat and meat products	206	20	332	2900
Milk and dairy products	195	90	970	1600
Products for non-standard diets, food imitates and food supplements	6	200	630	1700
Seasoning, sauces and condiments	82	100	295	2000
Starchy roots or tubers and products thereof, sugar plants	18	20	155	1200
Sugar and similar, confectionery and water-based sweet desserts	80	30	340	1000
Vegetables and vegetable products	259	40	335	2110
Water and water-based beverages	61	40	160	2019
Follow-on formulae, liquid	4	323	342	342
Follow-on formulae, powder	4	1100	1250	1250
Infant formulae, liquid	4	323	342	342
Infant formulae, powder	3	1250	1250	1250
Cocoa beverages	3	220	237	253
Coffee beverages	11	90	113	248
Coffee imitate beverages	4	187	187	187
Herbal and other non-tea infusions	11	212	212	212
Tea beverages	5	1335	1335	1335
Cocoa ingredients	2	600	600	600
Coffee imitate ingredients	6	100	100	100
Coffee ingredients	6	900	1800	4000
Herbal infusion materials (generic)	1	2000	2000	2000
Tea leaves derivatives and tea ingredients	7	70,000	95,000	95,000

*Dried fish 6000 μg/kg.

[20] https://portal.mintel.com/.

TABLE 5 Fluoride concentration (µg/kg or µg/L) in infant formula and follow-on formula used in the four dietary exposure assessment scenarios as described in this section and in Section 2.3.7.

	Dietary exposure assessment scenarios*					
	Basic			Water P95	Legal limit 1	Legal limit 2
Formula	LB	MB	UB			
IF/FF liquid	342	364	387	700	700	700
IF/FF, powder	1250	1250	1250	1250	1250	1250

*IF, infant formula; FF, follow-on formula; LB, lower bound; MB, middle bound; UB, upper bound.

Details of the composition data on fluoride concentrations at country level at the available FoodEx2 classification levels are provided in Table C.4 in Annex C.

2.2.2.6 | *Fluoride concentration in fluoridated salt*

Oral exposure to fluoride occurs through fluoridated table salt, generally in EU countries where water fluoridation is not implemented. The most important fluoride salts for human use are sodium and potassium fluoride, which are readily soluble in water. They are permitted for addition to foods salt according to Regulation (EC) No 1925/2006. At least up to 2006, many EU countries recommended the consumption of fluoridated salt at levels ranged from 200 to 250 mg F/kg depending on national regulations (Gotzfried, 2006), mostly in the form of sodium and potassium fluoride fluoridated iodised salt for the prevention of caries, especially in Austria, Croatia, the Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Lithuania, the Netherlands, Portugal, Slovakia, Sweden, Spain, as well as Switzerland (EFSA NDA Panel, 2013; EU Salt, 2006). There are countries with marginal sales, but there are also countries such as Germany with 65% and Switzerland with 88% market share in household salt sales (Gotzfried, 2006).

A value of fluoride concentration of 250 mg/kg in fluoridated salt (EFSA NDA Panel, 2013; EU Salt, 2006; Gotzfried, 2006) was used for the dietary exposure assessment to fluoride through the consumption of discretionary salt (see Sections 2.2.2.2 and 3.9.3).

2.2.2.7 | *Food supplements*

Currently, for food supplements the following are authorised sources of fluoride: calcium fluoride, potassium fluoride, sodium fluoride, sodium monofluorophosphate. No maximum amounts have been established so far for fluoride at EU level for food supplements or fortified foods, including specific sources. For use in food supplements, calcium fluoride and sodium monofluorophosphate were assessed at levels of 0.5–2 mg fluoride/day for children and adults (EFSA ANS Panel, 2008a, 2008b).

2.2.3 | Non-dietary sources of fluoride exposure

2.2.3.1 | *Oral care products*

The EU Cosmetics Regulation[21] covers the use of certain ingredients in cosmetic products. In the Cosmetics Directive 76/768/EEC, 18 fluoride compounds are listed which can be used in toothpaste as oral hygiene products:

- sodium/potassium/calcium/ammonium fluoride;
- sodium/potassium/calcium monofluorophosphate;
- aluminium/stannous fluoride;
- hexadecyl ammonium fluoride;
- 3-(N-hexadecyl-N-2-hydroxyethylammonio) propylbis (2-hydroxyethyl) ammonium difluoride;
- N,N′,N′-tris(polyoxyethylene)-N-hexadecylpropylenediamine dihydrofluoride;
- octadecenylammonium fluoride;
- sodium/potassium/magnesium/ammonium fluorosilicate;
- nicomethanol hydrofluoride.

Toothpaste may contain up to a maximum of 1500 mg fluoride/kg (0.15%). In European Union countries, about 90% of all toothpastes are fluoridated, up to a maximum level of 1500 mg fluoride/kg (EFSA NDA Panel, 2005). High fluoride toothpastes containing more than 1500 mg/kg fluoride (usually in the range of 2000–5000 mg/kg) are available on prescription for older children and adults at increased risk of caries (O'Mullane et al., 2016).

[21]Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.

The recommendations for the use of fluoride toothpastes in children from the updated policy guidelines established by the European Academy of Paediatric Dentistry (EAPD) in 2019 indicate a twice daily toothbrushing, with a concentration of fluoride at 1000 mg/kg from first tooth up to 2 years (recommended amount of 0.125 g) and 2–6 years (0.25 g) and with a concentration of fluoride at 1450 mg/kg from over 6 years (0.5–1.0 g) (Toumba et al., 2019). Other EU publications have reported representative samples of brands of toothpaste from retail markets with fluoride concentrations ranging from 800 to 1500 mg/kg (Nishikawara et al., 2006), 0–> 1350 mg/kg (Gupta et al., 2021), 500–1500 mg/kg (Borremans et al., 2008), 420–1399 mg/kg (Cochran et al., 2004a) and 500–1450 mg/kg (Djukic-Cosic et al., 2019).

2.2.3.2 | *Other fluoride dental health care products (gels, rinses, varnishes, tablets, lozenges, drops)*

Apart from the basic practice for caries prevention using recommended fluoridated toothpaste, other oral hygiene products such as fluoride gels (professional use; 5000–12,300 mg/kg) and rinses (50 mg/L on daily basis or 900 mg/L on weekly basis) are treatments included in the WHO list of essential dental medicines (WHO, 2023) and can be used especially in population groups, other than children < 6 years of age, assessed to be at increased risk of caries development (Toumba et al., 2019).

Professional applications of fluoride varnish (5% sodium fluoride) two to four times per year depending on caries risk is a WHO-recommended procedure for primary and secondary caries prevention. An advantage of the varnishes over other methods of professional fluoride application is that they are adhesive and hence should maximise fluoride contact with the tooth surface. Although the fluoride concentration is high (22,500 mg/kg), a small amount is needed. The nature of the varnish allows controlled and precise application to susceptible tooth surfaces. For young children (6 months-6 years old), around 0.25 mL is required while 0.5–1.0 mL is more than adequate for full adult permanent dentition (Adair, 2006).

Also, practices of using fluoride tablets/lozenges and drops for dental health care products that are regulated as drugs were first introduced before water fluoridation or effective fluoride toothpastes were widely available and recommended since the mid-1970s. It is noted that there is a general agreement within the EAPD that such practices of using fluoride tablets/lozenges and drops are only considered on medical prescription and on an individual basis for patients with a high risk of dental caries. The first recommendation addressed to these population groups or patients with higher risk of dental caries is to use a higher concentration of fluoridated toothpaste (more than 1500 mg/kg) (SCHER, 2011; Toumba et al., 2019).

A survey on the use of fluoride-containing tablets, with or without prescription, was carried out by EFSA in collaboration with EMA between April and May 2023. Responses were returned from 21 of the 29 EU/EEA (European Economic Area) countries that received the survey. Eight countries (Austria, Belgium, Czech Republic, Germany, Hungary, Iceland, Norway and Sweden) reported the use of fluoride-containing tablets. Recommendations for use of a daily dose and concentration of fluoride in tablets (0.25, 0.5, 0.75 or 1 mg) by children and adults differ between countries.

2.3 | Methodologies

2.3.1 | Hazard identification and characterisation

An adequate intake has been established for fluoride to account for its beneficial effects in preventing dental caries (EFSA NDA Panel, 2013). Hence, EFSA considers fluoride a nutrient, albeit not an essential nutrient. In the case of chemical substances that are regulated products and also nutrients, the Scientific Committee has proposed a harmonised approach for establishing a HBGV across different sectoral risk assessments (EFSA Scientific Committee, 2021). This approach is based on the concept of acceptable range of oral intake (AROI) defined in the 2002 report of the International Programme on Chemical Safety (IPCS) and World Health Organization (WHO), to represent the range of intakes of a nutrient at which a population has a minimal risk of nutrient deficiency and of toxicity. Since fluoride is not an essential nutrient, the concept of nutrient deficiency does not apply, however, inadequate intake is associated with increased risk of dental caries. The framework foresees approaches other than customary risk assessment to reduce the need for application of UFs which may result in HBGVs that cross into the range of nutritional needs (i.e. the upper tolerable intake level should not be lower than the adequate or recommended intake) (EFSA Scientific Committee et al., 2021; IPCS, 2002; SCF, 2002). The EFSA Statement on HBGV (2021) also proposes that exposure from all relevant sources should be assessed for nutrients that are also regulated products. This is in line with the terms of reference of the present mandate. The scientific principles and approach proposed in this Statement were taken into consideration within the scope of the current EC mandate with the aim of establishing health-based guidance values HBGVs protective of potential adverse effects of fluoride while maintaining its beneficial effects.

2.3.2 | Literature searches and screening

Literature search strategies were developed by information specialists at EFSA using PECO/PICO statements consistent with the inclusion and exclusion criteria described in the protocol (Annex B). The search strategy is further detailed in Appendix B of the protocol. Controlled vocabulary and free text search strings composed for each protocol sub-question are presented in Annex D of the opinion. All searches were conducted in two independent databases: PubMed and Web of

Science. After removal of duplicates, records collected were screened at title-abstract (T/A) and full-text (F/T) level by two screeners using the software Distiller SR®.

For evidence on the hazard properties of fluoride in humans and animals, a broad systematic literature search was performed in March 2022, covering literature published in English. Studies reporting on effects on adult and developmental neurotoxicity, effects on the thyroid system and effects on bone health were prioritised for systematic review according to the protocol. All studies were appraised for internal validity using customised Risk of Bias tool (see 2.3.4).

Three additional targeted searches were performed between October 2023 and January 2024 to identify any additional studies that may have been published on fluoride exposure and neurotoxicity (NT), bone health and thyroid. Specifically, the searches included (a) prospective human cohort studies on developmental neurotoxicity, for possibly inclusion in a meta-analysis; (b) specific terms for bone fractures and bone mineral density;[22] and (c) papers reporting effects on the thyroid specifically focused on fluoride exposure during pregnancy. In addition, searches for the prioritised endpoints of developmental neurotoxicity (DNT) and thyroid and bone health in both humans and animals were also extended to publication years before 2005 with no time limit, as foreseen in the protocol.[23] Studies identified were screened at title/abstract, at full/text level and selected papers were appraised for internal validity (RoB). However, findings reported in studies prior to 2005 were only considered as supporting evidence to the body of literature identified in the initial and targeted searches.

The initial search performed in March 2022 was repeated in January 2024 to capture new literature published since March 2022.

Studies that met the inclusion criteria were allocated to categories by reported endpoint and the intake at which adverse effects were reported by the authors was noted. Studies reporting adverse effects in humans at intake levels below 0.12 mg/kg bw per day, which is equivalent to the current UL for ages over 9 years (EFSA NDA Panel, 2005), were considered as most likely to have an impact on the HBGV and were prioritised for systematic review. Studies in animals reporting adverse effects below the equivalent dose (12 mg/kg bw per day; based on a default safety factor of 100) were also prioritised for systematic review (animal intake of fluoride from drinking water was extrapolated to dose in mg/kg bw per day using default extrapolation factors[24]) (EFSA Scientific Committee, 2012).

The search processes and strategies were documented and are reported in Annex D, including the date of the search, sources of information, search string for each bibliographic database and additional sources, and the number of records before and after de-duplication, screening and appraisal.

A systematic literature search strategy was performed as described in the protocol (for more details, see next section). Summary tables for the prioritised endpoints are provided in Appendices C and D. Summary tables for all other endpoints are provided as Annex E to the Opinion.

The results of the study selection process from all the literature searches performed are reported in the Tables 6 and 7. The list of studies excluded after full-text screening are documented, along with the reasons for excluding them, in DistillerSR.

EndNote® citation management software and DistillerSR® were used to manage the literature retrieved from bibliography databases and to support the screening process.

TABLE 6 Outcome of the human studies systematic literature search.

Identification			
Records identified	Web of Science - Core Collection ($n = 19,296$) PubMed ($n = 25,471$)		
Screening			
Studies included for T/A screening after duplicates removal	$n = 14,508$		
Studies included for F/T screening	$n = 1121$	Studies excluded at T/A level	$n = 13,387$ – 11,854 excluded according to protocol T/A inclusion/exclusion criteria – 1533 studies captured in the search 'Bone health before 2003'[20]

(Continues)

[22]The targeted search including specific terms for bone fractures and bone mineral density was run in October 2023 considering the time span 2003–2023 to identify any additional publication that may have been published during the drafting of the EFSA NDA Panel Opinion published in 2005.

[23]Studies on bone health published before 2003 were not further screened, since evidence reporting on this endpoint published before 2003 was thoroughly assessed by EFSA NDA Panel Opinion published in 2005.

[24]Default factors for conversion of water intake in 'mg/L' to dose in 'mg/kg bw per day': for rat, subacute 0.12, subchronic 0.09, chronic 0.05; for mouse, subacute 0.18, subchronic 0.15, chronic 0.09.

TABLE 6 (Continued)

Inclusion[25]			
Studies included at F/T level on prioritised endpoints	*n* = 138 Published after 2005 *n* = 133 Published before 2005 n = 5	Studies excluded at F/T level	*n* = 661 – 191 Reviews or meta-analysis – 78 Opinion papers (letters, editorials) – 141 Fluoride level (dose) or exposure not characterised – 141 Prevalence incidence only – 11 Non-oral route – 10 Lack of control group – 25 Underlying disease/condition – 134 No results on health effects – 2 Full text not in English – 230 Other[26]
Studies included at F/T level for data extraction and narrative description (other endpoints)	*n* = 319[27] – Skeletal fluorosis: *n* = 37 – Dental fluorosis: *n* = 164 – Developmental effects: *n* = 7 – Reproductive effects: *n* = 11 – Benefit: *n* = 89 – Other: *n* = 109		
Studies published after 2005 appraised for RoB and/or[28] included in the WoE	**Neurotoxicity and developmental neurotoxicity**: ***n* = 68** – Cohort studies: *n* = 15 – Cross-sectional studies: *n* = 53 **Effects on Thyroid**: ***n* = 28** – Cohort studies: *n* = 3 – Cross-sectional studies: *n* = 25 **Bone health**: ***n* = 37** – Bone fractures: *n* = 8 – Bone Mineral Density: *n* = 12 – Bone cancer: *n* = 9 – Osteoarthritis: *n* = 2 Other biomarkers of bone health: *n* = 12		
Studies published before 2005 appraised for RoB[29] but not included in the WoE	**Neurotoxicity and developmental neurotoxicity: *n* = 2** **Effects on Thyroid: *n* = 3**		

TABLE 7 Outcome of the animal studies systematic literature search.

Identification			
Records identified	Web of Science - Core Collection (*n* = 5447) PubMed (*n* = 4623)		
Screening			
Records included for T/A screening after duplicates removal	*n* = 4275		
Records included for F/T screening	*n* = 1056	Studies excluded at T/A level	*n* = 1336 Excluded according to protocol T/A inclusion/exclusion criteria

[25]The total number of studies included/excluded at F/T level for data extraction and narrative description or included at RoB appraisal/WoE level might not correspond to the algebraic sum of the studies included/excluded in each endpoint/study design/reason for exclusion sub-category. One study can report on multiple endpoints or can be excluded for multiple reasons.

[26]Abstract, 7; Animal study, 37; Case study, 4; Data analysis, 4; Diseased population, 2; Duplicate, 1; Ex vivo, 2; Image analysis, 2; In vitro study, 30; Non primary study, 6; Non toxicologically relevant endpoint, 29; Not on Fluoride, 4; No health effects, 17; Prior 2005 'other endpoints', 56; Protocol, 3; Retracted, 4; Risk Assessment Oopinion, 5; Route of administration not relevant, 4; Webpage, 2; Other, 3.

[27]Of these, only observational studies reporting on other endpoints (*n* = 240) were extracted (Annex H). Studies with a different study design (e.g. Intervention or Ecological) or reporting on benefit were not extracted.

[28]Not all studies on bone health were appraised for Risk of Bias (e.g. osteoarthritis, other biomarkers of bone health were not appraised).

[29]Studies identified through literature searches extended to publication years before 2005 with no time limit were appraised for RoB but not included in the WoE as they were considered not likely to change the outcome of the assessment.

TABLE 7 (Continued)

Inclusion[30]			
Records included at F/T level on prioritised endpoints	*n* = 98 Published after 2005 *n* = 78[31] Published before 2005 *n* = 20	Studies excluded at F/T level	*n* = 755 – 518 Single dose – 41 Review or meta-analysis – 14 Opinion paper – 6 F level (dose) or exposure not characterised – 5 Lack of control group – 10 Underlying disease/model – 46 No results on health effects – 211 Other[32]
Records included at F/T level for data extraction and narrative description (other endpoints)	*n* = 202 – Skeletal fluorosis: *n* = 19 – Dental fluorosis: *n* = 26 – Developmental effects: *n* = 31 – Reproductive toxicity: *n* = 47 – Oxidative stress: *n* = 51 – Benefit: *n* = 3 – Other: *n* = 155		
Records published after 2005 appraised and included in the WoE	**Neurotoxicity and developmental neurotoxicity**: ***n* = 39** – (D)NT behavioural endpoints: *n* = 29 – Endpoints at molecular, cellular, organ level: *n* = 38 **Thyroid effects**: ***n* = 5** **Bone health**: ***n* = 15**		
Studies published before 2005 appraised for RoB[33] but not included in the WoE	**Neurotoxicity and developmental neurotoxicity: 12** – (D)NT behavioural endpoints: *n* = 6 – Endpoints at molecular, cellular, organ level: *n* = 9 **Thyroid effects**: ***n* = 8**		

2.3.3 | Prioritisation of endpoints

A large volume of literature was identified through the broad systematic literature search conducted (Tables 6 and 7). Among the reported adverse effects, fluorosis remains a hallmark of well-established fluoride toxicity in bone in the context of high exposures to naturally occurring fluoride in water and food. The relationship between fluoride exposure in humans and evidence of dental and skeletal fluorosis has also been extensively characterised.

The current effort sought to determine whether there is evidence of more sensitive endpoints than dental and skeletal fluorosis that may warrant a reduction of the HBGV for fluoride. Selected endpoints include changes in bone homeostasis that may occur at intake levels below those associated with skeletal fluorosis. Neurotoxicity and developmental neurotoxicity (DNT) were selected as potential adverse effects of primary interest because of concerns raised in recent years, since the last EFSA risk assessment of fluoride and assessed by the National Toxicology Program (NTP). Lastly, the thyroid was selected as a potential target organ due to similarity between fluoride and iodide, suggesting potential competition and effects on the thyroid. It was also selected as an endpoint that, if affected by fluoride, may be related to the potential DNT effects of fluoride, considering that there is a well-established relationship between decreased thyroid function and adverse DNT effects (Appendix B). Figure 1 gives an overview of the life stages and population subgroups that are considered vulnerable to adverse outcomes in the prioritised health areas, as well as the time span relevant for the beneficial effects of fluoride.

There were also a considerable number of additional studies reporting other effects which contribute to the overall body of evidence but are considered unlikely to change the existing ULs. The number of remaining effects spanning all systems and domains constituted a large total volume of literature. This exceeded 180 human and 150 animal studies that reported various endpoints thus operational prioritisation was required (see Tables 6 and 7 and Appendix E). Other endpoints were not considered relevant in terms of challenging the existing ULs: endpoints less sensitive compared to fluorosis (e.g. kidney

[30]The total number of studies included/excluded at F/T level for data extraction and narrative description or included at RoB appraisal/WoE level might not correspond to the algebraic sum of the studies included/excluded in each endpoint/study design/reason for exclusion sub-category. One study can report on multiple endpoints or can be excluded for multiple reasons.

[31]During the RoB appraisal, 20 studies reporting on (D)NT behavioural endpoints were excluded from further consideration due to deficiencies in several bias domains (see Annex E, Table E.1.3). Three of these studies also reported on other endpoints and were included in the assessment.

[32]Abstract, 15; Correction note, 1; Disease model, 1; Duplicate, 2; Ex vivo, 15; Exposure not characterised, 2; In vitro, 53; Mechanistic, 51; Mixture effect, 2; No results on health effects, 7; No toxicologically relevant endpoint, 7; Not on Fluoride, 7; Other, 10; Prior, 2005; Other endpoint, 10; Retracted, 2; Review, 6; Route of exposure not relevant, 13; Sub-acute study, 3; Test species not relevant, 1.

[33]Studies identified through literature searches extended to publication years before 2005 with no time limit were appraised for RoB but not included in the WoE as they were considered not likely to change the outcome of the assessment.

effects); endpoints where the literature is limited and lacks reproducibility; endpoints which represent biochemical effects rather than adverse effects (e.g. changes in oxidative markers) that are not clearly linked to the adverse outcome.

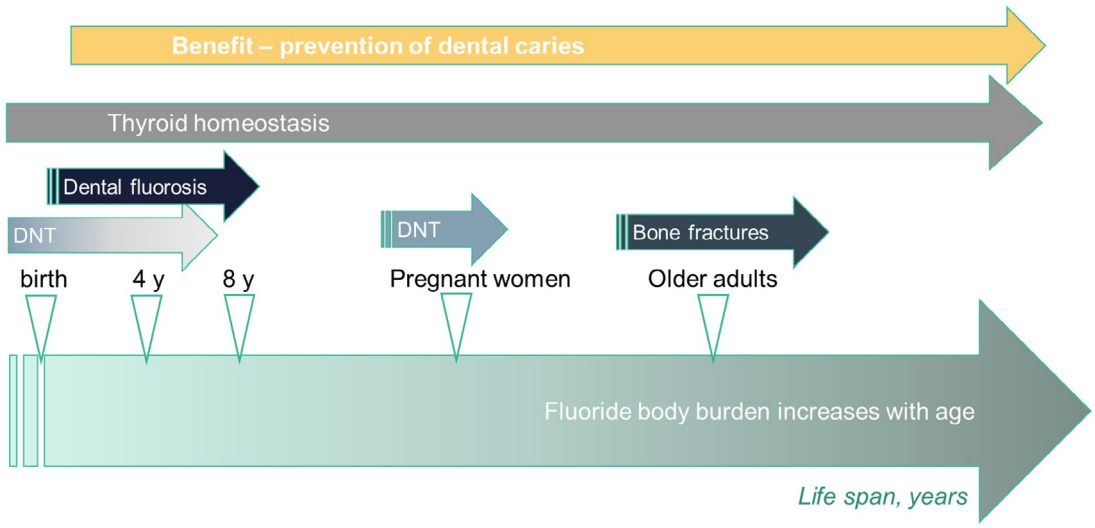

FIGURE 1 Life stages and subpopulation groups relevant to the prioritised endpoints of potential adverse effects of fluoride and to its beneficial effects.

2.3.4 | Literature appraisal for risk of bias (internal validity)

The Scientific Committee considered that it was important to assess the quality and internal validity of the studies reporting on the endpoints that may impact the existing ULs for fluoride. The internal validity of studies on neurotoxicity and DNT, thyroid and bone effects (bone mineral density and fractures) in humans[34] and experimental animals was therefore appraised using the NTP-OHAT risk of bias (RoB) tool (OHAT/NTP, 2019). Literature reporting on other toxicological endpoints and fluoride kinetics was evaluated narratively. Specific RoB questions and instructions were customised for each study design and for the context and scope of this assessment. An overall RoB judgement was attributed to each study according to pre-defined criteria (OHAT/NTP, 2019) with studies assessed to have low, moderate and high RoB allocated to Tier 1, 2 and 3, respectively. The criteria for study appraisal and rating instructions for the definition of the three tiers are presented in Appendix E.

For both animal and human studies exposure characterisation and the reliability of the endpoints assessed were among the key questions for RoB assessment. In animal studies, particular scrutiny was applied to the appraisal of neurobehavioural endpoints due to methodological requirements that can have large impact on the outcome. RoB criteria for neurobehavioural endpoints and histopathology were more stringent than the appraisal criteria for biochemical analyses, as subjective investigator interpretation has less impact on the outcome of the latter assays.

For the human studies, the RoB assessment included general criteria for assessing confounder control for the observational studies. A thorough assessment of the analytical methods used for quantifying fluoride exposure across studies was used to assess the reliability of exposure. This assessment was based on the review of analytical methods that was performed prior to conducting the RoB assessment (see Section 1.4.4).

2.3.5 | Data extraction

Data from studies reporting on prioritised endpoints were extracted in structured forms and discussed in detail in the text (systematic review). Data from eligible studies reporting on other endpoints were summarised briefly in the text (narrative review). All studies for the prioritised endpoints were also summarised in standardised evidence tables provided in Appendices C.1–C.8 for human studies and D.1–D.6 for animal studies. Standardised evidence tables for studies on all other endpoints are provided in Annex E.

2.3.6 | Hazard assessment

A weight of evidence approach was applied to assemble, weigh and integrate the evidence according to EFSA Scientific Committee guidance document (EFSA Scientific Committee, 2017a, 2017b, 2017c). Relevance of all studies was assessed

[34]Intervention studies and observational studies (prospective cohorts, case–control and cross-sectional studies).

during the literature screening process. Reliability was assessed through the structured appraisal for risk of bias, described above. The weight of evidence process also included assessment of the consistency of the evidence, including the biological relevance of the reported effect (EFSA Scientific Committee, 2017a, 2017b, 2017c), human relevance of animal studies and the generalisability of results obtained in a specific human population (EFSA Scientific Committee, 2022).

2.3.7 | Dietary exposure assessments

Dietary exposure to fluoride was assessed through four scenarios based on assumed fluoride concentration in water as described in Section 2.2.2.3.

Additionally, an estimate of the exposure to fluoride from the consumption of discretionary fluoridated salt based on literature data (see Section 2.2.2.2) was taken into consideration in the aggregated oral exposure to fluoride from all major identified sources (see Section 3.9.3).

To calculate the chronic dietary exposure to fluoride in each scenario, food consumption and body weight data at the individual level were obtained from the Comprehensive Database. The mean daily consumption at the individual level was combined with the mean concentration of fluoride, at the most detailed level of the FoodEx2 classification system available to calculate individual average daily exposures.

For consumption events concerning drinking water the LB, MB and UB concentrations derived from the EFSA occurrence database were used as indicated in Table 3.

For consumption events concerning food the same value was used for the LB, MB and UB scenarios as no information was available on the treatment of left censorship in the composition database. On the basis of distributions of individual average daily exposures, the LB, MB and UB mean and 95th percentile exposures were then calculated per survey and per age group.

The contribution of food categories at level 1 of the FoodEx2 classification and selected food categories at other FoodEx2 levels to the dietary exposure to fluoride, for each survey and age group, was calculated using the relevant LB mean exposure. Categories that contributed more than 10% to the exposure in the highest number of surveys per age group were considered a main contributor for that group.

As there is neither a specific FoodEx2 code available to report occurrence nor consumption data on ice-tea and while this was considered an important food source to be assessed in detail, the original food description of each consumed food was searched for the keyword 'ice-tea' and 'iced-tea' and linked to the concentration for ice-tea (value 1.1 mg/L) retrieved from Rodriguez et al. (2018). In addition, the eating events identified as referring to ice-tea were reclassified to tea beverages if they were reported under other food categories (e.g. soft drinks).

Dietary exposure related to the consumption of discretionary fluoridated salt was estimated separately using consumption estimates obtained from the literature and fluoride concentration in fluoridated salt from the literature.

2.3.8 | Non-dietary exposure assessment

2.3.8.1 | *Exposure assessment to fluoride from the use of toothpaste*

For toothpaste, the amount of fluoride ingested was calculated using four variables: the fluoride concentration of toothpaste, the amount of toothpaste used, the daily frequency of tooth brushing and the amount ingested after brushing and rinsing the teeth. Values used for these variables were extracted from literature and calculations are described in Section 3.9.3.

2.3.9 | Aggregated oral exposure to fluoride from all major sources

Water, food, discretionary salt and oral hygiene products (mainly toothpaste) were considered as the major regular daily exposure food and non-food sources of fluoride ion; medicinal prescription (e.g. gel, varnishes, rinses, tablets, lozenges) or supplementation (food supplement) were therefore not considered in this assessment.

To allow aggregated oral exposure estimates of fluoride from all major identified sources for the general healthy population, daily exposure estimates derived from the use of toothpaste were added to those estimated from the food and water consumption for the same survey and population groups expressed as a range (min-max) for the average and high levels (P95 consumers) at the MB scenario.

The approach used for estimating high percentiles of aggregated oral exposure from all major identified sources is based on the assumption that an individual might be a high-level consumer of one source only and would be an average consumer of the remaining sources. This method simply consists of adding at the country and survey level the highest high level of exposure from one source to the mean exposure values for the remaining sources. This approach was used by the Scientific Committee for its scientific opinion on carvones (EFSA, 2014) and was also used by the EFSA ANS Panel for the Food Additive Intake Model (FAIM) (EFSA, 2014).

3 | ASSESSMENT

3.1 | Fluoride kinetics

In this section a brief narrative description of the evidence on fluoride kinetics identified in the literature (with no time limits) is presented.

3.1.1 | Absorption

In rats, 80%–90% of ingested fluoride is absorbed from the gastrointestinal tract by passive diffusion; 20%–25% of total fluoride is absorbed from the stomach and the remainder from the proximal small intestine (Nopakun et al., 1989; Whitford, 1996). High concentrations of fluoride in the intestinal lumen promote its absorption (Rigalli et al., 2001). Gastric absorption of fluoride is determined by gastric acidity and content as well as the pattern of gastric emptying. In acidic pH, ionic fluoride is converted into non-ionic hydrogen fluoride (HF) which crosses biological membranes by diffusion, including gastric mucosa (Gutknecht & Walter, 1981), hence gastric absorption is inversely related to pH - the higher the gastric acidity (lower pH), the higher the fluoride absorption (Whitford & Pashley, 1984). The pKa of HF is approximately 3.4; thus, fluoride crosses cell membranes as HF in response to a pH gradient between adjacent body fluid compartments (Buzalaf & Whitford, 2011; Whitford, 1996).

In humans, fluoride is rapidly absorbed, with peak plasma fluoride concentrations reached after 20–60 min (Buzalaf et al., 2008; Whitford, 1996). Foods or beverages containing calcium, aluminium or magnesium can affect fluoride absorption from the diet. Fluoride salts soluble in water (sodium fluoride, sodium silicofluoride, fluorosilicic acid and sodium monofluorophosphate) are rapidly and almost completely absorbed. However, absorption can be reduced by the formation of insoluble complexes or precipitates with food components (EFSA NDA Panel, 2013). Simultaneous consumption of milk reduces the bioavailability of fluoride by 30% (Trautner & Einwag, 1989; Trautner & Siebert, 1986) and a mixed diet may reduce the absorption of fluoride from the diet by 47% (Shulman & Vallejo, 1990). Whether fluoride is naturally occurring or added and whether the water is hard or soft, does not affect fluoride absorption (Maguire et al., 2005; Whitford et al., 2008).

3.1.2 | Distribution

In animals, the short-term distribution kinetics of ^{18}F, with and without added carrier, has been studied in 12 soft tissues and femur following intravenous administration in rats. The highest tissue to plasma ratio of fluoride concentrations is found in femur (7.52), followed by kidney (4.16), liver (0.98), lung (0.83), spleen (0.70) and other tissues (Whitford et al., 1979). Fluoride concentration in the thyroid was not measured. The low value for brain-to-plasma concentration ratio (0.084) reported in this study may indicate that the blood–brain barrier in adult animals is relatively impermeable to fluoride (Whitford et al., 1990, 1979). For specific brain concentration measurements, Whitford et al. (2009) exposed post-weaning female rats (Sprague–Dawley, 1 week after weaning) to fluoride for 8 months through drinking water (0, 10, 25 and 50 mg/L, eight rats per group). Plasma and brain concentrations increased significantly with increasing fluoride dose. However, the brain-to-plasma ratio remained unchanged except at the highest dose (50 mg/L) where a significant decrease was observed, from 0.26 in control animals to 0.19 in treated animals. The short-term pharmacokinetics of fluoride were also investigated in dog, cat, rabbit, rat and hamster. The authors concluded that the pharmacokinetic parameters of fluoride in dogs are most similar to those in humans (Whitford et al., 1989). In dogs, during the first year of life, the fractional uptake of fluoride by bone gradually decreased to 50% at 1 year of age, with little changes thereafter (Ekstrand & Whitford, 1984; Whitford et al., 1990).

In humans, approximately 99% of the body burden of fluoride is found in the bone (Rao et al., 1995; Whitford, 1994). Fluoride can substitute hydroxyl ions in hydroxyapatite in bone and teeth (Grynpas, 1990). Fluoride in bone is not irreversibly bound; it can be mobilised over time by bone growth in the young, bone resorption and bone remodelling in the adult (Rao et al., 1995). Thus, even after a decrease in fluoride exposure, stored fluoride within bone tissue will be distributed slowly through the body. This was observed following defluoridation of a community water supply from 8 mg/L to 1 mg/L fluoride (Likins et al., 1956). In that study, urinary fluoride excretion decreased but remained higher than expected, indicating the release of fluoride stored in bones. The study also showed that the half-life of fluoride in bone for adults was 120 weeks, whereas it was 70 weeks for children.

Plasma fluoride is found in both inorganic and organic forms. Inorganic fluoride may be complexed with metals, whereas organic forms include fluoride adsorbed on (but not covalently bound to) plasma macromolecules (Ekstrand, 1996; Ekstrand et al., 1977; Whitford, 1996). The blood to plasma ratio has been estimated to be 1.3 (Rao et al., 1995).

The rate of distribution of fluoride from plasma to soft tissue is determined by the blood flow rate to different tissues and organs (Buzalaf et al., 2015). In a study with fluorosis patients, the mean cerebrospinal fluid (CSF) fluoride concentration was slightly lower in control individuals ($n = 32$, 0.17 ± 0.03 mg/L) compared to the fluorosis patients ($n = 40$, 0.20 ± 0.062 ppm) but not significantly different (Hu & Wu, 1988). The presence of fluoride in the brain of control individuals suggested that blood fluoride was in equilibrium with CSF also in non-fluorosis individuals. In another report (Singer et al., 1967), fluoride was detected in the CSF of 29 hospital patients without any information on their health status or on fluoride exposure.

The mean plasma to CSF ratio was 2.81 ± 0.3. Detection of fluoride in CSF suggests that fluoride crosses into the brain but there are currently no data to suggest that it accumulates in the brain. There are also no data concerning the mechanisms involved in the distribution of fluoride in the brain and how it crosses the blood–brain barrier (Żwierełło et al., 2023).

Fluoride circulating in maternal blood crosses the placenta and reaches the fetus (Castiblanco-Rubio & Martinez-Mier, 2022). The mean fluoride serum levels were 0.030 μg/mL (SD 0.015) in mothers, 0.018 μg/mL (SD 0.012) in cord blood and 0.038 μg/mL (SD 0.016) in neonates. The significantly lower fluoride levels in mixed cord serum, compared to maternal and neonatal serum, suggest placental sequestration of fluoride, indicating that cord serum fluoride may not accurately reflect fetal fluoride status (Shimonovitz et al., 1995). Amniotic fluid fluoride concentrations of pregnant women were studied in 47 different communities in Northern California and one in Montana, US. The amniotic fluoride concentrations were significantly higher in pregnant women living in communities adhering to the U.S. recommended water fluoride concentration (0.7 mg/L) compared with communities with less than 0.7 mg/L fluoride in drinking water (Abduweli Uyghurturk et al., 2020).

He et al. (2008) examined 16 fetuses aborted at 6–8 months, from Chinese mothers with dental fluorosis (DF), most of whom (87%) also had clinical skeletal fluorosis (SF) and 10 fetuses from healthy mothers living in an area with low concentrations of fluoride in water and food. The average concentration of fluoride was highest in bone tissue and lowest in brain tissue. Only in brain and femur (cells) was the fluoride concentration (significantly) higher in fetuses from endemic compared to control areas (brain cells: 31.7 vs. 23.2 ppm and femur cells: 129.8 vs. 60.5 ppm). Concentrations of fluoride in thymus, heart, liver, lungs, kidney, muscle, placenta, cartilage were not different between the groups.

Studies have shown that fluoride transfers into human milk. Mothers ($n = 125$; drinking water fluoride concentration was 0.3 mg/L) had a mean plasma fluoride concentration of 17 μg/L and a mean breast milk fluoride concentration of 6 μg/L, thus the exposure via breast milk would represent on average 2% of the exposure via drinking water (Sener et al., 2007). Fluoride concentration in breast milk was correlated with drinking water fluoride concentration and was found to be 2.06 and 2.32 μg/L in areas in Iran with water fluoride concentrations of 0.3–0.5 and 0.6–0.8 mg/L respectively (Faraji et al., 2014).

No data on fluoride concentrations in the thyroid of animals or humans was identified in papers retrieved through the systemic literature search.

3.1.3 | Elimination and excretion

Fluoride is eliminated from plasma in a biphasic manner by bone uptake and by renal clearance (Buzalaf et al., 2008; Whitford, 1996). Fluoride is also secreted in the saliva (Tóth et al., 2005). While approximately 36% and 55% of the fluoride dose is deposited in calcified tissues in healthy adults and children, respectively, about 60% in adults and 45% in children of ingested fluoride is excreted through the kidneys (Villa et al., 2010) and approximately 10%–20% is excreted via the faeces (EFSA NDA Panel, 2013). The renal clearance in adults varies between 35 and 45 mL/min, due to inter- and intra-individual variability of functional renal mass, glomerular filtration rate, urinary pH and flow rate (Whitford et al., 2008). In the weanling dog pup, renal elimination of fluoride was only 10% of the dose, whereas in the mature dog this fraction increased to about 50% (Ekstrand & Whitford, 1984; Whitford et al., 1990).

Overall, plasma and renal clearance of fluoride varies in humans depending on age. In adults, a plasma clearance mean value of 2.7 ± 0.4 mL/min per kg bw has been reported and ranged between 2.6 and 3.8 mL/min per kg bw, while renal clearance varied around 0.9 ± 0.4 mL/min per kg bw (mean ± standard error of the mean (SEM)). Plasma and renal clearances (mean ± SEM) were lower in elderly, with 2.1 ± 1.3 mL/min per kg bw (range: 1.7–5.1 mL/min per kg bw) and 0.6 ± 0.3 mL/min per kg bw respectively. Finally, in young infants (age range: 37–410 days and mean 192 days) plasma and renal clearances were higher, with values (mean ± SD) 6.8 ± 2.0 mL/min per kg bw and 1.1 ± 0.3 mL/min per kg bw, respectively (Ekstrand et al., 1994; Jeandel et al., 1992).

It has been suggested that the higher percentage of fluoride retention in bone in children compared to adults is probably due to a greater deposition of fluoride in growing bone in children, resulting from the larger surface area of loosely organised crystallites in the developing calcified tissues during growth (Whitford, 1999).

An overview of the available information on fluoride kinetics is shown in Figure 2.

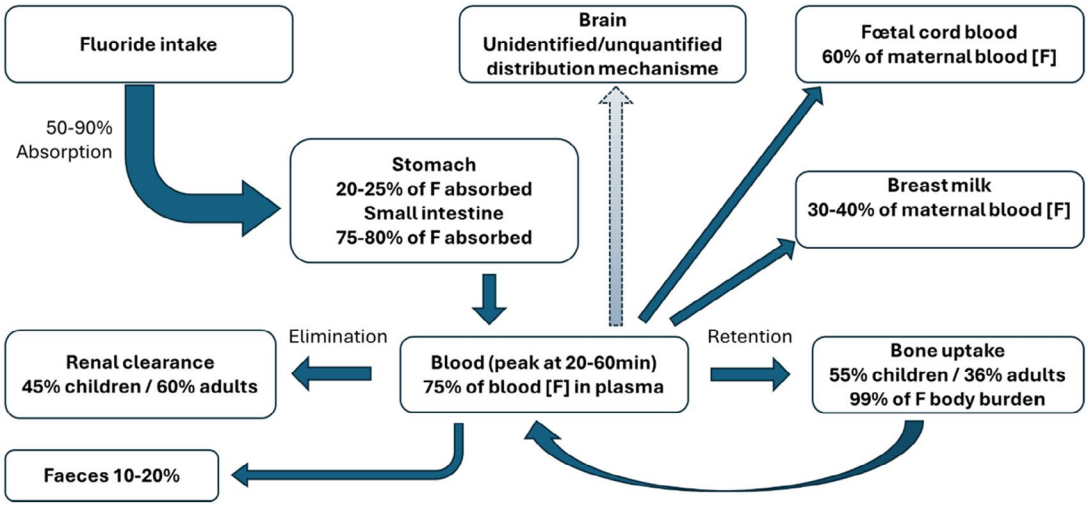

FIGURE 2 Overview of fluoride kinetic evidence in humans.

3.1.4 | Physiologically based kinetic models

A few physiologically based kinetic (PBK) models have been developed for fluoride. Rao et al. (1995) developed a PBK model for simulating the kinetics of chronic exposure to fluoride in both rats and humans. The model includes compartments representing plasma, kidney (including renal clearance), bone (as bone surface) and bone bulk (including bone clearance and bone return), liver, rapidly and slowly perfused tissues and lung. The gastrointestinal tract is a single compartment with zero-order absorption rate constants for drinking water and dietary fluoride. This model was validated using three independent datasets, namely the NTP (1990) drinking water rat study, the Maurer et al. (1990) dietary rat study and the Boivin et al. (1988) study on bone fluoride concentrations in humans receiving oral fluoride therapy. There are remaining uncertainties due to the absence of age specific kinetic data for young and elderly persons.

Jean et al. (2018) modified the previous PBK model (Rao et al., 1995) to model fluoride urinary excretion in infants. The same compartments were modelled, except that the gastrointestinal tract had been replaced with an infusion model into the liver and the bone model had been simplified in a perfusion limited compartment where blood flow rate was the limiting process. The modification of the model was intended to simulate fluoride kinetics in children in whom the balance between bone formation and resorption is in favour of formation, hence mobilisation of bone fluoride and its release in bloodstream was modelled with a single bone compartment instead of having a distinction between bone surface and bone bulk. An uncertainty of the model by Jean et al. (2018) is the application of an empirical factor of 0.43 to adjust the overestimated urinary fluoride excretion.

The PBK model proposed by Rao et al. (1995) was validated exclusively against bone fluoride concentrations, while the model by Jean et al. (2018) predicted urinary fluoride concentrations for children but faced limitations due to the use of an empirical factor that cannot be extrapolated to adults, including the elderly. Consequently, the mass balance approach emerges as a more robust method for modelling urinary fluoride concentrations following chronic exposure across different age groups. This approach incorporates the relevant urinary excretion fraction of fluoride (Villa et al., 2010) and accounts for daily urinary flow rates (Hays et al., 2018).

3.2 | Hazard assessment based on human studies

In this section the evidence of associations between fluoride exposure and health outcomes reported for the prioritised endpoints, including thyroid, DNT and bone health in humans is presented. An overview of the relationship between biomarkers of fluoride exposure with exposure based on drinking water concentrations reported in these studies is presented first to aid the interpretation of the findings. A summary of the evidence for other endpoints identified in the literature is also presented.

3.2.1 | Biomarkers of fluoride exposure

Biomarkers of fluoride exposure are critical for the interpretation of the exposure-health relationships reported in human observational studies. The relationship between fluoride intake and urine levels was previously assessed in the 2013 NDA Opinion (EFSA NDA Panel, 2013). This assessment was partly based on analyses of individual participants on total fluoride intake and fluoride concentrations in 24-h urine from several studies performed by Villa et al. (2010). Based on the data a linear relationship between total daily fluoride intake and fluoride concentrations in 24-h urine was

observed in both children ($n = 212$) and adults ($n = 283$). The daily fluoride intake in these studies ranged from 0.1 to 5.0 mg fluoride per day.

None of the human epidemiological studies identified for this assessment quantified total daily fluoride intake or collected 24-h urine. The primary measures of exposures used in those studies were water fluoride concentrations (wF), fluoride concentrations in spot urine (uF) or serum (sF), with many studies using more than one of these measures. To assess the relationship between these exposure measures, studies reporting associations between fluoride exposure with DNT or thyroid outcomes with at least two measures of fluoride exposure (i.e. wF, uF or sF) were examined. Extracted data from these studies are shown in Annex D. Except for the prospective studies on DNT, the spot uF concentrations were most often reported not adjusted for creatinine or specific gravity (wet weight).

A total of 39 papers from cross-sectional studies provided information on group mean values of wF, uF (morning or spot urine) or sF. These studies are summarised in Annex D.1. Most of these studies were conducted in children and adults form China and India and in areas where fluoride in drinking water was elevated. Studies from Turkey, Pakistan, Canada and Mexico were also included. The sample sizes in these studies ranged from 10 to 1636. In all cases the analytical measures used in these studies were considered appropriate (according to criteria described in Section 1.4.4). One paper was excluded (Ahmed et al., 2022) as the wF concentration was reported being ~70 mg/L, which is far above the exposure of interest for this assessment. The mean wF concentrations included in the analyses across groups ranged from 0.2 to 8.3 mg/L. Information on uF adjusted for creatinine or specific gravity was extracted from four prospective studies on neurodevelopment (8 datapoints) with mean wF concentrations between 0.1 and 9.4 mg/L.

Table 8 shows the Pearson correlations (r) between group mean wet weight concentrations[35] of fluoride in water versus urine (wF-uF), water versus serum (wF-sF) and urine versus serum (uF-sF) for all studies and stratified by wF concentrations. Category boundaries for fluoride concentration in drinking water between 0.7 and 1.0 mg/L were chosen because those concentrations are frequently used for caries prevention (EPA, CDC, (Dar & Kurella, 2024). The cutoff of 1.5 mg/L was chosen because that is the maximum concentration of fluoride added to drinking water allowed in the EU. To put these values in perspective, in EU countries fluoride levels in tap water vary between 0.01 and 5.8 mg/L in Ireland, 0.1–3.0 mg/L in Finland and 0.1–1.1 mg/L in Germany.[36]

TABLE 8 Pearson correlations (r) with 95% confidence interval (95%CI) between mean concentrations of fluoride in water (wF), urine (uF) and serum (sF), in mg/L.

	All studies[1]		wF ≤ 0.7		wF ≤ 1.0		wF ≤ 1.5		wF > 1.5 mg/L	
	n	***r* (95% CI)**	***n***	***r* (95% CI)**	***n***	***r* (95% CI)**	***n***	***r* (95% CI)**	***n***	***r* (95% CI)**
wF – uF	74	**0.79** (0.69, 0.87)	29	**0.00** (−0.37, 0.36)	37	**0.19** (−0.15, 0.48)	44	**0.36** (0.07, 0.59)	30	**0.63** (0.34, 0.81)
wF – sF	35	**0.62** (0.36, 0.79)	15	−**0.24** (−0.67, 0.31)	20	**0.22** (−0.25, 0.61)	23	**0.05** (−0.37, 0.45)	12	**0.71** (0.23, 0.91)
uF – sF	20	**0.81** (0.58, 0.92)	8	**0.66** (−0.09, 0.93)	10	**0.63** (0.00, 0.90	12	**0.84** (0.50, 0.95)	8	**0.71** (0.0, 0.94)

Abbreviations: sF, serum fluoride; uF, urinary fluoride; wF, water fluoride.

[1]A total 27 studies providing information on 74 different data points (or mean group values referred to as '*n*') were included for these analyses. These studies reported associations between either wF, uF or sF exposure with wither developmental neurotoxicity or thyroid hormones and can be found in Annex D.1.

As shown in Table 8 and graphically in Figure 3, a strong correlation was observed between mean concentrations of fluoride in drinking water and spot urine reported across studies (Pearson's $r = 0.79$, $n = 74$). The correlation between mean concentrations of fluoride in urine and in serum was also similarly strong ($r = 0.81$, $n = 20$), while the correlation between fluoride in water and serum was slightly weaker ($r = 0.62$, $n = 35$). When estimating the correlation at mean fluoride concentrations in water ≤ 1.5 mg/L, the correlation between fluoride in water and urine was still present but weaker ($r = 0.36$, $n = 44$). At lower water fluoride concentrations, the correlations between wF and uF was weaker ($r < 0.20$) and statistically non-significant. A similar pattern was observed for the correlation between wF and sF while the correlation between the two internal biomarkers of exposure, uF and sF, remained high ($r > 0.60$) and was largely independent of the wF concentration. In comparison, the correlation between fluoride in water and urine for the 8 group means that had been adjusted for creatinine or specific gravity was $r = 0.91$ (results not shown in Table 8).

[35]No adjustment for urinary creatinine or specific gravity.

[36]https://ec.europa.eu/health/scientific_committees/opinions_layman/fluoridation/en/l-2/1.htm.

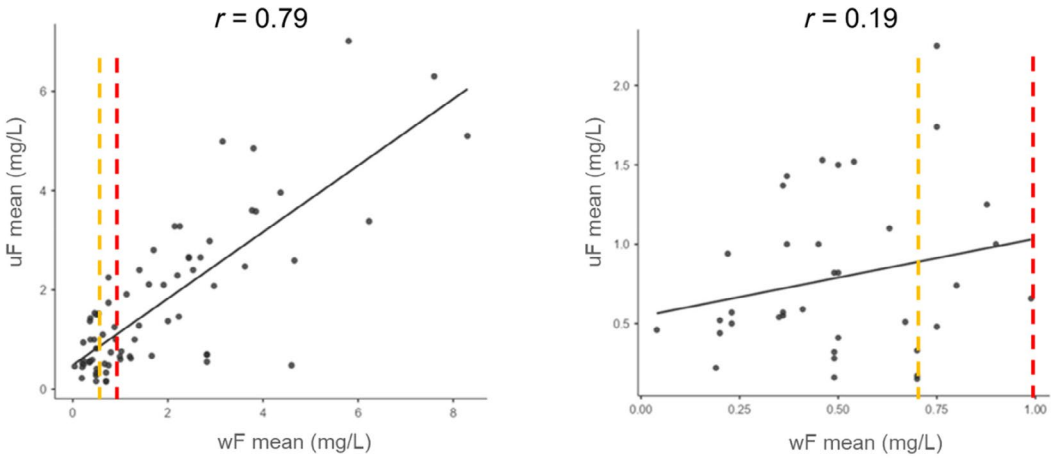

FIGURE 3 Scatter plot showing the correlation between fluoride concentrations in drinking water (wF, *x*-axis) and mean fluoride concentrations in spot urine (uF, *y*-axis) as reported in epidemiological studies reporting results on DNT or thyroid function (see Annex D). The scatter plot is shown for all studies (left panel) and studies with mean fluoride ≤ 1.0 mg/L (right panel). The red and orange dashed lines show the typical water fluoridation levels of 0.7 and 1.0 mg/L, respectively.

In some studies included in these analyses, concentrations in water were reported as ranges and the midpoint was then used. This may have led to lower precision for the estimated correlation coefficients. Furthermore, water fluoride concentrations may not accurately reflect actual exposure as participants in some studies may have used bottled water or received drinking water (partly or fully) from other sources. A more comprehensive assessment of actual drinking water and beverage consumption would therefore be expected to give higher precision. Such an assessment was performed in only one study from Canada including 512 pregnant women from areas with fluoridated (mean 0.59 mg/L) and non-fluoridated (mean 0.13 mg/L) drinking water (Green et al., 2019). Fluoride intake from drinking water was quantified by combining questions on tap and bottled water with concentration data. In the study, the correlation between individual fluoride intake and fluoride in urine (adjusted for specific gravity) was moderate and statistically significant ($r = 0.49$, $p < 0.001$). Furthermore, in the same study, despite the low concentration of fluoridated water, the mean urine concentrations of women exposed to fluoridated versus non-fluoridated drinking water was also significantly different (0.69 vs. 0.40 mg/L, $p < 0.001$).

In summary, living in an area with an elevated concentration of fluoride in drinking water results in higher spot urinary fluoride concentration. When comparing group means across studies when water concentrations are low (≤ 1.5 mg/L), the relationship between fluoride concentrations in water and spot urine becomes weaker or absent, most likely due to a relatively higher contribution of fluoride intake from foods and dental care products.

3.2.2 | Neurotoxicity and developmental neurotoxicity in humans

The body of evidence on neurotoxicity and developmental neurotoxicity in humans consists of several prospective cohort studies (15 publications) and a larger number of cross-sectional studies (53 publications). Many of the cross-sectional studies were conducted in populations exposed to relatively high levels of fluoride in drinking water compared to populations in Europe, while the prospective studies were conducted in populations with more comparable exposures. These differences need to be considered when interpreting results between these two streams of evidence.

3.2.2.1 | *Prospective studies*

The 15 publications identified described results from 7 different pregnancy cohorts examining neurobehavioural outcomes in children and one cohort examining neurotoxicity in adults. The majority of the studies ($n = 9$) were evaluated as low risk of bias (Tier 1) and six studies were evaluated as moderate risk of bias (Tier 2) (see Table 9). The neurodevelopment studies in children were birth cohorts in populations from Canada, Denmark, Mexico, New Zealand and Spain with a sample size ranging from 65 to 922 participants. Neurodevelopmental outcomes were assessed at offspring age ranging from 3 months to 38 years. The main characteristics for each publication, including measures of exposure, outcome and main results are summarised in Table D.2 in Annex D.

TABLE 9 Heat map for the risk of bias performed for human cohort studies on neurodevelopmental endpoints.[a]

Refid	Author	Year	Study design	TIER	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
216	Farmus, L.	2021	Cohort	1	+	+	–	++	+	+	+	+
10338	Goodman, C. V.	2022	Cohort	1	+	+	–	+	+	+	+	+
10239	Grandjean, P.	2023	Cohort	1	+	+	–	+	+	+	+	+
2376	Green, R.	2019	Cohort	1	+	+	–	++	+	+	+	+
10352	Ibarluzea, J.	2023	Cohort	1	+	+	–	+	+	+	+	+
722	Ibarluzea, J.	2022	Cohort	1	+	+	–	+	+	+	+	+
1664	Jimenez, L. V.	2017	Cohort	1	+	+	+	++	+	+	+	+
11982	Krzeczkowski	2023	Cohort	1	+	+	–	++	+	+	+	+
5224	Till, C.	2020	Cohort	1	+	+	–	+	+	+	+	+
723	Bashash, M.	2018	Cohort	2	–	+	–	+	+	+	+	+
724	Bashash, M.	2017	Cohort	2	–	+	–	+	+	+	+	+
184	Broadbent, J.	2015	Cohort	2	++	+	+	–	+	+	+	+
259	Cantoral, A.	2021	Cohort	2	+	+	–	–	+	+	+	+
3853	Goodman, C. V.	2022	Cohort	2	–	+	–	+	++	+	+	+
2044	Russ, T. C.	2020	Cohort	2	++	–	+	+	+	+	+	+

[a]The heatmap indicates whether the criteria stated in questions 1–8 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

3.2.2.1.1 | *Neurodevelopmental outcomes assessed in infancy and early childhood (≤ 2 years)*

A study from Mexico ($n = 65$) examined the association between maternal urinary fluoride concentration in pregnancy and offspring neurodevelopment evaluated through the Bayley Scale of Infant Development II between the age of 3 to 15 months (Jimenez et al., 2017). The mean (± standard error) fluoride concentrations in maternal urine were ~ 2.0 ± 1.0 mg/L in the 1st ($n = 65$) and 2nd ($n = 46$) trimester. The range of fluoride measured in drinking water was between 0.01 and 10.8 mg/L (mean ~ 3 mg/L). After adjustment for covariates each 1 mg/L increase in maternal urinary fluoride concentrations was associated with lower developmental scores ($\beta = -19$, $p < 0.05$ for both 1st and 2nd trimester samples).

Another study from Mexico City ($n = 103$) assessed the intake of dietary fluoride in the second and third trimester of pregnancy via food frequency questionnaire (Cantoral et al., 2021). Offspring neurodevelopment using the Spanish version of the Bayley Scales of Infant Development (Bayley-III) was assessed at 12 and 24 months. Median (25th, 75th percentile) maternal fluoride intake was 1.01 (0.73, 1.32) mg/day. Modest, but not statistically significant, inverse associations with offspring neurodevelopment endpoints were observed. The limitation of this study is the use of a food frequency questionnaire to quantify fluoride exposure without any validation against objective biomarkers. No information was provided on fluoride concentrations in drinking water.

3.2.2.1.2 | *Neurodevelopmental outcomes assessed in children aged > 2 years*

Several publications were identified from the *Maternal–Infant Research on Environmental Chemicals* (MIREC) birth cohort from Canada (Farmus et al., 2021; Green et al., 2019; Till et al., 2020). For the full cohort, a total of 2001 women in early pregnancy were recruited from several cities across Canada in 2008–2011. Neurodevelopmental outcomes were assessed when the children were 3–4 years of age in a sub-set of cohort participants ($n = \sim 600$) using the *3rd edition of the Wechsler Preschool and Primary Scale of Intelligence*. Maternal and offspring exposure to fluoride from drinking water was derived using information on fluoride levels in tap water by linking the postal code of each participant to their local water-treatment plant (Green et al., 2019; Till et al., 2020). Concentrations of fluoride were measured in maternal urine in samples collected in each trimester (Green et al., 2019) and in offspring urine collected at ages 1.9–4.4 years (Farmus et al., 2021).

In the MIREC studies, Green et al. (2019) examined the association between maternal exposure to fluoride from tap water ($n = \sim 400$) and maternal urinary concentrations of fluoride (mean of three samples, $n = 512$) with offspring IQ at age 3–4 years. Mean concentrations of fluoride in tap water of women exposed to fluoridated (38%) versus non-fluoridated water were 0.59 and 0.13 mg/L, respectively. Women from areas with fluoridated tap water had significantly ($p < 0.0001$) higher mean maternal urinary fluoride concentrations compared to those not exposed (0.7 vs. 0.4 mg/L). Fluoride intake from water and fluoride concentrations in spot urine were moderately correlated ($r = 0.49$, $p < 0.001$). Each 1 mg/day-increase in maternal fluoride intake from drinking water during pregnancy was associated with −3.7 (95% CI: −7.2, −0.2) lower IQ in the offspring. The association between maternal urine fluoride and IQ was consistent in terms of effect direction and magnitude but did not reach statistical significance [$\beta = -1.95$ (95% CI: −5.19, 1.28)]. A significant association between concentrations of fluoride in maternal urine and offspring IQ was reported in a later analysis [$\beta = -1.28$ (95% CI: −2.37, −0.18)] with a larger sample size ($n = 596$), extended to include also women who had provided fewer than three urine sample

(Farmus et al., 2021). When stratified by sex a significant association between maternal fluoride exposure with lower IQ was observed in boys, while no association was seen for girls (Farmus et al., 2021; Green et al., 2019).

Another study from the MIREC cohort (Till et al., 2020) examined fluoride intake in the offspring using both fluoride concentrations in tap water and fluoride intake from infant formula. Analyses were restricted to cohort participants who reported drinking tap water at home. Associations between offspring fluoride intake and IQ at age 3–4 years were assessed separately for infants who were breast-fed exclusively for more than 6 months ($n = 200$) and infants who were bottle-fed during the first 6 months ($n = 198$). In this study, fluoride intake of formula-fed infants (0.34 mg/day) living in an area with low fluoridated drinking water levels (0.59 ± 0.07 mg/L) was about three times higher compared to breast-fed infants (0.12 mg/day). Each 0.5 mg/L increase in water fluoride concentration was associated with −4.4 (95% CI: −8.3, −0.5) lower full-scale IQ in the formula-fed group, while no significant association was observed for the breast-fed group [β = −1.3; 95% CI: −5.4, 2.4]. However, each 0.5 mg/L increase in water fluoride concentration was associated with −9.3 (95% CI: −13.8, −4.8) and −6.2 (95% CI: −10.5, −1.9) lower performance IQ[37] in the formula-fed and breast-fed subgroup, respectively.

Three publications were identified from *the Early Life Exposures in Mexico to Environmental Toxicants* (ELEMENT) birth cohort (Bashash et al., 2018; Bashash et al., 2017; Goodman, Bashash, et al., 2022). In these studies, pregnant women were recruited from three hospitals in Mexico City that served low to moderate income populations. Women were asked to provide a spot urine sample in each trimester and 57% of women provided more than one sample. Neurocognitive outcomes were measured in the offspring at age 4 years ($n = 287$) using a standardised version of *McCarthy Scales of Children's Abilities* and IQ was assessed at age 6–12 years ($n = 211$) and *the Wechsler Abbreviated Scale of Intelligence*. ADHD-like symptoms were also assessed when the children were 6–12 years of age using the *Conners' Continuous Performance Test* and *Conners' Rating Scales-Revised* ($n = 213$). Fluoride in drinking water ranged between 0.2 and 1.4 mg/L and the main dietary source of fluoride was reported to be fluoridated salt (~ 250 mg fluoride/kg).

In the ELEMENT studies, maternal urinary fluoride concentrations were significantly associated with poorer neurocognitive outcomes at age 4 and age 6 to 12 years (Bashash et al., 2017). As an example, each 0.5-mg/L increase in maternal urinary fluoride was associated with −3.2 (95% CI: −5.4, −0.9) and −2.5 (95% CI −4.1, −0.6) lower offspring general cognitive index at age 5 and IQ scores between ages 6 and 12, respectively. Based on the scatter plot presented, the observed decrease in IQ seemed to occur at urinary concentrations above 1.0 mg/L. Maternal urinary fluoride concentrations were also associated with poorer cognitive performance and ADHD-like symptoms (Bashash et al., 2018). Finally, Goodman, Hall, et al. (2022) assessed the same associations as Bashash et al. (2017) with the addition of presenting results for verbal and non-verbal IQ at age 6–12 years as well as including more participants ($n = 278$ vs. 211). In that study 0.5 mg/L increase in urinary fluoride was associated with a − 2.1 lower full-scale IQ (95% CI: −3.5, −0.8); a − 2.6 change in non-verbal IQ (95% CI: −3.9, −1.4); and a more modest −1.3 change in verbal IQ (−2.6, 0.01).

A pregnancy cohort from Spain (INMA) assessed neurodevelopmental outcomes in the offspring at age 1 year ($n = 316$) using the *Bayley scale of infant development* and at 4 years ($n = 248$) using the *McCarthy Scales of Children's Abilities* (Ibarluzea et al., 2022). Maternal urine fluoride concentrations were collected in the first and third trimester of pregnancy and the mean of the two samples was used as measure of exposure. Mean fluoride concentration in drinking water was 0.8 mg/L (SD: 0.2) for those exposed to fluoridated drinking water compared to < 0.1 mg/L for those not exposed to fluoridated drinking water. Mean maternal urinary fluoride concentrations were 0.91, 0.62 and 0.43 mg/L for mothers reported to drink fluorinated drinking water ($n = 88$), bottled water ($n = 60$) or non-fluoridated drinking water ($n = 95$), respectively. No association was observed between maternal urinary fluoride concentration and infant neurodevelopmental scores at 1 year. Maternal urinary fluoride concentrations were associated with higher developmental scores at age 4 years in boys [β = 15.4 (95% CI: 6.3, 24.5), $n = 125$] but not girls [β = 0.19 (95% CI: −7.3, 6.9), $n = 124$]. In a separate study, Ibarluzea et al. (2023) assessed the association between prenatal maternal urinary fluoride and symptoms associated with attention-deficit/hyperactivity disorder (ADHD) at the age of 8 and 11 years (CRS-R-S; $n = 236$). Overall, no consistent association with ADHD symptoms was observed.

In a study of 837 participants from the Odense Child Cohort (OCC) in Denmark, Grandjean et al. (2023) examined the association between maternal urinary fluoride concentration in pregnancy with offspring IQ at age 7 based on the *Wechsler Intelligence Scale*. In contrast to the above-mentioned studies the participants were all exposed to non-fluoridated water (< 0.3 mg fluoride/L). A total of 453 participants provided spot urine samples during pregnancy while 384 mothers provided 24-h urine samples. The median fluoride concentration in all 837 urine samples was 0.52 mg/L (range, 0.08–3.04 mg/L). No association was observed between maternal urinary fluoride concentration with IQ in the offspring at age 7 years [β = 0.1 (95% CI: −1.1, 1.3) for each two-fold increase in exposure].

Finally, a study from New Zealand including 1037 children, born in 1972–1973 examined the association between community water fluoride exposure and use of oral care products at age 5 years with offspring IQ assessed at various ages up to 38 years (Broadbent et al., 2015). At age 5 years approximately 10% of participants were living in an area where they were exposed to fluoridated drinking water of 0.7–1.0 mg/L compared with those in the unexposed area (< 0.3 mg/L). Children's use of oral care products (0.5 mg fluoride tablets and toothpaste) was also recorded at age 5 years. No association between these two measures of fluoride exposure and IQ were observed. One limitation of this study is the lack of validation of exposure through use of objective biomarkers (i.e. fluoride concentrations in urine or serum).

[37] In this study total IQ (referred to in paper as full-scale IQ) was divided into performance IQ and verbal IQ.

Taken together, the exposure assessment in the above-mentioned studies was primarily based on maternal urinary fluoride concentrations in pregnancy supplemented by estimated fluoride intake in some studies. The urinary concentrations in these cohorts were partly overlapping. A clear separation was observed between participants exposed to low (< 0.3 mg/L) versus higher fluoride-containing water levels.[38] These studies had varying attrition rates and performed multiple analyses across a variety of IQ domains. In studies that reported an association with lower IQ, the effect size estimates ranged from small to moderate and effect direction was not fully consistent. A meta-analysis of the available prospective cohort studies was not performed due to the small number of studies with comparable effect estimates.

Of all the studies reviewed above, the studies from the MIREC cohort had the most robust exposure assessment because fluoride intake from drinking water was estimated using a questionnaire for water consumption linked with mean household fluoride concentration in drinking water (fluoridated 0.59 mg/L and non-fluoridated ~ 0.13 mg/L). Furthermore, the authors could confirm a correlation ($r = 0.49$, $p < 0.001$) between individual concentrations of fluoride in spot urine and fluoride intake from drinking water (Till et al., 2020). The significant association with lower IQ reported by (Bashash et al., 2017) in Mexican children exposed to fluoride in drinking water in the range of 0.2–1.4 mg/L is in agreement with these findings.[39]

The results from these two cohorts need to be interpreted in the context of lack of association reported in three other studies. That is, the INMA cohort where women were also exposed to low fluoridated (0.8 mg/L) and non-fluoridated (< 0.1 mg/L) drinking water (Ibarluzea et al., 2022) and more circumstantial evidence from New Zealand, due to lack of objective biomarkers of fluoride exposure, where women were also exposed to fluoridated (0.7–1.0 mg/L) and non-fluoridated (< 0.3 mg/L) drinking water (Broadbent et al., 2015). No association was also reported in the relatively larger (n = ~800) Odense cohort, where, unlike the two other studies (Broadbent et al., 2015; Ibarluzea et al., 2022), the participants were not exposed to fluoridated drinking water.

The significant positive association between urinary fluoride concentration and IQ in boys reported in the INMA study should be interpreted with some caution (Ibarluzea et al., 2022). When the association was analysed separately for sex, the number of participants was relatively small (n = 125 for boys and 124 for girls). With the association for girls suggesting a clear no association (slope ~ 0) and no previous report suggesting beneficial effects of fluoride on IQ, the positive association observed for boys in this study is most likely a chance finding. However, the study clearly indicates that in this population fluoride exposure in pregnancy was not adversely related to neurodevelopmental outcomes in the offspring.

In summary, the prospective studies reviewed above, conducted in populations exposed to low levels of fluoride in drinking water, do not allow for firm conclusions on a possible adverse association between exposure to fluoride and neurodevelopmental outcomes in children at the exposure ranges reported in these studies.

3.2.2.1.3 | *Neurotoxicity in adults*

One cohort study examined the association between exposure to fluoride from drinking water in adult life and dementia (Russ et al., 2020). The study included 6990 subjects (61% women) born in 1921 and whose archived record of IQ assessed at age 11 years was available and could be extracted in 2005. Fluoride concentrations in drinking water, obtained from the Drinking Water Quality Regulator for Scotland from 2005 to 2014 were used as measure of exposure. Prospective dementia cases were identified based on codes in the registry of dementia. Mean fluoride levels in drinking water were 0.05 mg/L (range 0.02–0.18 mg/L). All models were adjusted for IQ at age 11. In 2012, 1972 (28%) of the 6990 individuals had developed dementia. Higher mean fluoride levels in drinking water were associated with an increased risk of dementia with adjusted hazard ratio per 1-SD increase of 1.34 (95% CI: 1.28–1.41) for women and 1.30 (95% CI: 1.22–1.39) for men. In the absence of replication of these findings in another study, no firm conclusions can be drawn.

3.2.2.1.4 | *New evidence identified after January 2024*

For this assessment evidence identified through systematic literature review search published until January 2024 was included. The Scientific Committee is aware of more recent publications and preliminary findings from human studies (e.g. reported in conference abstracts) on the possible association between fluoride exposure and cognitive development in children. Although this evidence was not formally assessed, the results from these publications and ongoing initiatives are briefly described below.

Krzeczkowski et al. (2024) reporting on the MIREC study examined associations between prenatal fluoride exposure and visual acuity and heart rate variability (HRV) in 6-month-old infants (n = 435). In the adjusted analyses, water fluoride concentration was associated with poorer infant visual acuity (β = –1.51; 95% CI: –2.14,-0.88) and HRV (RMSSD; β = –1.60; 95% CI: –2.74, –0.46). Maternal fluoride intake was also associated with poorer visual acuity (β = –0.82; 95% CI: –1.35, –0.29) and HRV (RMSSD; β = –1.22; 95% CI: –2.15, –0.30). No significant associations were observed for maternal urinary fluoride.

Lee, Kim, et al. (2024) evaluated the association between the exposure to fluoridated tap water (defined as being born in a region where a water fluoridation programme was implemented) and neurodevelopment (Korean Developmental

[38] See Figure 1 of Grandjean et al. (2023).

[39] Comparison of fluoride exposure in the MIREC, ELEMENT and Odense Child Cohort cohorts can be found in the publication by Grandjean et al. (2023). In Figure 1 the maternal urinary concentrations are shown as box plot. Those results show that urinary concentrations among women in the ELEMENT and the MIREC cohorts that were exposed to fluoridated drinking water are largely comparable while those exposed no non-fluoridated water (MIREC and Odense) had lower urinary concentrations.

Screening Test for Infants and Children, K-DST) at 6 years of age in Korea ($n = 52,872$). No significant associations were observed for neurodevelopment. The study also assessed the association between exposure to fluoridated water and 16 paediatric disease entities (health insurance records); an association was observed for dental caries (HR, 95% CI; 0.76, 0.63–0.93), bone fractures (HR, 95% CI; 0.89, 0.82–0.93) and hepatic failures (HR, 95% CI; 1.85, 1.14–2.98).

Analysing data from a prospective cohort study in predominantly Hispanic women residing in Los Angeles, California, Malin et al. (2024) found that maternal urinary fluoride concentration in the prenatal period was significantly associated with adverse child neurobehavioural outcomes at age 3 years, as assessed through the Preschool Child Behaviour Checklist test. Study participants were residing in a predominantly fluoridated area and median maternal urinary fluoride concentration was 0.76 mg/L (IQR 0.51–1.19). A subgroup analysis for mothers having the lowest fluoride exposure was not presented.

Do et al. (2025) in a prospective cohort investigated the association between fluoride exposure in childhood and cognitive neurodevelopment in young adults ($n = 357$) using the WAIS-IV in Australia. Dental fluorosis was assessed and the percentage lifetime exposure to fluoridated water (%LEFW) during the first 5 y of life was estimated through data on socioeconomic factors, oral health behaviours and residential history (exposure categories; 0%LEFW, > 0% to < 100%LEFW and 100%LEFW). No statistically significant associations were reported.

In addition, two preliminary reports in the form of abstracts were presented at the European Young and Early Career Conference of the International Society for Environmental Epidemiology, held in June 2024 in Rennes, France. In a cohort study carried out in England Lee, Hamilton, et al. (2024), the average exposure to drinking water fluoride at birth (0.2 ± 0.23 mg/L) was not associated with cognitive and developmental outcomes at ages 5–7 years. In another cohort study carried out in Northern Sweden Mariza Kampouri et al. (2024), no association was found between urinary fluoride concentrations of mothers in gestational week 29 and child cognition of 4 year old children.

Moreover, three cross-sectional studies were identified. Liu et al. (2024) evaluated the association between water fluoride and iodine levels and thyroid function and intellectual development in school-age children in China ($n = 399$). The median (IQR) urine fluoride concentration was 1.29 (0.89, 1.84) mg/L. Overall, and among the multiple analyses performed, no statistically significant associations were reported between urine fluoride and IQ. Xia et al. (2025) also evaluated the relationship between urinary fluoride levels, urinary iodine levels and IQ in school children in China ($n = 711$). The median UF level was 1.39 mg/L. A statistically significant unadjusted correlation estimate was reported for urinary fluoride and IQ (beta, 95% CI; −3.34, −4.24, −2.43). Singhal et al. (2025) assessed the relationship between fluoride level in urine and drinking water and IQ in children aged 8–12 years in India ($n = 300$). Urine fluoride was ≥ 1 ppm in 231 participants (79%). Urine fluoride was inversely associated with IQ.

It is concluded that this new evidence does not change the conclusions of the current assessment based on studies published until January 2024.

3.2.2.2 | *Cross-sectional studies on neurotoxicity in humans*

A total of 53 publications from cross-sectional studies were assessed for risk of bias (Table 10). Of these at least five publications were based on the same study population of 7–13 year old children from Tianjin China (see Table C5). Around half of all publications were assessed to be of high risk of bias (Tier 3), mostly due to no- or limited confounder control. Around a quarter of the publications were of low risk of bias (Tier 1) and another quarter of moderate risk of bias (Tier 2).

TABLE 10 Heat map for the risk of bias performed for human cross-sectional and case–control studies on neurodevelopmental endpoints.[a]

Refid	Author	Year	Study design	TIER	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
93	Choi, A.	2015	Cross-sectional	1	++	+	++	++	+	+	+	+
271	Cui, Y.	2018	Cross-sectional	1	+	++	+	+	++	+	++	++
10337	Godebo, T. R.	2023	Cross-sectional	1	+	+	+	+	+	+	+	+
1512	Rocha-Amador, D.	2007	Cross-sectional	1	+	+	+	+	+	+	+	+
1798	Rocha-Amador, D.	2009	Cross-sectional	1	+	++	+	+	++	+	++	++
3361	Sebastian, S. T.	2015	Cross-sectional	1	+	+	+	+	+	+	+	+
4055	Soto-Barreras, U.	2019	Cross-sectional	1	+	+	+	+	+	+	++	+
910	Wang, M.	2020	Cross-sectional	1	+	+	+	+	+	+	++	++
160	Wang, S.	2021	Cross-sectional	1	+	+	+	+	+	+	++	+
2079	Xiang, Q. Y.	2011	Cross-sectional	1	+	+	+	+	+	+	+	+
428	Yu, X.	2021	Cross-sectional	1	+	+	+	++	+	+	++	++
909	Yu, X.	2018	Cross-sectional	1	+	+	+	++	+	+	+	+
632	Zhang, S.	2015	Cross-sectional	1	+	+	+	++	++	+	+	+

TABLE 10 (Continued)

Refid	Author	Year	Study design	TIER	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
432	Zhao, L.	2021	Cross-sectional	1	+	+	+	+	+	+	+	+
6637	Zhou, G. Y.	2021	Cross-sectional	1	+	++	+	++	++	+	++	+
5221	Adkins, E. A.	2022	Cross-sectional	2	+	+	+	-	+	+	+	+
10256	Ali, M.	2023	Cross-sectional	2	+	-	+	+	+	+	+	+
789	Ding, Y.	2011	Cross-sectional	2	+	-	+	+	+	+	+	-
10323	Do, L.G.	2023	Cross-sectional	2	+	++	+	-	+	++	+	+
10330	Feng., Z.	2022	Cross-sectional	2	+	+	+	+	+	-	+	+
178	Li, M.	2016	Cross-sectional	2	+	+	+	+	+	-	++	+
10609	Lin, Y.Y.	2023	Cross-sectional	2	+	+	+	-	+	+	+	+
2399	Riddell, J. K.	2019	Cross-sectional	2	+	++	+	++	+	-	++	+
4052	Saxena, S.	2012	Cross-sectional	2	+	-	++	+	+	+	++	-
4098	Seraj, B.	2012	Cross-sectional	2	+	+	+	-	+	+	++	-
62	Wang, S.	2007	Cross-sectional	2	+	-	+	+	+	+	+	-
430	Xu, K.	2020	Cross-sectional	2	+	+	++	-	+	+	+	+
10249	Ahmad, M.S.	2023	Cross-sectional	3	-	-	-	+	+	-	+	-
4046	Aravind, A.	2016	Cross-sectional	3	+	-	+	-	+	+	+	+
5216	Barberio, A. M.	2017	Cross-sectional	3	+	+	+	+	-	-	+	+
790	Cui, Y.	2020	Cross-sectional	3	+	--	+	-	+	+	+	+
3509	Das, K.	2016	Cross-sectional	3	+	--	+	+	++	+	++	-
10319	De la Cruz, J. T.	2022	Cross-sectional	3	+	-	+	+	+	+	+	-
6339	Eswar, P.	2011	Cross-sectional	3	+	-	+	-	+	+	+	+
8189	Hong, F. G.	2008	Cross-sectional	3	+	-	+	+	+	-	+	+
6401	Karimzade, S.	2014	Cross-sectional	3	+	-	+	-	+	-	+	-
10583	Kaur, D.	2022	Cross-sectional	3	+	--	+	+	+	-	+	-
4361	Li, J.	2008	Case–control	3	-	--	+	-	+	+	+	+
8185	Liu, S. L.	2008	Cross-sectional	3	+	-	+	+	+	-	+	-
6211	Mondal, D.	2016	Cross-sectional	3	-	-	+	+	+	+	++	+
8113	Mustafa, D. E.	2018	Cross-sectional	3	+	--	+	-	-	-	+	-
3027	Nagarajappa, R.	2013	Cross-sectional	3	+	--	+	-	+	+	+	+
3094	Poureslami, H. R.	2011	Cross-sectional	3	+	-	+	-	+	+	+	-
9277	Qin, L. S.	2008	Cross-sectional	3	+	-	+	-	++	+	+	-
1214	Razdan, P.	2017	Cross-sectional	3	+	-	+	+	+	-	+	-
2970	Ren, C.	2021	Cross-sectional	3	+	-	+	+	+	-	++	-
2304	Trivedi, M. H.	2012	Cross-sectional	3	+	--	+	+	+	-	+	-
4096	Trivedi, M. H.	2007	Cross-sectional	3	-	--	+	+	+	-	+	-
10819	Valdez-Jiménez, L.	2023	Cross-sectional	3	+	--	+	+	+	-	+	-
1466	Wang, A.	2022	Cross-sectional	3	+	-	+	-	+	+	+	+
8748	Wang, G. J.	2008	Cross-sectional	3	+	-	+	+	+	-	-	+
11068	Xia, Y.T.	2023	Cross-sectional	3	+	-	+	-	+	-	+	+
4362	Yang, Y. K.	2008	Cross-sectional	3	-	--	+	+	+	+	+	-
6236	Yani, S. I.	2021	Cross-sectional	3	+	--	+	+	+	-	+	+

[a]The heatmap indicates whether the criteria stated in questions 1–8 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

In terms of the populations covered, most of the cross-sectional studies (see Tables C.3 and C.4) were from China ($n = 24$) and India ($n = 11$) while other studies ($n = 18$) were conducted across several countries including countries in North America, Asia and Africa. Most of the studies pertained to paediatric populations in primary school settings. Two studies were conducted in adults and one study recruited subjects across all age groups.

The intake assessment was predominately based on drinking water, most often sampled across areas with low to high fluoride concentrations in drinking water. In two-thirds of the studies the exposure was complemented with other exposure measures, such as fluoride concentrations in urine or serum.

The outcomes assessed fell into six broad categories. When not counting repeated publications from the same study population, 37 studies[40] addressed children's intelligence. There were also 12 studies covering different cognitive outcomes in children (other than IQ) including general cognition, behaviour (including ADHD) and school performance. These studies are summarised in Tables C.3 and C.4 and briefly described below.

3.2.2.2.1 | *Studies on intelligence*

A total of 27 out of 37 studies (70%) reported some associations between higher fluoride exposure and lower IQ in children (Table D.3 in Annex D). Associations with lower IQ were reported in most of the studies regardless of their RoB status. For example, seven out of eight Tier 1 studies reported some association with lower IQ, while the corresponding number for the Tier 3 studies was 15 out of 22 studies.

Even though most of the cross-sectional studies (70%), regardless of risk of bias, reported an inverse association between fluoride exposure and IQ, the main concerns about these studies relate to (a) uncertainties around whether the measured exposure at the time of outcome assessment accurately reflects past exposures and (b) concerns around suboptimal adjustment for confounders. To address these questions, studies were grouped into 4 different categories based on information provided in each study about how timing and duration of exposure was assessed combined with information on confounder control.

Group 1: Information on duration of exposure provided and some confounder control (8 studies; 12 publications).

Group 2: Information on duration of exposure provided but no confounder control (9 studies).

Group 3: No information on duration of exposure but some confounder control (6 studies).

Group 4: No information on duration of exposure and no confounder control (14 studies).

Below a short summary of these studies is provided.

Group 1: In this group there were eight studies (12 publications) in which duration of exposure was assessed to have occurred throughout the lifetime of participants.

For one of these studies there were five publications, all Tier 1, reporting on the same group of 7–13 year old children recruited in 2015 in Tianjin, China (Wang et al., 2020, 2021; Yu et al., 2018; Yu et al., 2021; Zhou, Zhao, et al., 2021). These different publications were largely focused on examining the possible gene–environment interactions for fluoride. All studies consistently reported, through different analyses, association between higher fluoride exposure and lower offspring intelligence. As an example, among 709 children in one of these studies, a 4.1-point lower IQ score (95%CI: 1.5, 6.7) was observed for children who had been exposed to high (> 1.6 mg/L) versus low (≤ 0.3 mg/L) fluoride concentrations in drinking water (Wang et al., 2021). In that study, comparable estimates were also observed for urinary fluoride concentrations.

Another study (Tier 1) from Tianjin in China reported 2.4 points lower IQ score (95%CI: 0.2, 4.6) for each 1-mg/L increase in urinary fluoride concentration among 180 children aged 10–12 years (Zhang, Zhang, et al., 2015). Similar correlations were also observed for fluoride concentrations in water and serum. The limitation of this study was that only age and gender were included for confounder control. Parental education did, however, not differ significantly between the high (wF ~ 1.4 mg/L) and low (wF ~ 0.6 mg/L) fluoride recruitment area. Similarly, another study from Tianjin reported a relatively strong inverse association [β = −6.0 (95%CI: −9.7, −2.2] with IQ in 567 children aged 6 to 11 years (Zhao et al., 2021). The effect size reported in this study is somewhat unexpected as the observed urinary fluoride concentrations were moderately elevated (25th to 75th percentile of 0.7 to 1.5 mg/L).

Another study (Tier 1) recruited 6–10 year old Mexican children ($n = 132$) who had been living in their recruitment area since birth and were exposed to drinking water with mean fluoride concentrations of either 0.8, 5.3 or 9.4 mg/L. In that study an approximately 10-point lower IQ was observed with each 10-fold increase in fluoride exposure[41] (Rocha-Amador et al., 2007).

Similarly, a study (Tier 1) of 10–13 year old children from India ($n = 405$) reported around 6 points lower IQ in children who had been life-long residents in an area with high (2.0 mg/L) compared to low (0.4 mg/L) fluoride concentrations in drinking water ($p = 0.03$) (Sebastian & Sunitha, 2015). The IQ scores of children who were living in another area with a mean water fluoride concentration of 1.2 mg/L did not significantly differ ($p = 0.36$) from those living in the low fluoride area. Due to the recruitment strategy parental education and occupation was reported to be comparable for the three water fluoride areas.

Concerning studies with moderate risk of bias (Tier 2), a study recruiting 293 Iranian children aged 6–11 years, who had been life-long residents of 5 villages from which they were recruited, reported 3.9 points lower IQ for each 1 mg/L increase in fluoride in drinking water in the adjusted analyses (Seraj et al., 2012). The water fluoride concentrations of recruitment sites ranged from 0.8 to 5.2 mg/L.

[40] 5 publications from Tinjian (see Table D.3 in Annex D) were counted as one study.

[41] Exposure was in log10 scale, therefore the regression slope reflects change in IQ for each 10-fold increase in fluoride concentration in drinking water.

In contrast, a Tier 2 study of 12 year old children from India ($n = 171$) showed a higher intelligence grade for children who had been life-long residents in areas with higher compared to lower concentrations of fluoride in drinking water (< 1.5 vs. > 4.5 mg/L) (Saxena et al., 2012).

Finally, a Tier 3 study of 8–12 year old children from Jiangsu, China ($n = 721$), found that children living in an area with high (1.89 mg/L) versus low (0.73 mg/L) water fluoride concentration had 6.8 times higher odds (95% CI 3.2, 14.5) of having an IQ below 90 points (Xia et al., 2023). In linear regression analyses for the high fluoride area this corresponded to 4.08 points lower IQ for each 1 mg/L increase in urinary fluoride concentrations.

In summary, 7 out of the 8 cross-sectional studies (12 publications) with participants who were exposed throughout their lifetime and after adjustment for confounders, reported an association between higher fluoride concentrations in drinking water and lower IQ.

Group 2: Nine additional studies were identified in which exposure during several years (most studies since birth) was one of the inclusion criteria but no adjustments for covariates were made. In all these studies participants were recruited from areas with low (mean range: 0.2–2.0 mg/L) or high (2.4–6.8 mg/L) fluoride concentrations in drinking water (Ali et al., 2023; Eswar et al., 2011; Nagarajappa et al., 2013; Qin et al., 2008; Razdan et al., 2017; Soto-Barreras et al., 2019; Trivedi et al., 2007; Valdez-Jiménez et al., 2023; Wang et al., 2007). One of these studies was evaluated as Tier 1 (Soto-Barreras et al., 2019), two were evaluated as Tier 2 (Ali et al., 2023; Wang et al., 2007) while all other studies were evaluated as Tier 3. All studies except three (Eswar et al., 2011; Qin et al., 2008; Soto-Barreras et al., 2019) reported lower IQ with higher fluoride concentrations in drinking water.

Group 3: A total of 6 studies (2 of Tier 3, 2 of Tier 2 and 2 of Tier 1) with varying fluoride concentrations in drinking water (range < 0.3–4.0 mg/L) were identified in which covariate adjustment was performed but information on duration of residence in the recruitment area was not reported (Cui et al., 2020; Cui et al., 2018; Feng et al., 2022; Karimzade et al., 2014; Lin et al., 2023; Xiang et al., 2011). Three of these studies reported an association between lower IQ and higher fluoride exposure while three (Cui et al., 2020; Feng et al., 2022; Lin et al., 2023) found no association.

Group 4: A total of 14 studies were identified that did not report duration of residence in the recruitment area and did not perform covariate adjustment. However, in some studies the authors noted that subjects from the recruitment area had similar characteristics, including socioeconomic status. Of these 14 studies, 1 study was of moderate risk of bias (Tier 2) (Ding et al., 2011), while the other studies were all Tier 3 (Ahmad et al., 2021; Aravind et al., 2016; Das & Mondal, 2016; De la Cruz et al., 2022; Hong et al., 2008; Kaur et al., 2022; Liu et al., 2008; Mondal et al., 2016; Poureslami et al., 2011; Trivedi et al., 2012; Wang et al., 2008; Yang et al., 2008; Yani et al., 2021). Of these 14 studies, 10 studies reported associations with lower IQ at higher fluoride water concentrations.

In summary, the main limitation for most of the cross-sectional studies was lack of clarity about the characteristics of study participants including parental education and socioeconomic status. These factors are well known predictors of child intelligence and should (ideally) be accounted for. Adjustment for these factors was only performed in a few studies. For the cross-sectional studies another limitation is missing information on the duration and timing of exposure to fluoride. This limitation was addressed in several studies (from groups 1 and 2) which recruited participants that had been long-term residents (often lifelong) in areas with low or high fluoride concentrations in drinking water. In those studies, assuming that exposure occurred during a sensitive window of development, seems plausible.

Overall, the cross-sectional studies suggest that living in an area with elevated water fluoride concentrations is associated with lower performance on intelligence tests by children. On their own these studies are suggestive but not robust as a standalone line of evidence to derive a health-based guidance value. However, the evidence provided in these studies contributes to the overall weight of evidence, which is integrated in Section 3.5.

3.2.2.2.2 | *Other neurodevelopmental outcomes*

A total of 12 cross-sectional studies from Canada, USA, Mexico, China, Sudan, India, Ethiopia and Australia assessed associations between fluoride exposures and different cognitive outcomes, other than IQ, in children and adults. The main characteristics and key results from these studies are summarised in Table D.4 in Annex D. A brief summary is provided below.

Two studies from China with adults 60 years or older, assessed associations with cognitive impairment using two different screening tools.[42] One of the studies (Ran et al., 2021) observed a higher prevalence of cognitive impairment (46% vs. 15%) among subjects ($n = 544$) exposed to high ($n = 272$, > 2.0 mg/L) versus low ($n = 172$, < 0.8 mg/L) fluoride in drinking water. In contrast, the other study found no association for either urinary fluoride concentrations (mean: 1.1 mg/L, $n = 511$) or water fluoride intake (2.2 vs. 3.6 mg/day) (Li et al., 2016).

In children, two cross-sectional studies from the Canadian Health Measure Survey examined associations between fluoride concentrations in water (10th–90th percentile: 0.01–0.65 mg/L) and urine (mean 0.51 mg/L, SD: 0.39) with parental- or self-reported ADHD diagnosis or ADHD-like symptoms (SDQ-score). These studies were partly overlapping as the former included 2221 participants aged 3–12 years, while the latter included 1877 participants aged 6–17 years. No association was observed in the study of the 3–12 year old participants (Barberio et al., 2017), while positive associations with both

[42]The Mini Mental State Examination or the Montreal Cognitive Assessment-Basic instruments.

self-reported ADHD diagnoses and ADHD-like symptoms (SDQ-score) were reported in the study including the 6–17 year old children (Riddell et al., 2019).

Six other studies in children evaluated associations with different neurodevelopmental endpoints. In a study from the US a positive association between urinary fluoride concentration (mean: 0.89 mg/L) and internalising symptoms, as assessed by BASC-2,[43] was observed for 286 children around the age of 12 years (Adkins et al., 2022). The association corresponded to 2.9-fold increased odds (95% CI: 1.2, 6.9) of having an internalising composite T-score[44] in a clinically 'at-risk' range. No associations with symptoms of depression or anxiety were observed. A study from China (Wang et al., 2022) examined the association between urine fluoride concentrations (mean (SD): 1.5 (0.9) mg/L) and children's behavioural outcomes in 325 children aged 7–13 years using the Conners' Parent Rating Scale-Revised. Each 1.0 mg/L increase in urinary fluoride concentration was associated with 1.97 higher odds (95% CI: 1.19, 3.27) of being classified as having psychosomatic problems. In that study, no association was observed for conduct problems, learning problems, impulsive–hyperactive, anxiety or ADHD index. A study from Mexico with 80 children aged 6–11 years exposed to varying concentrations of fluoride, lead and arsenic (Rocha-Amador et al., 2009) reported a negative correlation between urinary fluoride (5.6 mg/g creatinine) and immediate recall score[45] ($r = -0.27$; $p < 0.05$). A small ($n = 51$) study from China in children (mean age ~7 years) found no association between water fluoride (range: 1.0–4.1 mg/L) and urinary fluoride (mean 1.6 mg/L) concentrations with different cognitive scores assessed using the Wide Range Assessment of Memory and Learning, finger tapping tasks and the grooved pegboard test (Choi et al., 2015). A study from Ethiopia ($n = 74$, age range 5–14 years) found an inverse association between water fluoride (> 8–15.5 mg/L) and urinary fluoride concentrations and children's drawing scores while a positive association was found between water fluoride concentration and the number of errors in the CANTAB PAL[46] tasks (Godebo et al., 2023). Finally, in a follow-up study from Australia's National Child Oral Health Study 2012–2014, children aged 5–10 years at baseline were contacted again after 7–8 years. No association between exposure to fluoridated water[47] during the first 5 years of life and altered measures of child emotional and behavioural development and executive functioning was found (Do et al., 2023).

A study from China assessing neonates 1–3 days old reported a lower mean (36.5 vs. 38.3) neurobehavioural assessment score for neonates born in areas with high (range: 1.7–6.0 mg/L) as compared to low (0.2–1.5 mg/L) fluoride concentrations in drinking water (Li et al., 2008). Without further follow-up testing the interpretation of a lower score at this age is difficult. Similarly, the finding of a negative correlation between mean school grades and mean concentrations of fluoride in drinking water (0.1–2.1 mg/L) across 16 rural areas in Sudan is difficult to interpret due to lack of socioeconomic background of children living in these areas (Mustafa et al., 2018).

In summary, although some associations with various cognitive outcomes were reported in studies in children, lack of replication using similar assessment tools makes it difficult to draw a conclusion on a possible association between fluoride concentrations in drinking water or urine of children and cognitive outcomes other than IQ in children. The available evidence for an association between fluoride exposure and cognitive decline in adults and ADHD-like symptoms (or diagnoses) in children is inconsistent.

3.2.2.2.3 | *Evidence on neurotoxicity and developmental neurotoxicity from literature published before 2005*

Among the studies identified through the targeted search on Neurotoxicity and Developmental Neurotoxicity prior 2005, two studies that reported on neurobehavioural outcomes in children were appraised for internal validity (RoB). These included one Tier 2 cohort study and one Tier 3 cross-sectional study. Both studies addressed behavioural problems in children (Table 11).

TABLE 11 Heat map for the risk of bias performed for human studies on neurodevelopmental endpoints.[a]

Refid	Author	Year	Study design	TIER	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
12887	Shannon	1986	Cohort	2	+	+	+	-	+	+	+	+
12831	Morgan	1998	Cross-sectional	3	+	+	+	-	+	-	+	+

[a]The heatmap indicates whether the criteria stated in questions 1–8 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

In a study on 917 children aged 7 to 11 years from Boston (US), Morgan et al. (1998) examined associations between history of fluoride exposure, including years exposed to fluoridated water, with child behaviour using the Child Behaviour Checklist. No association between previous history (parental report) of fluoride exposure with behavioural problems was observed. Similarly, no association with behavioural problems as measured by Conners and Rutters behaviour scales was

[43]Behaviour Assessment System for Children – Second Edition (BASC-2).

[44]Covering anxiety, depression and somatisation.

[45]Assessed by the Rey–Osterrieth Complex Figure (ROCF) test.

[46]Validated Cambridge Neuropsychological Test Automated Battery's (CANTAB) Paired Associate Learning (PAL), which examines memory and new learning.

[47]Measured as percent lifetime exposed to fluoridated water (%LEFW) estimated from residential history and post code wF levels in tap water.

observed in a study of 1028 children from New Zealand[48] (Shannon et al., 1986). In that study, duration of exposure to fluoridated drinking water up to the age of 7 years was used as measure of exposure.

Overall, the Scientific Committee concludes that these older publications and their inclusion in the weight of evidence would not change the conclusion of this assessment.

3.2.3 | Thyroid function and thyroid disease

The thyroid gland is part of the endocrine system and regulates multiple physiological functions, including the cardiovascular- and digestive system, muscle function, bone health and embryonic brain development. The impact of maternal thyroid homeostasis disturbance on offspring neurodevelopmental outcomes is well established and is receiving attention (Gilbert et al., 2012; Goodman & Gilbert, 2007; Haddow et al., 1999; Hall et al., 2023; Jansen et al., 2019; Korevaar et al., 2016; O'Shaughnessy et al., 2019; Zoeller, 2007; Zoeller & Rovet, 2004). Details of this relationship are described in the OECD adverse outcome pathways (AOP) Wiki.[49] An assessment of possible effects of fluoride on neurodevelopment would not be complete without evaluation of possible thyroid-mediated effects. Background information on the role of the thyroid during pregnancy is provided in Appendix B.

In addition to possible thyroid-mediated effects on neurodevelopment, a disturbance of the hypothalamus–pituitary–thyroid (HPT) axis is a concern in itself, since it represents a toxicological burden that compromises an organism's capacity to compensate for the multitude of additional stressors. Hence, the evidence on thyroid status has been reviewed as a separate prioritised health effect (endpoint).

Thyroid homeostasis is regulated by a complex feedback loop involving the hypothalamus and the pituitary that maintains the concentrations of the two main thyroid hormones, thyroxine (T4) and triiodothyronine (T3). The hypothalamus-released thyrotropin-releasing hormone (TRH) stimulates the pituitary gland to release thyroid-stimulating hormone (TSH). This, in turn, stimulates the synthesis and release of T4 and, to a lesser extent, T3, through iodination of tyrosine residues on thyroglobulin catalysed by thyroid peroxidase (TPO). Iodine uptake in the thyroid is mediated by the sodium-iodide symporter (NIS). When (F)T4 is low in the blood, the hypothalamus is triggered to release TRH and activate the pituitary to release TSH. TSH stimulates iodine uptake into the thyroid gland via the NIS to augment production and release of T4 and T3 into the bloodstream. Any of these elements can be targets of endogenous and exogenous chemical agents resulting in disturbance of thyroid homeostasis (Gilbert et al., 2020; Noyes et al., 2019; Zoeller, 2007, 2021).

In the assessment of fluoride effects on the thyroid, human data are available for blood concentrations of TSH,[50] T4 and T3, which are standard clinical markers of thyroid function. Generally, a modest change in TSH in an individual without changes in (F)T4 and (F)T3 is not considered adverse but an adaptive response to a perturbation of thyroid homeostasis. Iodine deficiency[51] was assessed as a possible effect modifier for the interpretation of results from human studies when assessing potential effects of fluoride on thyroid function. For additional information, see Appendix B.

3.2.4 | Assessing the relationship between fluoride exposure and thyroid hormones in humans

Twenty-eight publications were identified that assessed fluoride concentrations in water or urine with thyroid function or thyroid volume. Although these studies covered results from four prospective pregnancy cohorts and studies with case–control sampling, almost all analyses were cross-sectional, meaning that exposure and outcome were assessed around the same time period. Eight papers were judged to be of low risk of bias (Tier 1), while 10 papers were of moderate (Tier 2) and 10 papers were of high risk of bias (Tier 3). The outcome of the study appraisal is presented in Table 12 (see Annex D, Table D.6 for more details).

Results from three pregnancy cohorts were presented in 4 publications from Canada ($n = 2$), Sweden and Thailand.

TABLE 12 Heat map for the risk of bias performed for studies on effects on the thyroid.[a]

RefID	Author	Year	Study design	Tier	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
571	Du, Y.	2021	Cross-sectional	1	+	+	+	+	+	+	+	+
910	Wang, M.	2020	Cross-sectional	1	+	+	+	+	+	+	+	+
5219	Malin, A. J.	2018	Cross-sectional	1	+	+	+	+	+	+	+	+
632	Zhang, S.	2015	Case–control	1	+	+	+	+	+	+	+	+

(Continues)

[48]This is the same cohort study as in Broadbent et al. (2015).

[49]https://aopwiki.org/aops.

[50]Generally accepted range for individual blood TSH concentrations: 0.4–4.0 mUI/L; for FT4: 9.0–24.0 pmol/L (0.6992–1.8645 ng/dL); for TT4: 64–154 nmol/L (4.9720–11.964 μg/dL).

[51]On a population basis, iodine deficiency is defined by the WHO as median urinary iodine concentration (ul) below 100 μg/L. A urinary concentration between 100 and 200 μg/L represents adequate iodine nutrition, whereas concentrations ≥ 300 μg/L present a risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease) (WHO, 2013). In pregnancy, the recommended threshold for ul is 150 μg/L (WHO, 2013).

TABLE 12 (Continued)

RefID	Author	Year	Study design	Tier	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
11281	Hall, M.	2023	Cohort	1	+	+	+	+	++	+	+	+
11285	Hall, M.	2024	Cohort	1	+	+	+	+	+	+	+	+
10358	Kampouri, M.	2022	Cohort	1	+	+	+	+	+	+	+	+
11576	Xu, K.	2022	Cross-sectional	1	+	+	+	+	+	+	+	+
339	Karademir, S.	2011	Cross-sectional	2	+	-	+	+	+	+	+	-
429	Barberio, A. M.	2017	Cross-sectional	2	++	-	+	+	+	+	++	+
790	Cui, Y.	2020	Cross-sectional	2	-	+	+	+	+	+	+	+
3316	Zulfiqar, S.	2019	Cross-sectional	2	+	-	+	+	+	+	+	+
3527	Khandare, A. L.	2017	Cross-sectional	2	+	-	+	+	+	+	+	+
3841	Ahmed, I.	2022	Cross-sectional	2	+	-	+	+	+	+	+	+
4037	Zulfiqar, S.	2020	Cross-sectional	2	+	-	+	+	+	+	+	+
5949	Kheradpisheh, Z.	2018	Cross-sectional	2	+	+	+	-	+	+	+	+
630	Wang, Y.	2022	Cross-sectional	2	-	+	+	+	+	+	+	+
3858	Khandare, A. L.	2018	Cross-sectional	2	+	-	+	+	+	+	+	+
3087	Singh, N.	2014	Cross-sectional	3	-	-	+	+	+	+	+	-
4362	Yang, Y. K.	2008	Cross-sectional	3	-	-	+	+	+	+	+	+
5101	Shaik, N.	2019	Cross-sectional	3	-	-	+	+	+	+	+	+
5393	Kumar, V.	2018	Cross-sectional	3	-	-	+	+	+	+	+	+
6399	Kutlucan, A.	2013	Cross-sectional	3	-	-	+	+	+	+	+	-
4834	Susheela, A. K.	2005	Cross-sectional	3	-	-	+	++	++	+	++	+
4038	Yasmin, S.	2013	Cross-sectional	3	-	-	+	+	+	+	+	+
2215	Peckham, S.	2015	Cross-sectional	3	+	-	+	-	+	+	+	+
-	Ray, D.	2012	Cross-sectional	3	-	-	-	+	+	+	+	-
10416	Somporn, R.	2023	Cross-sectional	3	-	-	-	-	+	+	+	-

[a]The heatmap indicates whether the criteria stated in questions 1–8 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

Associations between fluoride concentrations in drinking water or urine and thyroid hormones in children were reported in 16 studies carried out in Turkey ($n = 2$), China ($n = 5$), India ($n = 7$) and Pakistan ($n = 2$). Associations between fluoride concentrations in drinking water or urine and thyroid hormones in adults were reported in 7 studies from Canada ($n = 2$), Pakistan ($n = 1$), India ($n = 2$), Iran ($n = 1$) and Sweden ($n = 1$). In several of these publications iodine status as reflected by urinary concentrations was also assessed.

The Scientific Committee noted in some studies possible errors in units reported for TSH and thyroid hormones. For further detail, see Annex D, Table D.7.

Due to possible differences in thyroid concentrations in children and adults, studies in children and adults are assessed separately below.

3.2.4.1 | *Studies in children*

Most studies assessed the association between fluoride and thyroid hormones by comparing subjects living in endemic fluorosis areas with subjects living in control areas where fluorosis was absent or less prevalent. All studies were carried out in Asia except for two studies that were carried out in Turkey. From these studies mean water fluoride (wF), urinary fluoride (uF) and serum fluoride (sF) concentrations and the mean concentrations of thyroid hormones were extracted (Annex D, Table D.7) and assessed for correlations (see Section 3.2.4.3).

In a Tier 1 study of 571 children from Tianjin, China, aged 7–13 years, Wang et al. (2020) reported that for every 1 mg/L increase of urinary fluoride concentration significantly lower concentrations were observed for both T4 (slope: −0.09 ng/L, $p = 0.02$) and FT4 (slope: −0.09 ng/L, $p = 0.02$) and significantly higher concentrations of TSH (slope: 0.11 μIU/L, $p = 0.01$). For water fluoride concentrations the corresponding associations were in the same direction but did not reach significance for T3 and FT4. In this study median (10th, 90th percentile) concentration in drinking water and spot urine samples were 1.0 mg/L (0.4, 2.9) and 0.4 (0.07, 3.11), respectively.

Zhang et al. (2015) (Tier 1) compared 84 children aged 10–12 years from an endemic fluoride area (median in drinking water ~ 1.4 mg/L) with 96 children of the same age from a control area (median 0.6 mg/L) in Tianjin, China. The mean urinary fluoride concentration was more than double in the endemic area compared to the control area (2.40 vs. 1.10 mg/L).

The mean TSH concentration was significantly higher in the children from the endemic compared to children from the control area (3.1 vs. 2.6 mIU/L, $p = 0.03$) and the mean T4 concentration was also lower (85.7 vs. 90.8 ng/mL, $p = 0.06$) while concentrations of T3 were similar (2.22 vs. 2.15 ng/mL, $p = 0.29$).

Karademir et al. (2011) (Tier 2) compared children from endemic fluorosis areas with children living in a non-endemic fluorosis region in Turkey. At recruitment the children were between 8 to 16 years of age. Endemic fluorosis was defined as: (1) living in the endemic fluorosis region since birth, (2) having dental fluorosis, (3) consuming drinking water with a fluoride level >1.2 mg/L and (4) urinary fluoride concentration > 0.6 mg/L. Children were further divided according to severity of dental fluorosis (Dean index). The control group ($n = 26$) had a lower median urinary fluoride concentration (0.20 mg/L) while the concentrations were 0.74 mg/L to 0.90 mg/mL in children grouped to have very mild to moderate ($n = 22$) or severe ($n = 13$) dental fluorosis, respectively. Children classified as having severe dental fluorosis had significantly lower FT4 compared to the control group (0.96 vs. 1.11 ng/dL, p = 0.03) while FT4 did not differ between those with milder fluorosis and controls. No significant difference was observed for TSH and FT3 among the three groups.

In a Tier 2 study of 498 children aged 7–12 from Tianjin in China Cui et al. (2020) grouped the children according to urinary fluoride concentrations of < 1.6, 1.6–2.5 or ≥ 2.5 mg/L. The corresponding mean TSH concentrations of 2.81, 2.82 and 3.29 mIU/L, respectively, were not significantly different ($p = 0.29$).

In a Tier 2 study of 842 children from India, aged 8–18 years, (Khandare et al., 2017) examined the association between living in an endemic versus non-endemic fluorosis area on TSH and thyroid hormone concentrations. A total of 445 children were recruited from the control area where the mean fluoride concentrations in water and urine were 1.1 and 1.9 mg/L, respectively. The mean urinary concentrations among the 397 recruited children from the endemic area were 3.28 mg/L and the water concentrations ranged from 1.8 to 3.8 mg/L. Children in the endemic fluoride area had a *lower* mean TSH concentration compared to the low fluoride area, (2.9 and 3.4 mIU/L, $p < 0.05$) and slightly but not significantly lower mean T3 and T4 concentrations.

In another Tier 2 study, Khandare et al. (2018) compared thyroid hormones in 685 children from India aged 8–14 years and exposed to low, medium and high concentrations of fluoride in drinking water (0.877, 2.53, 3.77 mg/L, respectively). Children with increased exposure to fluoride in drinking water (0.877 vs. 3.77 mg/L) had significantly higher mean TSH concentrations (1.66 vs. 2.65 µIU/mL respectively), lower mean concentrations of T3 (2.17 vs. 1.34 µg/L) and higher mean T4 concentrations (113 vs. 133 µg/L). In addition, thyroid hormones were also measured in children exposed before to a high concentration of fluoride in drinking water (4.515 mg/L) but who were exposed for the last 5 years to drinking water with < 1.0 mg fluoride/L due to a change in water supply. This group had lower mean TSH and lower mean T4 than the children in the three groups studied. The T3 concentration was (1.46 µg/L).

Zulfiqar et al. (2019) (Tier 2) studied 134 children, aged 7–18 years, with similar social-economic conditions and living since birth in an endemic fluorosis area or in a non-endemic fluorosis area in Pakistan. Children in the endemic area ($n = 74$) had been exposed to a mean fluoride concentration in drinking water of 4.7 mg/L, while those in the non-endemic area ($n = 60$) were exposed to a mean water fluoride concentration of 0.5 mg/L. Mean concentrations of FT3 and FT4 did not differ significantly between groups but mean TSH was significantly higher in the endemic area compared to the non-endemic area (3.2 vs. 2.4 mIU/L). The authors reported that 22% children in the endemic area had well-defined thyroid hormonal aberrations while the corresponding percentage was not reported for the non-endemic area.

In a later study Zulfiqar et al. (2020) (Tier 2) compared 7–18 year old children from a different endemic fluorosis area ($n = 130$) using again the same children living in the non-endemic area as controls ($n = 60$). The water fluoride concentrations in the endemic area was now considerably higher (6.2 mg/L vs. 0.5 mg/L). The mean concentrations for fluoride in urine were 3.4 and 1.5 mg/L in the endemic and non-endemic area, respectively. Compared to the non-endemic area mean TSH concentration was higher (4.4 vs. 2.4 mIU/L, $p < 0.001$), FT3 was lower (5.1 vs. 5.7 pmol/L, $p < 0.001$) and FT4 was also lower (1.6 vs. 17.6 pmol/L, $p = 0.10$) although the difference for FT3 and FT4 did not reach statistical significance. The authors reported that 80% children in the endemic area had well-defined thyroid hormonal aberrations while the corresponding percentage was not reported for the non-endemic area.

For 6 studies rated Tier 3, increased mean concentrations of TSH were reported in 5 studies when comparing children from areas with high versus low exposure to fluoride in drinking water (Kumar et al., 2018; Shaik et al., 2019; Singh et al., 2014; Susheela et al., 2005; Yang et al., 2008; Yasmin et al., 2013). Increased FT3 and T3 concentrations were reported in 2 and 3 studies, respectively. Results on FT4 and T4 were inconsistent and an overall comparison of thyroid hormone profiles is hampered by the fact that results on other thyroid hormones than TSH were not consistently measured across studies.

In summary, when comparing groups of children living in areas with low and high(er) water fluoride concentrations, higher mean TSH concentrations were reported in 10 out of 14 studies. The same consistent pattern was also observed in studies measuring fluoride concentrations in urine. Studies that reported an increased mean TSH ($n = 9$) when comparing groups of children exposed to low versus high fluoride in drinking water, reported higher ($n = 5$) or equal ($n = 1$) mean concentrations of FT3, higher ($n = 3$) or lower ($n = 1$) concentrations T3 and higher concentrations T4 ($n = 3$). For FT4, the pattern was not consistent ($n = 3$ higher and $n = 3$ lower).

3.2.4.2 | *Studies on fluoride in children including iodine measurements*

Five publications in children, two from the same study population had information on iodine status while examining the association between exposure to fluoride and thyroid hormones. A brief summary of these studies is provided below. Background information on iodine sufficiency and insufficiency is provided in Appendix B.1.

Du et al. (2021) (Tier 1) examined the association between fluoride exposure and thyroid hormones in 7–12 year old children in China ($n = 446$) without thyroid-related diseases and with sufficient iodine status as reflected by a mean (SD) urinary iodine concentration of 396 (191) μg/L. An increase of 1-SD in urinary fluoride concentration [mean (SD): 1.45 mg/L (0.88)] was associated with 0.22 cm^3 (95% CI: 0.14, 0.31) higher thyroid volume. No association was observed between urinary fluoride concentrations and TSH, TT3 or TT4.

Examining effect modifications of CREB1 gene polymorphisms, similar results for thyroid volume were observed but now a significant inverse association was reported between urinary fluoride and TT4 (Xu et al., 2022) (Tier 1). The slightly different findings from these two publications (Du et al., 2021; Xu et al., 2022) may be explained by differences in covariate adjustment, including use of interaction terms for urinary fluoride and iodine, between the two studies.

In another study, thyroid hormones and TSH were assessed in 413 Chinese children from Tianjin, aged 7–12 years, in areas with drinking water fluoride concentrations ranging between 0.24 and 3.81 mg/L (Wang et al., 2022) (Tier 2). Mean urinary fluoride concentrations in children with DF were significantly higher (1.5 mg/L, $n = 141$) compared to those without DF (0.78 mg/L, $n = 272$). Both groups were iodine sufficient as reflected by mean urinary iodine concentrations > 100 μg/L. No significant differences in FT3 (6.6 vs. 6.5 pmol/L), FT4 (17.3 vs. 18.4 pmol/L) or TSH (2.75 vs. 2.90 mIU/L) were observed between children with or without DF. However, mean thyroid volume was lower in children with DF compared to those without DF (2.52 and 3.63 mL, respectively).

Kutlucan et al. (2013) (Tier 3) compared children, 10–15 years old in Turkey varyingly exposed to fluoride in drinking water that were grouped in terms of having low (≤ 0.6 mg/L, mean 0.22 mg/L) and high (> 0.6 mg/L, mean: 0.48 mg/L) urinary fluoride concentrations. Mean urinary iodine concentrations were comparable between the groups (93 and 98 μg/L, $p = 0.08$). Absolute thyroid gland volumes did not differ between the two fluoride exposure groups (8.6 vs. 8.7 mL, $p = 0.62$). However, the results on thyroid volume need to be interpreted in the context that height, weight and BMI were all lower in the high fluoride group and not accounted for in statistical analyses.

In a Tier 3 study of children (< 16 years of age) in China living in an area with high fluoride (3.0 mg/L) and high iodine (1.1 mg/L) concentrations in drinking water ($n = 23$) were compared with children ($n = 33$) living in an area with low fluoride (0.5 mg/L) and low iodine (0.13 mg/L) concentrations in drinking water (Yang et al., 2008). Urinary fluoride in the high versus low fluoride areas was 2.1 versus 0.8 mg/L and the corresponding numbers for urinary iodine were 818 versus 212 μg/L. Radioactive iodine uptake in the thyroid gland was reduced in children from the high fluoride/high iodine area and mean serum TSH concentration was significantly higher compared to children from the control area (3.4 vs. 0.8 mIU/L, $p < 0.01$). Mean T4 concentration was non-significantly lower (148 vs. 128 ng/dL) in the high exposure area while no meaningful difference was observed for T3.

In summary, all except one of the above-mentioned studies (Yang et al., 2008), were conducted in populations with sufficient iodine status (urinary iodine concentration > 100 μg/L). As a result, no clear conclusions can be drawn on the role of iodine as effect modifier for the possible association between fluoride exposure and thyroid hormones.

3.2.4.3 | *Correlations between fluoride exposure markers and thyroid hormones*

Based on the mean concentrations of fluoride measured in water, urine or serum as reported in each study (often several exposure groups) reviewed in Sections 3.2.4.1–2 the relationship between fluoride exposure and thyroid hormone concentrations was further examined across studies. This was done to assess a possible dose–response relationship between markers of fluoride exposure, TSH and thyroid hormones. Such comparison, although somewhat limited due to heterogeneity of populations and study design, allows for comparison across broader exposure ranges than reflected in individual studies. The extracted data used for these analyses can be found in Annex D.7.

Based on these data the simple linear correlation between mean water, urinary and serum fluoride concentrations and the mean concentrations of thyroid hormones were examined and are presented in Table 13.

TABLE 13 Correlations between mean concentrations of fluoride in drinking water (wF), urine (uF) or serum (sF) and thyroid hormones in children (< 18 years) reported across studies reviewed in Sections 3.2.4.1–2.

	TSH	FT4	T4	FT3	T3
	n[a] = 24	$n = 10$	$n = 15$	$n = 12$	$n = 15$
wF	0.55 (0.19, 0.78)[b]	0.03 (−0.61, 0.65)	0.47 (−0.05, 0.79)[c]	0.42 (−0.20, 0.80)	0.10 (−0.43, 0.59)
	$n = 27$	$n = 15$	$n = 13$	$n = 17$	$n = 13$
uF	0.60 (0.29, 0.80)[b]	0.24 (−0.31, 0.67)	0.38 (−0.22, 0.77)	−0.05 (−0.51, 0.45)	−0.18 (−0.66, 0.42)
	n = 16	n = 9	n = 7	$n = 11$	n = 7
sF	0.46 (−0.05, 0.78)[c]	−0.27 (−0.79, 0.48)	−0.16 (−0.82, 0.67)	0.10 (−0.53, 0.66)	0.49 (−0.42, 0.91)

[a] n refers to number of datapoints (mean values).
[b] $p < 0.05$.
[c] $p < 0.10$.

Despite fewer datapoints than available when assessing markers of fluoride exposure (see Section 3.2.1) a moderately strong positive and significant correlation with serum TSH was observed for mean water fluoride concentrations ($r = 0.55$, $p < 0.01$) and mean urinary fluoride concentrations ($r = 0.60$, $p < 0.01$). No clear association was observed for other thyroid

hormones, which is largely in line with findings from individual studies. However, the lower number of datapoints available for analyses of thyroid hormones limits a strong conclusion from this analysis.

The scatter plots for the association between water and urinary fluoride concentration with TSH and FT4 are shown in Annex D.7. To further examine the relationship between water and urinary fluoride concentrations and serum TSH, water and fluoride concentrations were divided into three roughly equal groups plotted against mean TSH concentrations (Figure 4). Overall, these plots show substantially higher TSH on average (from ~2 mUI/L to ~3 mUI/L) when water fluoride concentrations increased from ≤ 0.7 mg/L to around 2 mg/L and a similar increase is observed for urinary fluoride concentrations above 2 mg/L (compared to concentrations < 2 mg/L). These changes appear to occur at relatively high exposures, as a concentration above 2 mg/L is well above the concentration reported in most studies from EU and North America assessing neurodevelopment, as reviewed in Section 3.2.2.

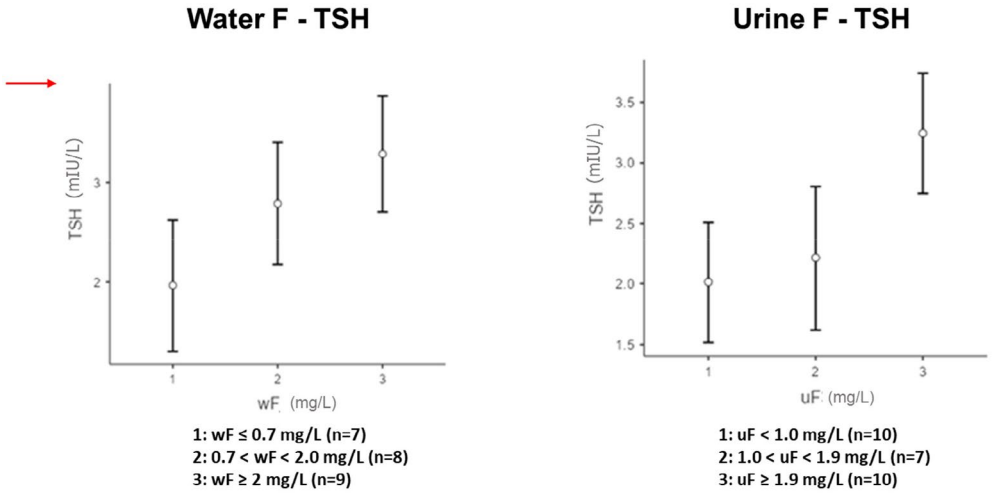

FIGURE 4 Left panel: Pooled data of mean water fluoride (wF, mg/L) and group mean TSH concentrations (mIU/L); right panel: Pooled data of mean urinary fluoride (uF, mg/L) and group mean TSH concentrations (mIU/L). Ranges of TSH values (bars) represent means of TSH concentrations as reported within different studies for each exposure category. Red arrow: Upper optimal TSH in individuals, 4 mIU/L.

3.2.4.4 | *Studies in adults*

Studies assessing the association between fluoride exposure and thyroid hormones in adults are briefly summarised below. Further information can be found in Table D.7. in Annex D. The number of studies identified for adults were too few to conduct similar analyses as for children shown in Section 3.2.4.3.

Cross-sectional studies

Using the Canadian Health Measures Survey (2012–2013), Malin et al. (2018) (Tier 1) examined the association between urinary concentration of fluoride and possible effect modification of iodine status in a sample of around 1000 participants who provided blood and urine samples. Overall, urinary fluoride concentrations [median (10th, 90th percentile): 0.74 mg/L (0.34, 1.73)] were not associated with TSH ($p = 0.43$). However, for those participants who were classified as iodine deficient, based on urinary iodine concentration ≤0.38 µmol/L (18%), urinary fluoride concentrations were positively associated with TSH [slope: 0.35 mIU/L (95% CI: 0.06,0.64) for each 1-mg increase in uF]. No association was observed among those classified as being iodine sufficient.

Kheradpisheh et al. (2018) (Tier 2) studied 198 cases with hypothyroidism and 213 controls from Iran. Subjects were grouped according to low (0–0.03 mg/L) or high (0.3–0.5 mg/L) fluoride concentration in drinking water. Living in the high versus low fluoride area was not associated with hypothyroidism [OR = 1.03 (95%CI: 0.70, 1.52)]. Both cases and controls living in the high fluoride area had significantly higher TSH compared to those living in the low fluoride area.

Across-sectional survey from UK linked data on fluoride in drinking water across England (2012–2013) with prevalence of hypothyroidism as reported in registries based on GP registered patient numbers in 2013 (Peckham et al., 2015) (Tier 3). Approximately 10% of the population covered in this analysis were living in areas with community water fluoridation schemes (> 0.7 mg/L). Compared to areas exposed to drinking water (0.2–0.3 mg/L) the prevalence of hypothyroidism was significantly higher in areas with medium (> 0.3–≤ 0.7 mg/L) and high (> 0.7 mg/L) water fluoride content. The corresponding prevalence odds ratios, adjusted for sex, age (< 40 vs. ≥ 40 years) and index of multiple deprivation were 1.4 (95% CI: 1.1, 1.7) and 1.6 (1.4, 1.9), respectively.

Finally, a study from India compared thyroid hormones among 63 adults living in an endemic fluorosis area in India (drinking water range 0.6–7.2 mg/L, mean: 2.8 mg/L) with 39 adults living in a control area with lower fluoride concentration in water (range: 0.3–0.8 mg/L, mean 0.5 mg/L). TSH and T4 concentrations were not significantly different between

males or females living in the control versus endemic area. Mean concentrations of T3 were, however, significantly higher in both males (1.9 vs. 1.1 pg/dL) and females (1.9 vs. 1.1 pg/dL) living in the endemic versus control area, respectively (Yasmin et al., 2013) (Tier 3).

Overall, these studies in adults provide some evidence for a link between fluoride exposure and thyroid function. However, compared to the studies in children the findings are less clear largely due to the limited number of studies and the heterogeneity in study design.

Pregnant women

Studies from three pregnancy cohorts were identified reporting associations between maternal fluoride exposure and thyroid hormone concentrations during pregnancy.

Hall et al. (2023) (Tier 1) classified 1508 women from the MIREC cohort as euthyroid ($n = 1301$), subclinical hypothyroid ($n = 100$) or primary hypothyroid ($n = 107$) based on their thyroid hormone levels in the first trimester. Mean (SD) concentrations of fluoride in drinking water and urine were 0.41 mg/L (0.26) and 0.59 mg/L (0.42), respectively. Each 0.5 mg/L increase in drinking water fluoride concentration was associated with a 1.65 (95% CI: 1.04, 2.60) higher odds of primary hypothyroidism. However, a non-significant association was observed between urinary fluoride and primary hypothyroidism [OR: 1.00 (95% CI: 0.73, 1.39].

In a later study from the same cohort, Hall et al. (2024) (Tier 1) examined the association between the fluoride concentration in water and urine and fluoride intake with thyroid hormones among 1876 women from the MIREC cohort. Water and urinary fluoride concentrations were not associated with TSH. However, each 0.5 mg/L increase in fluoride concentrations in drinking water was inversely associated with FT4 [−0.04 ng/mL (95%CI: −0.08, −0.00), $p = 0.04$]. A non-significant trend was also observed with higher TT4 [3.9 ng/mL (95% CI: −0.5, 8.3), $p = 0.09$]. The same associations for urinary fluoride concentrations were in both cases not significant.

Data from the Swedish NICE birth cohort with 583 mother–child examined the association between maternal urinary fluoride concentrations with maternal thyroid hormones (both samples collected in week 29 of gestation (Kampouri et al., 2022). The median (5th, 95th percentile) maternal urinary fluoride concentration was 0.71 mg/L (0.31, 1.90). In linear regression analyses maternal urinary fluoride concentrations were not significantly associated with TSH, FT4 or FT3. However, maternal urinary fluoride concentrations were significantly associated with higher FT3 to FT4 ratio (slope per 1 mg increase in uF: 0.007, $p = 0.047$).

In a study of 152 women from Thailand living in an area with endemic fluorosis, Somporn et al. (2023) (Tier 3) examined the association between maternal urinary fluoride concentrations with serum FT3 and TSH. In this study maternal urinary fluoride concentrations were non-significantly correlated with higher serum TSH (Pearson $r = 0.21$) and non-significantly inversely associated with FT3 ($r = -0.18$). No information on fluoride concentrations in urine was provided in this study except that it was reported that participants were selected based on having urinary fluoride concentrations above 0.7 mg/L.

3.2.4.5 | *Evidence on effects on the thyroid from literature published before 2005*

Among the studies identified through the targeted search on thyroid effects prior 2005, three cross-sectional studies were appraised for internal validity (RoB). These included two Tier 1 studies and one Tier 3 study (Table 14).

TABLE 14 Heat map for the risk of bias performed for human studies on effects on the thyroid.[a]

Refid	Author	Year	Study design	TIER	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
12611	Jooste	1999	Cross-sectional	1	+	+	+	+	+	+	+	+
12653	Day	1972	Cross-sectional	1	+	+	+	+	+	+	+	+
12632	Gedalia	1963	Cross-sectional	3	-	-	+	+	+	-	+	-

[a]The heatmap indicates whether the criteria stated in questions 1–8 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

A number of studies investigated (endemic) goitre in relation to iodine deficiency or concentrations of fluoride and iodine in drinking water of lifelong residents, mainly children in South Africa (Jooste et al., 1999), Israel (Gedalia & Brand, 1963) and Nepal (Day & Powell-Jackson, 1972). Treatment with fluoride for cases of hyperthyroidism was described by (Galletti & Joyet, 1958).

No relationship was found between fluoride in drinking water and mild goitre prevalence (5%–18%) in four towns with either a low (0.3–0.5 mg fluoride/L) or near optimal (0.9–1.1. mg/L) fluoride in the water. However, the prevalence of goitre was higher (28% and 29%) in the two towns with high levels of fluoride in the water (1.7–2.6 mg/L). These results indicated that either a high fluoride level in the water or another goitrogen, other than iodine deficiency, may have been responsible for these goitres (Jooste et al., 1999).

Also, in Israel a high percentage of goitre was observed in areas with low concentrations of iodine in drinking water. However, when iodine was adequate, high concentrations of fluoride did not appear to have an effect on thyroid function

(Gedalia & Brand, 1963), and wide variations in goitre prevalence were not attributable to differences in iodine intake. Goitre prevalence was found to correlate with fluoride content ($r = 0.74$; $p < 0.01$) and hardness ($r = 0.77$; $p < 0.01$) of the water in each village. The effects of fluoride and water hardness were independent (Day & Powell-Jackson, 1972).

Overall, the Scientific Committee concludes that a deeper analysis of these older publications and their inclusion in the weight of evidence would not change the outcome of assessment based on the more recent literature.

3.2.4.6 | *Summary*

An association between water and urinary fluoride concentrations and higher TSH was seen across multiple studies comparing groups of people living in areas with low versus high fluoride concentrations in drinking water. Based on those analyses it appears that changes in TSH are occurring at fluoride concentrations in drinking water above those observed in most European populations, or urinary fluoride concentration around 2 mg/L or higher. No consistent pattern was observed for other thyroid hormones, which may relate to smaller number of studies and differences across studies in terms of which thyroid hormones were measured (i.e. FT3, T3, FT4, T4). At relatively high exposure there were some indications of higher thyroid volume in children. The few studies measuring urinary iodine were largely inconclusive in terms of effect modification by iodine status.

Although an increased TSH concentration may be of concern, it is not known on a population basis at what magnitude this becomes an adverse health effect. Life stage is an important determinant, with pregnant women and children considered the most vulnerable for thyroid dysfunction.

For studies in adults, including pregnant women, the data on the effect of fluoride on thyroid function are too limited to draw any conclusions.

3.2.5 | Bone health in humans

3.2.5.1 | *Previous EFSA conclusions on skeletal outcomes*

In 2005 the EFSA NDA Panel established an UL for fluoride of 7 mg per day (0.1 mg/kg bw per day) for children and adults 15 years or older (EFSA NDA Panel, 2005). This value was derived from a randomised controlled trial (RCT) in 202 postmenopausal women (Riggs et al., 1990; Riggs et al., 1994) who were treated with sodium fluoride ($n = 101$, 75 mg/day of NaF, equivalent to 34 mg/day of fluoride) or calcium as comparator (n = 101, 1.5 g/day). Over a follow-up period of 3 years women in the fluoride group experienced a significant higher rate of non-vertebral fractures compared to the calcium group [relative risk of 3.2 (95% CI, 1.8–5.6)]. An UF of 5 was applied and a UL of 0.12 mg/kg bw per day was established.

The NDA Panel noted that these findings were partly supported by other experimental studies where side effects in the form of lower limb pain occurred at a significantly higher frequency at fluoride doses higher than 0.4 mg/kg bw per day (Kleerekoper et al., 1991; Meunier et al., 1998; Riggs et al., 1990, 1994, 1982). These findings were interpreted as '*indicative of incomplete fractures of the bone*' (EFSA NDA Panel, 2005). Although reports of higher fracture risk with higher fluoride exposure were reported in some observational studies, they were less consistent, possibly due to lower exposures and inclusion of younger participants who were generally not at risk of low impact trauma.

Based on findings from RCTs it is also reasonably well established that fluoride treatment increases bone mineral density. The NDA Panel noted that a systematic review and meta-analysis of different trials published until 1998 found that fluoride treatment in middle-aged or older adults increased bone mineral density of the lumbar spine by 8% and 16% after 2 and 4 years of treatment, respectively ($n = 6$ studies) (Haguenauer et al., 2000). More modest increase in bone mineral density of the hip was observed of 3% ($n = 3$ studies) and 5% ($n = 2$ studies) after 2 and 4 years of treatment, respectively. A larger effect size for lumbar spine is not unexpected, as results from clinical trials with antiresorptive agents[52] also showed a larger increase in the bone mineral density of the lumbar spine compared to hip after 1 year of treatment (Hochberg et al., 2002). The results from the above-mentioned systematic review (Haguenauer et al., 2000) were used as support for the UL established for fluoride (EFSA NDA Panel, 2005). The meta-analysis concluded that after 4 years of treatment the relative risk of non-vertebral fractures, based on four studies, increased by 1.85 (95% CI 1.36–2.50). That is, the increased risk of fractures appeared to coincide with increase in bone mineral density following fluoride treatment. Due to the high doses used in these RCTs (Haguenauer et al., 2000), all above the existing UL of 7 mg/day for adults, interpreting their findings to effects that may occur at lower environmental exposure, is subject to high uncertainty.

Regarding the well-established effect of skeletal fluorosis, the EFSA NDA Panel did not consider this outcome as a basis for deriving a UL mainly due to uncertainties at what exposure this effect would occur (EFSA NDA Panel, 2005). However, it was noted that based on studies from India the prevalence of skeletal fluorosis was 4.4% at water fluoride levels of 1.4 mg/L and 63% at water fluoride levels of 6 mg/L. Crippling fluorosis was consistently found in villages with more than 3 mg fluoride/L (EFSA NDA Panel, 2005). Studies on bone cancer were also reviewed and it was concluded that '*no increased risk of developing cancer at the observed fluoride dose levels can be deduced*'.

[52]Osteoporosis is characterised by bone resorption exceeding bone formation and antiresorptive agents are used to reduce or reverse that imbalance.

For children the EFSA NDA Panel (2005) established a UL corresponding to 0.1 mg fluoride/kg bw per day based on *'occurrence of less than 5% of moderate forms of dental fluorosis'* as reported in studies by Dean and Fejerskov (Dean, 1942; Fejerskov et al., 1996).

3.2.5.2 | *Studies on bone health since 2003*

A total of 39 studies were identified that were either published after 2003 or not included in the assessment of a UL for fluoride (EFSA NDA Panel, 2005). Of these, 9 studies examined associations with bone cancer, 8 studies examined associations with risk of fractures, 11 studies examined associations with bone mineral density, 2 studies examined associations with osteoarthritis and 12 studies associations with other biomarkers of bone health. A summary of these studies is provided below.

3.2.5.2.1 | *Bone cancer*

A total of 9 studies on bone cancer were identified. Six studies were case–control studies on cancer (Archer et al., 2016; Bassin et al., 2006; Hayes et al., 2021; Kharb et al., 2012; Kim et al., 2011; Sandhu et al., 2011) that differed slightly in their selection of controls and exposure assessment. In addition, there were two cross-sectional studies and one ecological study[53] examining the association between community water fluoridation concentrations and incidence of bone cancer (Blakey et al., 2014; Crnosija et al., 2019; Young et al., 2015). The outcome of the study appraisal is shown in Table 15. A tabulated summary of these studies can be found in Annex D.

TABLE 15 Heat map for the risk of bias performed for studies on bone cancer.[a]

RefID	Author	Year	Study design	Tier	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
5226	Archer, N. P.	2016	Case–control	1	+	+	+	+	+	+	+	+
572	Blakey, K.	2014	Cross-sectional	2	+	+	++	-	+	+	+	+
2009	Bassin, E. B.	2006	Case–control	2	+	+	+	-	+	+	+	+
68	Kim, F. M.	2011	Case–control	3	-	+	-	+	++	-	+	+
5308	Kharb, S.	2012	Case–control	3	+	--	+	+	+	-	+	+
8446	Sandhu, R.	2011	Case–control	3	-	--	+	-	+	-	+	-
145	Hayes, C.	2021	Case–control	3	+	+	+	-	+	-	+	+
414	Crnosija, N.	2019	Cross-sectional	3	+	--	+	-	+	+	+	+

[a]The heatmap indicates whether the criteria stated in questions 1–8 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

One case–control study from the USA measured fluoride concentration in bone tissue among 137 osteosarcoma cases and 52 control patients who had benign tumours or non-neoplastic conditions (Kim et al., 2011). Another larger study from the USA, consisting of 261 osteosarcoma cases and 494 controls who had a malignant bone tumour (other than osteosarcoma) used self-reported measures of fluoride exposure, i.e. living in a fluoridated area or previous use of supplemental or topical fluoride (Hayes et al., 2021). Neither of these studies found an association with osteosarcoma. Two smaller studies (10 and 25 cases and 10 and 25 controls, respectively) from India did however find higher serum fluoride concentrations among cases with osteosarcoma compared to healthy controls (Kharb et al., 2012) or controls with bone-forming tumours other than osteosarcomas (Sandhu et al., 2011). The limitation of these two studies from India is that no information on characteristics of cases and controls was provided and no covariate adjustment was performed.

Two comparable case–control studies among US children and young adults (age < 20 years) reported opposite findings on cancer risk. In a study of 103 osteosarcoma cases and 215 matched controls (patients at the same orthopaedics department), Bassin et al. (2006) observed a positive association between exposure to fluoride via drinking water and osteosarcoma among males but not females (odds ratios ranging between 2–4 depending on age). However, another similar but larger study of 308 cases using two groups of controls who had other cancer diagnoses ($n = \sim 600$ each) found no association between previous drinking water exposure and osteosarcoma (Archer et al., 2016).

Two large cross-sectional population-based studies from the US found no association between fluoride intake with incidence of osteosarcoma, Ewing sarcoma or other cancers extracted from health records (Blakey et al., 2014; Crnosija et al., 2019). Both studies used, as proxy for exposure, concentrations of fluoride in drinking water based on monitoring

[53]Risk of Bias appraisal was not done for studies with ecological design.

data from water suppliers, which were linked with the participants' area of residency. The main limitations of these studies are limited confounder control and no validation of exposure through use of objective biomarkers.

Finally, one ecological study from UK found no association between exposure to fluoride via drinking water with incidence of osteosarcoma or other cancers (Young et al., 2015).

In summary, as most studies were of high risk of bias (see Table 15) and reported divergent findings. It is concluded, in line with the previous opinion (EFSA NDA Panel, 2005), that available evidence does not support an association between fluoride exposure and bone cancer.

3.2.5.2.2 | *Bone fractures*

One RCT from 2003 reporting results on fractures in relation to fluoride supplementation, was identified that was not included in the previous opinion on fluoride (EFSA NDA Panel, 2005). This study was judged to be of high risk of bias (Tier 3, see Table 16). In addition, 4 observational studies with all but one of them judged to be at high risk of bias (Tier 3), 2 ecological studies and one case series reporting results on fractures were identified.[54] A tabulated summary of these studies can be found in Annex D.

Of note is the fact that no new (since 2003) RCTs were identified. This may reflect that fluoride treatment is no longer recommended in many clinical guidelines.

TABLE 16 Heat map for the risk of bias performed for studies on bone fractures.[a]

RefID	Author	Year	Study design	Tier	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
433	Helte, E.	2021	Cohort	1	+	+	+	+	++	+	+	+
68	Kim, F. M.	2011	Case–control	3	-	+	-	+	++	-	+	+
4534	Sowers, M.	2005	Cross-sectional	3	-	-	+	+	+	-	+	-
1596	Nasman, P.	2013	Cohort	3	++	-	++	-	+	+	+	+

[a]The heatmap indicates whether the criteria stated in questions 1–8 or 1–9 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

The RCT by von Tirpitz et al. (2003) recruited young women (mean age 37 years) with Crohn's disease[55] who were assigned to either calcium and vitamin D (800 mg and 1000 IU respectively, $n = 12$) or same treatment plus sodium fluoride (25 mg/day or 11.3 mg fluoride /day, $n = 36$) with a follow-up period of 27 months. The fluoride group took a 3-months break from treatment after 12 months. No new vertebral fractures were observed in the treatment groups over the follow-up period. No information on non-vertebral fractures was reported. With few subjects in each treatment group, no conclusions on fractures can be drawn from this study.

The one observational study judged to be of low risk of bias (Helte et al., 2021) examined the association between fluoride exposure, assessed via urinary fluoride concentration ($n = 4306$) and estimated intake from diet and drinking water through questionnaires ($n = 4072$), and incident fractures in postmenopausal women from Sweden (Helte et al., 2021). Information on fractures was obtained from the Swedish National Board of Health and Welfare's National Patient Register (NPR). The incident cases of all fractures, major osteoporosis fractures and hip fractures over a follow-up period of 9.3 years were 859, 529 and 187, respectively. Women had been exposed to drinking water with fluoride concentrations ranging up to 1.0 mg/L; the majority had been exposed to concentrations of ~1.0 mg/L. Drinking water, coffee and tea accounted for around 80% of fluoride intake (mean ± SD: 2.2 mg/day ±0.9) and estimated fluoride intake was modestly correlated with fluoride concentration in urine ($r = \sim 0.4$). Fluoride intake from diet/drinking water was positively associated with incident hip fractures with a hazard ratio of 1.6 (95% CI: 1.1, 2.3) when comparing the highest to lowest (median 2.9 vs. 1.4 mg/day) tertiles of exposure. The hazard ratio for urinary fluoride concentration was 1.50 (95% CI: 1.04, 2.17) when comparing the lowest to the highest tertiles of exposure (median 0.7 vs. 1.6 mg/g creatinine). For both measures of exposure, there was a modest association with major osteoporosis fractures that did not reach statistical significance. Associations for all fractures were non-significant and the effect estimate was close to null (indicating no risk).

All other studies were judged to be at high risk of bias. Of those, a prospective cohort study ($n = 1300$) recruiting 20–90 year old females from a population in the US with low to high drinking water fluoride concentrations (1.0 or 4.0 mg/L) found no association with fractures (Sowers et al., 2005). It is worth noting that the women recruited were highly diverse in terms of age (20–90 years) and that the number of fracture cases was small (only 33 for osteoporosis fractures and 41 for non-osteoporosis fractures).

In a registry-based cohort study from Sweden including all individuals born between 1900 and 1919 ($n = 473,277$), no association was observed [hazard ratio: 0.98 (95% CI: 0.93, 1.04)] between drinking water exposure (≥ 1.5 vs. < 0.3 mg/L) assessed through area of residency, with incident hip fractures occurring until end of 2006 (Nasman et al., 2013).

[54]Risk of Bias appraisal was not done for studies with ecological and case series designs.

[55]Osteoporosis is a common complication in subjects with Crohn's disease.

A case–control study from the US compared previous history of fractures among 137 osteosarcoma cases and 52 controls (Kim et al., 2011) and found no association between bone fluoride concentrations and history of fractures [odds ratio: 1.33 (95% CI: 0.56–3.15)]. Given the small study size (on fractures) and focus on a population with osteosarcoma, no firm conclusions on fracture risk can be drawn from this study.

In a study of ecological design, Young et al. examined the association between drinking water exposure to fluoride and hip fractures across different areas with and without water fluoridation in UK (England) (Young et al., 2015). The prevalence of hip fractures in a given area was extracted from hospital records. No association of fluoridated areas and hip fractures was observed.

An ecological study comparing rates of fractures in 6–10 year old children across different states in the US with or without community water fluoridation found higher rates of both bone forearm fractures and femur fractures among children living in states with high percentage of communities with fluoridated drinking water (Lindsay et al., 2023).

Finally, a case series study from France examining 60 cases of insufficiency fractures admitted to a hospital between 1989 and 1997, reported that 6 patients had received fluoride treatment; no control group was used (Soubrier et al., 2003). Due to the nature of this study (retrospective analyses of fracture cases), no risk of bias was performed for this study but for sake of completeness, its results are noted.

In summary, there is some evidence from a sufficiently large study from Sweden that relatively low fluoride exposure might be associated with higher risk of hip fractures in postmenopausal women. This appears to occur at dietary intake levels of ~3 mg/day (or urinary fluoride levels of ~1.6 mg/g creatinine). Fluoride exposure was assessed through diet and drinking water and in postmenopausal women, who are known to be at considerably higher risk of experiencing fractures. The findings from the study indicate effects of fluoride on bone at levels below those reported in previous RCTs that formed the basis of the existing UL for fluoride based on fractures occurring in postmenopausal women after 4 years fluoride treatment (EFSA NDA Panel, 2005; Riggs et al., 1990). The remaining observational studies do not provide strong support for or against these findings owing to methodological limitations as reflected by their risk of bias evaluation.

3.2.5.3 | *Evidence on osteoarthritis*

Two studies assessed the association between fluoride exposure and osteoarthritis. These studies were not appraised for risk of bias. Sowanou et al. (2022) in a case–control study in China evaluated the association between urine fluoride concentrations (measured after the diagnosis of osteoarthritis) and knee or elbow osteoarthritis ($n = 372$, born and raised in the study area). The water fluoride concentrations for the communities under study ranged from 0.9 to 2.3 mg/L (mean 1.5 mg/L) and the mean urine fluoride concentration in the control group was 2.4 (SD 1.2) mg/L. In the fully adjusted model, each 1 mg/L increase in urine fluoride concentration was associated with an increased odds for osteoarthritis (OR = 1.27, 95% CI: 1.06–1.52). In the adjusted per quartile analysis, a statistically significant association was observed for the comparison between the 1st (< 1.6 mg/L) and the 4th (> 3.3 mg/L) quartile of urine fluoride concentration (OR, 2.46; 95% 1.34, 4.57).

Meng et al. (2023) performed a cross-sectional study in China ($n = 1128$) to assess the association between water and urinary fluoride concentrations and knee osteoarthritis. The median water and urine fluoride concentrations in the control group were 0.34 mg/L (IQR; 0.23, 0.76) and 1.17 mg/L (IQR; 0.66, 2.12), respectively. A statistically significant association was observed for water fluoride concentration (per 1 mg/L increase; OR 1.32; 95% CI 1.16, 1.50) as well as in the per quartile analysis for the 4th versus 1st quartile (OR 2.03 (95% CI 1.41, 2.9). For the urine fluoride concentrations, the observed association was also statistically significant (per 1 mg/L increase; OR 1.21; 95% CI 1.12, 1.31).

Overall, the available evidence on the association of fluoride exposure and osteoarthritis is limited and firm conclusions cannot be drawn.

3.2.5.4 | *Bone mineral density*

One RCT and 12 observational studies on bone mineral density were identified (Table 17). The latter represents findings from 5 different cross-sectional or longitudinal cohorts.

TABLE 17 Heat map for the risk of bias performed for studies on bone mineral density.[a]

RefID	Author	Year	Study design	Tier	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
433	Helte, E.	2021	Cohort	1	+	+	+	+	++	+	+	+
2405	Levy, S. M.	2009	Cohort	2	+	+	-	-	+	+	+	+
2406	Levy, S. M.	2018	Cohort	2	+	+	-	-	+	++	+	+
4365	Levy, S. M.	2014	Cohort	2	+	+	-	-	+	++	+	+
RefID	Author	Year	Study design	Tier	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
86	Gao, M.	2020	Cross-sectional	1	+	+	+	+	+	+	+	+
2578	Godebo, T. R.	2020	Cross-sectional	1	+	+	+	+	+	+	+	+

TABLE 17 (Continued)

RefID	Author	Year	Study design	Tier	Q1. Compare	Q2. Confound	Q3. Attrition	Q4. Exposure	Q5. Outcome	Q6. Temporal	Q7. Report	Q8. Statistics
10015	Saha	2021	Cross-sectional	1	+	+	++	+	+	++	+	+
3887	Khandare, A. L.	2007	Cross-sectional	3	+	-	-	+	+	-	+	+
6606	Chachra, D.	2010	Cross-sectional	3	-	-	+	+	+	+	+	+
9588	Topuz, O.	2006	Cross-sectional	3	+	+	+	+	+	-	+	-
10069	Kodsup, P.	2022	Cross-sectional	3	+	-	+	+	+	-	+	+
9501	Yildiz	2003	Cross-sectional	3	-	-	+	+	+	+	+	+

[a]The heatmap indicates whether the criteria stated in questions 1–8 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

The above-mentioned RCT reported results on fractures in 68 young women (mean 36–37 years) with Crohn's disease, randomised to treatment with either calcium citrate (800 mg/day) and vitamin D3 (1000 IU, $n = 12$) or the same treatment plus sodium fluoride (25 mg/day or ~ 11 mg fluoride, $n = 36$) (von Tirpitz et al., 2003). The T-score for the lumbar spine in the fluoride group increased significantly from -1.95 ± 0.15 to -1.36 ± 0.18 over 2 years of follow-up. This increase in T-score was significantly higher than what was observed in the control group that received calcium and vitamin D supplements only (-1.58 ± 0.08 at baseline to -1.33 ± 0.11 at endpoint).

Concerning the observational studies, several studies in children and adolescents were identified from the longitudinal *Iowa Bone Developmental Study* (US). In that study self-reported fluoride intake from food and drinking water were assessed every 4–6 months and ranged (mean (SD)) from 0.54 mg/day (0.20) to 0.81 mg/day (0.41) depending on age and sex. Bone mineral density and other measures of bone structure were assessed via DXA-scanning of the whole body and lumbar spine roughly every 3 years. Around 70% of participants was reported to have had access to 'optimally fluoridated water' (Levy et al., 2018), without defining that further. In one of the studies, the authors examined the correlation between fluoride intake with bone mineral density and content at ages < 3, 3–6, 6–8.5 and 8.5–11 years ($n = 385$–418) (Levy et al., 2009). After adjustment for covariates, no clear association with bone mineral density or other related outcomes was observed. The same conclusions were reached in another study ($n = 424$) examining the same correlations in 0–11 year old children through different analyses (Levy et al., 2018). Similarly, no clear findings were reported in two later studies with follow-up until age 15 ($n = 358$) and 19 years ($n = 324$) (Levy et al., 2014; Saha et al., 2021).

In the Swedish Mammography cohort (Helte et al., 2021) associations between fluoride exposure (estimated intakes and urinary concentrations) were also examined in relation to bone mineral density. The mean (SD) bone mineral density of the lumbar spine and hip (femoral neck) were 1.12 (0.20) and 0.87 (0.12) g/cm^3, respectively. After adjustment for covariates the mean bone mineral density of the lumbar spine and hip were significantly higher corresponding to around 1.2% and 1.0% increase from the mean value when comparing the highest versus lowest tertile of creatinine adjusted urinary fluoride excretion (1.9 vs. 0.7 mg/g creatinine). The corresponding results for highest versus lowest tertile of dietary fluoride intake were 1.7% and 0.9% for lumbar spine and hip, respectively (3.2 vs. 1.3 mg fluoride/L drinking water).

A cross-sectional study from Canada compared fluoride content and structural and mechanistic properties of bone samples from the hip (femoral head) collected from subjects undergoing hip-replacement (Chachra et al., 2010). The recruited participants had either been living in Toronto ($n = 53$) and exposed to fluoridated water (1 mg/L) or in Montreal ($n = 39$) and had not been exposed to fluoridated water. The mean concentration of bone fluoride among those exposed and not exposed to fluoridated water was 1033 and 643 μg/g, respectively (or 61% higher, $p < 0.0001$). The bone mineral density was also significantly higher among those exposed compared to those not exposed to fluoridated water (0.90 vs. 0.75 g/mc^3 or 20% higher, $p < 0.05$). Furthermore, fluoride content in the bone was weakly but significantly associated with lower ultimate compressive stress (a measure of bone strength), which explained ~5% of the total variability. However, some care should be taken when interpreting these findings as age was not accounted for in these analyses.[56] Furthermore, the proportion of bone samples from males was higher (49%) in the fluoridated versus non-fluoridated area (38%) and this may partly explain the 20% difference in bone mineral density observed between the two regions.

A study from Turkey compared 45 postmenopausal women living in an area with a mean (SD) fluoride concentration in drinking water of 2.74 (0.64) mg/L to 41 women from a control area exposed to fluoride in drinking water of 0.53 (0.06) mg/L (Yıldız et al., 2003). The women in the exposed area were included based on the following criteria, (1) had been living in the area since birth, (2) having teeth indicating dental fluorosis, (3) having consumed water with fluoride concentrations > 1.2 mg/L and having urinary fluoride > 1.5 mg/L. Bone mineral density of the lumbar spine, femoral neck and trochanter were assessed by DXA scanning. Significantly higher bone mineral density of the lumbar spine (1.08 vs. 0.86 g/cm^2 or 26%), femoral neck (0.68 vs. 0.60 g/cm^2 or 13%) and trochanter (0.65 vs. 0.56 g/cm^2 or 16%) were observed in women with skeletal fluorosis from the exposed area versus women from the control area. No significant differences in age, BMI and clinical

[56]Bone strength decreases with age while fluoride content of the bone most likely increases.

markers of nutrition status (serum levels of total protein, albumin, prealbumin and transferrin) were observed between the exposed and control women.

Several other studies were conducted using surrogate measures of bone mineral density such as speed of sound. In addition, those measures were taken in other areas than the lumbar spine and hip which complicates interpretation. This limits comparison with the studies on bone mineral density described above.

A cross-sectional study from China conducted in 1124 adults with mean age (SD) of 47.8 years (8.8) examined the association between urinary fluoride concentration and bone mineral density measured in the left calcaneus using an ultrasound bone densitometer (Gao et al., 2020). The participants were recruited from two areas with low and elevated fluoride in drinking water as defined by urinary fluoride concentration (≤ 1.6 vs. > 1.6 mg/L). No association between urinary fluoride concentration and bone mineral density in the left calcaneus was observed. In contrast, a comparison of 12 subjects from India who had been exposed to drinking water with 4.5 mg/L fluoride, reported significantly higher bone mineral density of the femoral neck (43%) compared to 12 subjects from a control area (fluoride in drinking water < 1.5 mg/L) (mean age of 53 years) Khandare et al., 2007).

A study from Ethiopia conducted in a population exposed to fluoride in drinking water ranging between 0.3 and 15.5 mg/L assessed speed of sound measurements of the cortical radius, tibia and phalanx in 341 subjects aged 10–70 years (Godebo et al., 2020). In terms of interpreting these results, lower speed of sound has been associated with net bone loss, abnormal mineralisation and collagen formation, or altered microarchitecture (Hoffmeister et al., 2002; Wang et al., 2014). In adjusted analysis, each 1 mg/L increase of fluoride in drinking water was associated with 15.8 m/s (95% CI: –21.3 to –10.3), lower tibial speed of sound. When the exposure was divided in categories the decrease in speed of sound appeared to occur at relatively high exposures (> 6 mg fluoride/L) with non-significant differences observed between lower (< 2 mg/L) and more moderate (2–6 mg/L) exposure. A later publication from the same study showed higher concentrations of Ca, Mg, B and Sr with higher concentrations of fluoride in drinking water (Kodsup et al., 2022).

Another study from Turkey recruited 122 subjects from an endemic fluorosis area and 117 controls and compared heel broadband speed of sound and urinary C-terminal telopeptide of type I collagen between cases and controls stratified by age and sex (Topuz et al., 2006). Overall, no clear differences were observed between the two areas for speed of sound measurement while the CTX measures where ~ 1 and ~ 2 standard deviations higher for men and women living in the fluorosis area compared to controls of the same age and sex.

In summary, in line with previous observations from RCTs (Haguenauer et al., 2000) the overall evidence from the above studies suggests that fluoride exposure even at relatively low water concentrations is associated with increase in bone mineral density. This conclusion is supported by the relatively large study from Sweden (Helte et al., 2021) where around 1% increase in bone mineral density of hip and lumbar spine in postmenopausal women was observed at exposures corresponding to water fluoride concentrations of ~ 1 mg/L. In the context of such low water fluoride concentration, it is noted that the bone content of fluoride is 61% higher in bone samples of Canadian subjects exposed to fluoridated (with 1 mg/L) than subjects consuming non-fluoridated water (Chachra et al., 2010). At high life-long exposure (mean water concentration of 2.7 mg/L) a higher increase in bone mineral density of the hip (13%–16%) was reported in middle-aged women from Turkey (Yıldız et al., 2003). This is around twice the effect size seen at higher doses in short-term (1–3 year) RCTs (Haguenauer et al., 2000), suggesting that duration of exposure, and not just dose, is an important factor. Taken together these results suggest that there may be a continuous increase in bone mineral density occurring from relatively low exposures (from drinking water of ~ 1 mg fluoride/L).

No firm conclusions can be drawn from the other studies due to differences in age or recruited participants (Levy et al., 2018, 2009, 2014; Saha et al., 2021) or more proxy measures of bone mineral density used (Gao et al., 2020; Godebo et al., 2020; Khandare et al., 2007; Kodsup et al., 2022).

3.2.5.5 | *Other biomarkers of bone health*

Biomarkers of bone turnover are markers of bone formation (from osteoblasts) and bone resorption (from osteoclasts and collagen breakdown products). Bone turnover involves bone resorption followed by bone formation, complicating the interpretation of these biomarkers. While these markers change substantially following treatment with osteoporosis medication, the interpretation of smaller changes becomes more difficult when assessing exposures to other factors with potentially smaller effects.

Three RCTs and 9 observational studies were identified that reported associations (or effects) of fluoride exposure in relation to biomarkers of bone turnover or other biomarkers of bone health. These studies were not appraised for risk of bias.

Although only one RCT, reviewed above (von Tirpitz et al., 2003), was identified as relevant for fractures and bone mineral density,[57] two other RCT studies are included in this section. Those studies (Morabito et al., 2003; Reginster et al., 2003) reported results of treatment with antiresorptive agents alone or in combination with fluoride. As such, they provide some information on the effect of fluoride on biomarkers of bone reabsorption in subjects taking antiresorptive agents, thus not allowing for assessment of the independent effect of fluoride during treatment.

[57]For the two studies included here both groups were treated with antiresorptive agents which makes it impossible to draw any meaningful conclusions on fractures or bone mineral density. However, comparison between those treated with antiresorptive agent or antiresorptive agent plus fluoride allows for some conclusion on possible effects of fluoride on biomarkers of bone health.

In the above-mentioned RCT in women (mean age 37 years) with Crohn's disease (von Tirpitz et al., 2003) randomised to treatment with either calcium and vitamin D (800 mg and 1000 IU daily, respectively; $n = 12$) or the same treatment plus sodium fluoride (25 mg/day or 11.3 mg fluoride, $n = 36$) non-significant but consistently higher osteocalcin concentrations (6 vs. 4 ng/mL after 27 months of treatment) were observed in the fluoride group compared to controls. No marked differences were observed for carboxy-terminal cross-linked type I collagen telopeptide and osteoprotegerin. The low and uneven sample size between controls ($n = 12$) and fluoride-treated persons ($n = 36$) is a limitation of this study.

In another RCT, Morabito et al. (2003) randomised 40 postmenopausal osteoporotic women to either pamidronate (45 mg/day) or the same treatment plus sodium fluoride (50 mg/day, 22.6 mg fluoride) with follow-up of 3 years. Relative to the pamidronate group, those receiving co-treatment with fluoride had significantly higher serum concentrations of osteocalcin (0.86 ± 0.11 vs. 0.72 ± 0.11 pmol/mL), bone-alkaline phosphatase (bone ALP) (34.3 ± 6.5 vs. 29.3 ± 5.3 μg/L) and insulin growth factor-1 (14.7 ± 1.4 vs. 12.2 ± 1.1 nmol/L) after 27 months of treatment. Furthermore, these biomarkers reduced over the treatment period in the pamidronate-treated subjects while co-treatment with fluoride significantly increased these biomarkers.

Comparable results were observed in a much larger RCT of 596 women (mean age 62 years) who had either osteopenia or osteoporosis and were randomised to receive for 18 months monofluorophosphate alone or monofluorophosphate plus raloxifene (Reginster et al., 2003). The fluoride treatment corresponded to around 20 mg fluoride per day. All subjects also received calcium (1000 mg/day) and vitamin D (500 IU/day). After 18 months of treatment cross-linked type I collagen telopeptide and bone specific ALP increased significantly by 13% and 27%, respectively in the fluoride group relative to those treated with fluoride plus raloxifene.

Most observational studies were conducted in areas with high drinking water concentrations (> 2.0 mg/L) comparing those subjects with controls exposed to lower water concentrations (< 1.0 mg/L).

Studies comparing children (Gupta et al., 2008; Khandare et al., 2017), young adults (Koroglu et al., 2011) or postmenopausal women (Goudu & Naidu, 2013; Ravula et al., 2012) with high versus low fluoride exposure from drinking water consistently found higher parathyroid hormone (PTH) of subjects in the high water fluoride area. In several but not all of these studies the exposed subjects had confirmed skeletal fluorosis and the mean difference in PTH between those more highly exposed versus controls ranged from 20% to > 100% depending on exposure. One study in pregnant women reported higher PTH in pregnant women exposed to low (0.5 mg/L) versus high (2.7 mg/L) fluoride from drinking water (Thippeswamy et al., 2021). A higher prevalence of women with sub-optimal vitamin D status among the women with lower fluoride exposure may however have confounded this comparison.

Several studies also reported on serum ALP in children, pregnant women and adults recruited from areas with high and low fluoride concentrations in drinking water. These studies did not measure bone specific ALP. The reported findings are difficult to interpret with one study reporting no differences (Ravula et al., 2012; Yıldız et al., 2003) and another reporting a negative correlation between fluoride exposure and serum ALP (Manjunathappa, Devegowda, Mysore, Vishwanath, & Narayana, 2023) in pregnant women. Others reported significantly higher serum ALP with higher fluoride exposures (Goudu & Naidu, 2013; Ravula et al., 2012). One study measured bone specific ALP and observed significantly higher levels with higher fluoride exposure (Khandare et al., 2007). In addition, two studies reported divergent findings on serum phosphate levels (Koroglu et al., 2011; Manjunathappa et al., 2023).

In summary, the above-mentioned studies, particularly findings from the RCTs, are in line with previous observations that fluoride influences bone formation and metabolism. Lack of studies examining these relationships at low fluoride concentrations limits interpretation of the above-mentioned findings.

3.2.6 | Dental fluorosis

The causal relationship between fluoride exposure and dental fluorosis is well established. New studies that became available since the previous EFSA assessment were screened but were not found to challenge existing evidence. Therefore, the evidence on dental fluorosis was not appraised for risk of bias and only a summary is provided here.

Dental fluorosis is a condition causing changes in the tooth's enamel appearance resulting from hypomineralisation, as a consequence of excess intake of fluoride during tooth-forming years, referred to as primary and permanent dentition phases.

The crowns of the primary anterior teeth are mineralised before birth and are not affected by dental fluorosis under low fluoride conditions. However, in areas with very high concentrations of fluoride in drinking water, there is evidence of primary tooth fluorosis at the cervical parts of the crowns, possibly as a result of pre- and postnatal exposure. Dental fluorosis in primary posterior molar teeth may occur seldomly as a result of postnatal exposure.

The mineralisation of the permanent teeth starts at birth. A later fluoride exposure will appear as fluorosis lower down on the teeth. The risk of dental fluorosis is highest before the age of 3 years (mostly of very mild or mild form at the rim of the teeth) and for the first and second molars by the age of 8 years, with negligible risk thereafter. Mild dental fluorosis is the first clinical indicator of fluoride activity at doses where the beneficial effects may begin to overlap with the changes to the enamel. Mild fluorosis is more difficult to diagnose and may be missed or overlooked. Very mild and mild forms of dental fluorosis have an aesthetic effect and have been associated with a decrease in the prevalence of dental caries. Whether an aesthetic effect of mild fluorosis may be considered adverse is subjective, as it may result in psychological effects but no physical adverse effects on teeth. Diagnosis of moderate or severe fluorosis is less equivocal. Adverse effects

on the teeth integrity and function are associated with moderate to severe fluorosis. In addition, the aesthetic impact of moderate fluorosis is considerably more visible and unpleasant than that of the milder forms and it is not reversible unless with professional aesthetic treatment.

The NDA Panel established in 2005 the UL for young children (1–8 years) based on the prevalence of moderate to severe dental fluorosis of less than 5% in populations of children ingesting 0.08–0.12 mg fluoride/kg bw per day, as described by Dean (1942). The Panel considered moderate dental fluorosis, which is characterised by brown staining and minute pitting of teeth, to be an adverse effect. The UL was set at 0.1 mg/kg bw per day (1–3 years: 1.5 mg/day and 4–8 years: 2.5 mg/day).

The study by Dean (1942) assessed the prevalence of dental fluorosis in 5824 children across several states and communities in the US with drinking water fluoride concentrations up to 14.1 mg/L. This study was also the basis of the assessment conducted by U.S. EPA (2010) and more recently by Health Canada (2023). An overview of these assessments and the resulting HBGVs is presented in Table 18.

The data presented in Dean (1942) on the characterisation of the beneficial and adverse effects of fluoride dental health are still considered robust data to this day with respect to fluoride exposure due to minimal confounding by sources of fluoride other than drinking water (Health Canada, 2023). The data from Dean (1942) are supported by later studies, as presented in more detail in the assessments conducted by the NDA Panel (2005) and the U.S. EPA (2010). Taken together, these studies showed that prevalence of dental fluorosis, in populations of comparable ethnicity and socioeconomical status exposed to different drinking water fluoridation levels, was very low or absent in areas with 'optimal' fluoride concentrations in drinking water (up to 1 mg/L). The studies also showed that severe fluorosis consistently occurred at fluoride concentrations around 2 mg/L. However, in their assessment, the U.S. EPA (2010) noted that later studies from the US conducted in the 1950s and 1960s observed both moderate and severe fluorosis in permanent residents with drinking water levels between 1 and 2 mg/L (prevalence of severe fluorosis ≤ 3%) (Driscoll et al., 1983, 1986; Galagan & Lamson Jr., 1953; Heifetz et al., 1988; Horowitz et al., 1984; Richards et al., 1967; Selwitz et al., 1995, 1998). These studies were conducted across fewer water districts and are therefore less suitable for modelling.

At the time of the Dean study, food was the only other source of fluoride. The contribution of fluoride from food was not characterised, which is a source of uncertainty (see uncertainty assessment section). The absence of fluoridated dental care products at the time of the Dean study is not subject to uncertainty. Additional fluoridation sources, including dental care products, appeared in the early 1970's. Studies after this period show that approximately 10% moderate dental fluorosis (and < 1% severe grade) occurs at water fluoride levels twice the optimal fluoridation level (i.e. at 2 mg/L) and above (Heifetz et al., 1988; Selwitz et al., 1995).

TABLE 18 Overview of previous assessments by other agencies of dental fluorosis data from the Dean study.

Dental fluorosis assessments	Adversity grade of DF	Prevalence, (%)	BMCL (or reference point, water fluoride) (mg/L)	HBGV (mg/kg bw per day)	Includes fluoride from food
IOM (1997)	Moderate	(5)	(2)	0.1 (0.08–0.12)	No
NDA Panel (2005)	Moderate	(5)	NA	0.1	No
US EPA (2010)	Severe	0.5	1.87	0.08	Yes (0.01 mg/kg bw per day)
Health Canada (2023)	Moderate	1	1.56	NA (UF not determined)	TBD: Water allocation factor of 0.5

A total of 172 studies published after 2005, were identified that reported on fluoride intake and dental fluorosis. Of those, most studies reported prevalence of dental fluorosis in areas in Asia and Africa with high levels of naturally occurring fluoride in drinking water and a high prevalence of dental fluorosis.

Some studies suggest that dental fluorosis occurs at lower drinking water fluoride concentrations. As an example, in a study by Ding et al. (2011) carried out in China with 331 children, aged 7–14 years exposed to fluoride in drinking water with a range of 0.23–2.84 mg/L (mean 1.31 mg/L), dental fluorosis was assessed using Dean's index. A clear dose–response relationship was reported between urinary fluoride and dental fluorosis. Mean urine fluoride concentrations of 0.80, 1.13, 1.11, 1.31 and 1.46 mg/L in children were associated with, respectively, no ($n = 136$), questionable ($n = 54$), very mild ($n = 74$), mild ($n = 39$) and moderate fluorosis ($n = 28$). None of the children had severe fluorosis. The urine fluoride levels associated with moderate fluorosis would correspond to drinking water concentrations < 2 mg/L. Similar observations have been reported in earlier studies (published before 2003).

Although out of scope of this mandate, it is noted that the caries preventive effect of fluoride does not result from the incorporation of fluoride ions into the enamel but rather from the interaction of fluoride ions in the local interface between the enamel, the dental microbiota and saliva in a dynamic interplay. This results in slower (reduced) enamel demineralisation in the acidic oral environment occurring after a meal and enhanced re-mineralisation when the oral pH rises again. Hence, regular and multiple daily application of low fluoride concentration in this interface is the current professional recommendation (Toumba et al., 2019).

3.2.7 | Other endpoints

A number of additional outcomes and endpoints have been investigated in the human studies about the effects of fluoride exposure. The most common investigated endpoints have been connective tissue abnormalities, endocrine alterations and disease (other than those affecting the thyroid), abnormalities in vitamin D status, alterations in blood chemistry, lipid peroxidation and genotoxicity, metabolic abnormalities, increased blood pressure levels and risk of hypertension, and birth defects. In general evidence supporting a positive association between fluoride exposure and such endpoints is sparse, limited and inconsistent, with little evidence of meaningful effect sizes and dose–response relations. In addition, such studies have limitations and biases that affect their internal validity. Overall, little evidence of a positive association between habitual levels of fluoride exposure occurring in the human and such other endpoints has been provided to date, and such evidence is therefore not considered to be suitable for further consideration.

A few studies have reported effects of fluoride exposure during pregnancy, other than the prioritised health effects discussed above (DNT, thyroid, bone effects), and are summarised here. Fluoride effects on birth size and gestational age at birth were assessed in the Swedish NICE birth cohort with 583 mother–child pairs (Kampouri et al., 2022). Every 1 mg/L increase of maternal uF was associated with a mean increase in birth weight of 84 g, length of 0.41 cm, head circumference of 0.3 cm and with increased odds of being born large for gestational age. The fluoride-related associations with increased size at birth were not explained by changes in maternal TSH concentrations.

Aghaei et al. (2015) found in a study of 35 communities in Iran with 492 infants, that birth length and weight were positively correlated with drinking water fluoride when fluoride levels were < 0.7 mg/L but not when water fluoride levels were > 1.5 mg/L.

In the MIREC study, maternal fluoride intake and water fluoride concentration was associated with reduced visual acuity and alterations in cardiac function of children. However, no statistically significant associations were observed between maternal urinary fluoride concentration adjusted for specific gravity and visual acuity or cardiac function (Krzeczkowski et al., 2024).

Goyal et al. (2020) examined 600 pregnant women with a gestational age of less than 20 weeks and with urinary fluoride levels > 1.5 mg/L; 67% of the women were anaemic. When divided in groups based on urinary fluoride concentrations, adverse fetal outcomes were detected for 46% in group 1 (urinary fluoride concentration 1.0–1.9), 76% in group 2 (urinary fluoride concentration 2.0–2.9) and 97% in group 3 (urinary fluoride concentration ≥ 3 mg/L). Eighty-one women had serious adverse fetal outcomes (fetal miscarriage, abortion or congenital abnormalities) with the highest percentage in group 3 (40 cases). Women with high urinary fluoride concentrations (≥ 3 mg/L) had a strong association with pregnancy complications, i.e. anaemia, miscarriage, abortion and still-birth.

In summary, associations have been reported between urinary fluoride concentration and birth weight, length, head circumference (Kampouri et al., 2022), miscarriage and stillbirths (urinary fluoride concentration > 3 mg/L) (Goyal et al., 2020).

3.3 | Hazard assessment based on experimental animal studies

3.3.1 | Neurotoxicity and developmental neurotoxicity

3.3.1.1 | *Appraisal of (D)NT behavioural endpoints in experimental animals*

All 59 studies that were selected as relevant for the assessment underwent an initial screening for risk of bias (RoB) and 20 of the 59 studies were excluded from further consideration due to deficiencies in several bias domains (see Annex E, Table E.1), noted as questions 1–9 (Q1–Q9) (Appendix E). Among the remaining 39 studies, 29 studies included (D)NT behavioural data and these were analysed in detail for RoB specifically relevant for behavioural data (Q7 & Q9) This staged approach increased efficiency of RoB assessment, such that data with major limitations based on other RoB criteria did not undergo the detailed behavioural Q7 and Q9 analysis. More details on the appraisal criteria and reasoning for behavioural endpoints can be found in Annex E.1. Moreover, 19 of the 39 studies reporting effects at molecular, cellular and organ level were also appraised. Studies reporting evidence of histopathology were appraised according to specific criteria for the quality of tissue processing and documentation and quality of the results through the reported images (detailed appraisal is presented in Annex E.2). The RoB scoring results for (D)NT behavioural endpoints are shown in Table 19.

The RoB scores of studies reporting on neurotoxicity (NT) and developmental neurotoxicity (DNT) endpoints are based on specific and stringent scoring criteria applied to behavioural tests (Table 19; Annex E.1). Criteria that have particular impact on the assessment of behavioural endpoints include randomisation of animals into test groups (Q1); blinding of research personnel to the test groups when recording of data, when this is not fully automated or clearly reported (Q4); and the measures to avoid stress and systematic bias from animal handling which directly impacts behaviour during the test (Q7). Importantly, the sequence of allocating animals to the behavioural tests should be counterbalanced between dose groups over the duration of the test and historical negative and positive control data should be available to show the laboratory's proficiency (Q7). Moreover, analysis with appropriate statistics is particularly important for the usually complex assays (Q9).

TABLE 19 Heat map for the risk of bias performed for studies reporting on (D)NT behavioural endpoints in rodents.[a]

Refid	Author	Year	Tier	Q1* Randomisation	Q2 allocation	Q3* conditions	Q4* blinding	Q5 attrition	Q6* exposure	Q7* outcome	Q8 reporting	Q9 statistics
306	Cao, K.	2019	2	+	+	+	+	++	++	-	++	-
1033	Jiang, C.	2014	2	+	+	+	+	+	+	-	++	-
1581	McPherson, C. A.	2018	2	+	+	+	+	++	++	-	++	+
1949	Ran, L. Y.	2021	2	+	+	+	+	+	+	-	+	+
3197	Wang	2023	2	+	+	+	++	+	+	-	-	-
45	Agustina, F.	2019	3	+	+	+	-	+	+	-	+	+
169	Bartos, M.	2019	3	-	+	++	-	++	+	-	++	+
170	Bartos, M.	2018	3	-	+	+	-	++	+	-	++	-
168	Bartos, M.	2015	3	-	+	+	-	+	+	-	++	++
2997	Bittencourt	2023	3	+	+	+	-	+	-	--	-	+
366	Chen, J.	2018	3	-	+	++	+	+	++	-	++	-
421	Chioca, L. R.	2008	3	-	+	+	++	++	-	-	++	-
3050	Han	2022	3	+	+	+	+	-	+	--	++	--
851	Han, H. J.	2014	3	+	+	+	+	+	-	-	++	-
3118	Ma	2023	3	+	+	-	+	+	+	--	-	-
1746	Niu, Q.	2018	3	-	+	+	+	-	-	-	++	-
1919	Pulungan, Z. S. A.	2018	3	+	+	+	++	++	-	-	++	+
2297	Sun, Z.	2018	3	-	+	+	+	-	-	-	+	-
2489	Wang, D.	2021	3	-	+	+	+	+	-	-	++	-
2595	Whitford, G. M.	2009	3	+	+	+	-	+	+	-	++	-
3214	Xiang	2024	3	+	+	+	++	++	+	--	++	--
2775	Yuan, J.	2019	3	-	+	+	-	++	++	-	-	-
3239	Zhang	2023	3	+	+	+	+	+	+	--	++	--
3240	Zhang	2023	3	+	+	+	-	++	+	-	++	--
2813	Zhang, C. Z.	2020	3	-	+	+	+	+	+	-	++	-
2810	Zhang, C.	2022	3	-	+	+	+	++	+	-	++	-
3250	Zhao	2022	3	-	+	+	-	+	+	--	++	+
2877	Zhao, Q.	2019	3	-	+	+	+	-	+	-	++	-
2910	Zhou, G.	2021	3	-	+	+	+	-	+	-	++	-

[a]The heatmap indicates whether the criteria stated in questions 1–9 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

The methods and measurements applicable to assessments at molecular, cellular and organ level are less prone to experimental bias compared with the behavioural assessments. Generally, biochemical measurements are less subjective, while blinding for histopathology is usually not feasible (the control group is assessed first as a reference). Therefore, the quality criteria for analyses reported in these studies are generally met to a greater degree and result in lower risk of bias for these endpoints (not shown).

The conduct of rodent behavioural studies requires a particularly high level of expertise and training and especially detailed reporting of methods (NAFTA, 2016). Behavioural methods may test for different nervous system functions that are characterised by different maximum effect sizes (i.e. dynamic ranges), and hence different test sensitivity. The test sensitivity also depends on the specific protocol. For learning and memory tests the difficulty of the task influences the dynamic range. Importantly, the handling of animals before and during the test may affect the variability within the experiment and consequently the dynamic range of the output data. Furthermore, the handling may introduce a systematic bias when the testing sequence is not counterbalanced over all dose groups and throughout the handling time. Consequently, for the interpretation of data from behavioural studies, available historical negative and positive control data from the same laboratory are very important and explicitly required by the regulatory standard test guidelines (NAFTA, 2016; OECD TG426, 2007; OECD TG443, 2018). These historical data are needed to assess the laboratory's proficiency to produce negative or positive results with their specific protocols with their specifically trained personnel (Crofton et al., 2004).

In addition, it is important to understand possible confounders for the behavioural outcomes, such as effects of the test substance on vision or motor activity, which impede performance in the behavioural tests and may be misinterpreted as adverse outcomes of learning and memory. In addition, effects on DNT that occur at dose levels where significant

reduction of body weight is also observed, may be confounded by general toxicity. In this case, DNT effects would not be a sensitive endpoint that would impact the overall HBGV, since severe body weight effects occur at high doses that are not relevant for establishing the HBGV. Nevertheless, it is uncertain how specific DNT effects versus general toxicity in the rodent translate to specific or general toxicity effects in humans. Therefore, understanding the differences between doses inducing general toxicity from those inducing specific DNT effects may impact the overall uncertainty and risk assessment.

Finally, the complex testing protocols, often including several nested and repeated measures, and the possibility of non-monotonic dose–response relationships for neurotoxicological effects require a particularly careful selection of statistical approaches, i.e. to reduce the multiple testing error and optimise the sensitivity of the method (Holson et al., 2008).

Consequently, a detailed assessment of these specific RoB aspects for behavioural data was carried out, with a focus on the above-mentioned RoB aspects, relating to appraisal questions Q1, Q4, Q7 and Q9.[58] In conclusion, all behavioural data were classified as tier 2 or 3 (medium or high risk of bias; Table 19). More details are presented for the appraisal of the tier 2 studies in Annex E.1, Table E.1.1 for developmental exposure and E.1.2 for exposure of adult animals.

With regards to characterisation of animal exposure (Q6), it is noted that, with the exception of four studies, the F content in feed was not reported. This constitutes a relevant uncertainty. Standard feed for experimental animals may contain between 15 and 19 mg F/kg feed (5001 - Laboratory Rodent Diet and 5008 - Formulab Diet). This corresponds to approximately 1.5 mg/kg bw per day in rats and 3.3 mg/kg bw per day in mice (for subchronic exposure), based on EFSA default conversion factors (EFSA Scientific Committee, 2012). Therefore, the effects observed in experimental animal studies must be associated with the total F intake, rather than only the F received through the selected route of exposure (mostly drinking water). Similarly, the concurrent control groups cannot be assumed to provide zero fluoride exposure and baseline measures of outcomes in all the studies described below are not responses in the absence of fluoride (unlike other test substances). The total dose was calculated and is reported in the Annex E.7 for all animal studies, based on the standard rodent feed, unless explicitly measured and reported in a given study. However, the impact on BMD modelling results (including or excluding F content in diet) appeared minor (see Appendix D.2).

3.3.1.2 | *Behavioural (D)NT endpoints in experimental animals*

Of the 39 studies identified for further evaluation, 29 reported on (D)NT behavioural endpoints and are summarised in Annex E.3, Tables F.3.1 for exposures during development and F.3.2, for exposure in adult animals. Of those, 20 studies reported also on endpoints at molecular, cellular, organ level (see Section 3.3.1.3). The Tier 2 studies were selected as the primary source of evidence on the potential effects of fluoride on neurobehavioural endpoints and are presented in the text below.

Developmental life stage

In developing Sprague–Dawley rats, Morris Water Maze tests were conducted with fluoride exposure via the drinking water throughout two generations in two studies. In one study (Jiang et al., 2014), exposure to fluoride at dose levels of 0, 1, 2 and 4 mg/kg bw per day continued from the parental premating phase, through the gestation and lactation phases until postnatal day (PND) 60 (calculated total doses of 1.5, 2.5, 3.5 and 5.5 mg/kg bw per day). Relative to the baseline F intake from feed within all dose groups (1.5 mg/kg bw per day) a statistically significant increase of latency time and the total distance swam and decreased time on the platform and distance travelled were reported at all doses with a LOAEL of 2.5 mg/kg bw per day, the lowest dose tested. There was no effect on swimming speed. A NOAEL could not be identified from this study.

In another study (McPherson et al., 2018), exposure to fluoride at dose levels of 0, 0.9 and 1.8 mg/kg bw per day started at gestation day (GD) 4 and lasted until PND 60. In this study, the animals were maintained on a low F feed (3.24 mg/kg) resulting in baseline intake of 0.3 mg/kg bw per day (total doses of 0.3, 1.2 and 2.1 mg/kg bw per day, respectively). This study included several other behavioural tests beyond the Morris Water Maze (see Annex E.3). No adverse effects were observed; the NOAEL was 2.1 mg/kg bw per day, the highest dose tested.

For both these studies the main limitation is the lack of historical negative and positive control data. However, the Jiang et al. (2014) study is additionally limited critically in that it was not reported whether the testing sequence was counterbalanced between dose groups over time. All limitations, including more minor ones, are summarised in detail in Annex E.1 and these can explain the different outcomes between these two studies.

Adult life stage

In adult rats (Ran et al., 2021; Wang et al., 2023) or mice (Cao et al., 2019), Morris Water Maze Tests were conducted with fluoride exposure for up to 12 weeks. In rats, fluoride was tested at doses of 0, 0.45, 4.5 and 9 mg/kg bw per day (total doses of 1.5, 2, 6 and 10.5 mg/kg bw per day (Ran et al., 2021) or 0, 2, 4, 6 mg/kg bw per day (total doses of 1.5, 3.5, 5.5 and 7.5 mg/kg bw per day (Wang et al., 2023). Both rat studies administered fluoride via drinking water. In mice, fluoride was

[58] Appraisal questions refer to the following study aspects: Q1 refers to random allocation of animals in treatment groups; Q4 to blinding of personnel to the allocation of animals to treatment groups during the conduct of testing; Q7 to the reliability of outcome assessment methodologies; and Q9 to appropriate statistical analysis for the endpoint assessed. See Appendix G for details.

administered by gavage at doses of 0, 0.03, 0.3 mg/day, corresponding to 0, 1, 10 mg/kg bw per day in males (30 g bw) and 0, 1.5 and 15 mg/kg bw per day in females (20 g bw). When F intake from food is taken into account total dose levels are 1, 2 and 11 mg/kg bw per day in male and 1, 2.5, 16 mg/kg bw per day, in female mice (Cao et al., 2019). Statistically significantly slower learning was observed in the rat studies with LOAEL of 10.5 mg/kg bw per day (Ran et al., 2021) and 3.5 mg/kg bw per day (Wang et al., 2023). Reliable data for the memory phase were only available within the latter study and these were also adversely affected relative to the reference group (1.5 mg/kg bw per day). From these data a NOAEL of 6 mg/kg bw per day can be derived from Ran et al. (2021), and a value < 3.5 mg/kg bw per day from Wang et al. (2023). In mice, statistically significantly slower learning as well as reduced memory performance was observed with a NOAEL of 2 mg/kg bw per day and a LOAEL of 11 mg/kg bw per day (in males) (Cao et al., 2019).

The LOAELs at which effects were observed are in the same order of magnitude in these three studies, although they were derived from different species (rat vs. mouse) and different exposure routes (drinking water vs. gavage). All three studies lacked negative and positive control data and several critical aspects for the study conduct and statistical analyses were not reported (see Annex E.1).

The differences in outcomes in two rat studies can be partly explained based on methodological differences (see Annex E) and the evidence from the behavioural Morris-Water-Maze tests can be integrated. Taken together, these data indicate that adverse effects may be expected in rats from ~3.5 mg/kg bw per day and this LOAEL lies within the NOAEL to LOAEL range of the mouse study, i.e. 2–11 mg/kg bw per day in Cao et al. (2019).

The assessment of experimental animal (D)NT behavioural outcomes does not rely on data from tier 3 studies ($n = 17$) due to limitations that compromise the reliability and interpretation of the findings (see Annex E.1). However, several of these studies met tier 2 quality requirements regarding molecular, cellular, organ data, even though behavioural assessment did not. The NOAEL and/or LOAEL values related to the (D)NT behavioural findings of these 17 tier 3 studies were compared with the values derived from McPherson et al. (2018) and Jiang et al. (2014) for DNT, and to Ran et al. (2021), Wang et al. (2023) and Cao et al. (2019) for NT in a combined graphical representation as part of the weight of evidence (Figure 5).

This plot shows that including these tier 3 behavioural data would not change the overall conclusion in terms of the most likely NOAEL and LOAEL for adverse (D)NT behavioural effects (see Figure 5 below).

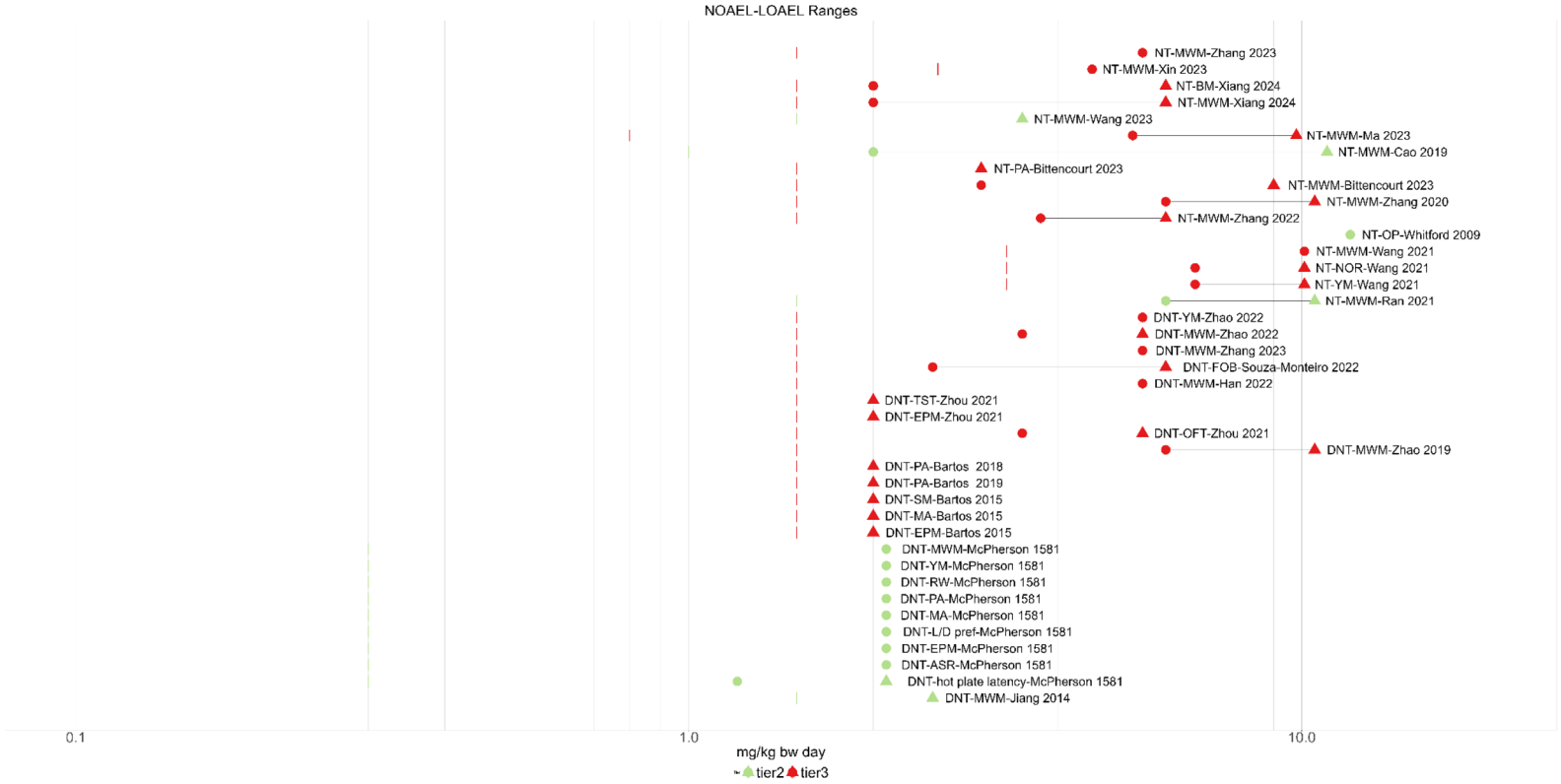

FIGURE 5 F-background in controls from feed (vertical lines), NOAELs (circles) and LOAELs (triangles) from all available behavioural studies, including tier 2 (green) and tier 3 (red) data. Tier 3 studies shown here presented limitations in the reporting of the specific aspects critical for behavioural endpoints (blinding; outcome assessment; statistics). Studies that were rated as tier 3 also for non-behavioural endpoints are not shown. NOAEL-LOAEL ranges are labelled by study type (DNT vs. NT) and study reference. AA, Active Avoidance; ASR, Acoustic Startle Response; EPM, Elevated Plus Maze; HPL, Hot Plate Test; LDP, Light–Dark-Preference; MA, Motor Activity; MWM, Morris Water Maze; NOR, Novel Object Recognition Test; OFT, Open Field Test; OP, Operand Procedure; PA, Passive Avoidance; RW, Running Wheel; SMD, Sensorimotor Development; TST, Tail Suspension Test; YM, Y-Maze; YRM, Y or radial arm maze.

In summary, based on the behavioural assessments alone (Morris water maze performance), a lowest LOAEL of 2.5 mg/kg bw per day can be derived for DNT based on Jiang et al. (2014). An overall NOAEL to LOAEL range of 2–11.3 mg/kg bw per day can be derived for NT based on Cao et al. (2019) and this covers also the NOAEL-LOAEL range from Ran et al. (2021) and the LOAEL from Wang et al. (2023). However, all DNT and NT behavioural data identified are obtained from studies with limitations in reporting for one or more of the key quality criteria (see Annex E.1). Therefore, the potential (D)NT behavioural effects of fluoride must be interpreted in conjunction with additional lines of evidence presented below, such as evidence from in vivo molecular, cellular, organ measurements, as well as mechanistic in vitro data (see Section 3.3.4.1) and human epidemiological data.

3.3.1.3 | *Appraisal of (D)NT molecular, cellular and organ level endpoints in experimental animals*

Assessments of endpoints at molecular, cellular and organ level relevant to (D)NT were reported in 38 studies. These included 7 tier 1 studies, 19 tier 2 studies and 12 tier 3 studies (Table 20). Among these, 28 studies also reported effects on (D)NT behavioural endpoints which are discussed above. The additional endpoints included brain weight measurement, histological examination of the brain and specific brain regions, immunohistochemistry, proteomics, transcriptomics, mitochondrial function, biomarkers of oxidative stress and gene expression studies. In addition, 10 studies were assessed (of which 2 Tier 1 studies, 5 Tier 2 studies and 3 Tier 3 studies), which reported only measurements at molecular, cellular and organ level. The RoB appraisal for molecular, cellular and biochemical evaluations is presented in Table 20, while the RoB appraisal for histopathological and morphological evaluations is presented in Annex E.2. For a summary of the 26 tier 1 and 2 studies reporting evidence molecular, cellular and organ level with or without (D)NT behavioural assessments, see Section 3.3.1.4. Tier 3 studies were taken into consideration as supporting evidence. These studies are summarised in Annex E.3.

TABLE 20 Heat map for the risk of bias performed for studies reporting on endpoints at molecular, cellular, organ level in rodents.[a]

Refid	Author	Year	Tier	Q1* Randomisation	Q2 allocation	Q3* conditions	Q4* blinding	Q5 attrition	Q6* exposure	Q7* outcome	Q8 reporting	Q9 statistics
306	Cao, K.	2019	1	+	+	+	+	++	++	+	++	++
1033	Jiang, C.	2014	1	+	+	+	+	+	+	+	++	+
1581	McPherson, C. A.	2018	1	+	+	+	+	++	++	+	++	++
1949	Ran, L. Y.	2021	1	+	+	+	+	+	+	+	+	+
2401	Trivedi, M. H.	2012	1	+	+	+	+	++	+	+	++	+
2684	Yan, N.	2016	1	++	+	+	+	+	++	++	++	+
2910	Zhou, G.	2021	1	+	+	+	+	-	+	+	++	+
45	Agustina, F.	2019	2	+	+	+	+	+	+	-	+	+
170	Bartos, M.	2018	2	+	+	+	+	++	+	-	++	+
169	Bartos, M.	2019	2	+	+	++	+	++	+	-	++	+
2997	Bittencourt	2023	2	+	+	+	+	+	-	+	++	+
851	Han, H. J.	2014	2	+	+	+	+	+	-	+	++	-
3118	Ma	2023	2	+	+	-	+	+	+	+	+	++
1758	Niu, R. Y.	2015	2	+	+	++	+	++	-	+	++	++
1919	Pulungan, Z. S. A.	2018	2	+	+	+	++	++	-	+	++	+
3150	Ran	2023	2	+	+	-	+	+	+	+	+	++
2359	Teng, Y.	2018	2	-	+	+	++	++	+	++	++	++
3214	Xiang	2024	2	+	+	+	+	+	+	-	++	++
3216	Xu	2023	2	+	+	+	+	+	+	-	++	++
2761	Yu, Q. L.	2019	2	+	+	+	+	++	-	+	++	-
2775	Yuan, J.	2019	2	-	+	+	+	++	++	+	++	-
3239	Zhang	2023	2	+	+	+	+	+	+	-	++	++
3240	Zhang	2023	2	+	+	+	+	-	+	-	++	+
2810	Zhang, C.	2022	2	-	+	+	+	++	+	+	++	+
3250	Zhao	2022	2	+	+	+	+	+	+	-	+	+
2877	Zhao, Q.	2019	2	-	+	+	+	-	+	+	++	+
168	Bartos, M.	2015	3	-	+	+	-	+	+	-	++	++
366	Chen, J.	2018	3	-	+	++	+	+	++	-	++	++
421	Chioca, L. R.	2008	3	-	+	+	++	++	-	+	++	+
3050	Han	2022	3	+	+	+	+	-	+	--	++	+
1040	Jiang, P.	2019	3	-	+	+	+	++	-	-	++	+

TABLE 20 (Continued)

Refid	Author	Year	Tier	Q1* Randomisation	Q2 allocation	Q3* conditions	Q4* blinding	Q5 attrition	Q6* exposure	Q7* outcome	Q8 reporting	Q9 statistics
1720	Narayanaswamy, M.	2010	3	-	+	+	+	++	-	-	++	++
1746	Niu, Q.	2018	3	-	+	+	+	-	-	-	++	+
2297	Sun, Z.	2018	3	-	+	+	+	-	-	+	+	-
3187	Tang	2023	3	-	+	+	+	+	+	-	+	+
3197	Wang	2023	3	+	+	+	+	-	+	--	++	+
2489	Wang, D.	2021	3	-	+	+	+	+	-	-	++	+
2813	Zhang, C. Z.	2020	3	-	+	+	+	+	+	-	++	-

[a]The heatmap indicates whether the criteria stated in questions 1–9 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

3.3.1.4 | *Molecular, cellular, organ endpoints in experimental animals*

While many of the papers observed changes in activities of enzymes, alterations in signalling pathway proteins or changes in expression of specific proteins, these were generally descriptive. Studies on general toxicity measuring body weight gain and/or organ weight showed variable results. One DNT and one NT study reported lower postnatal absolute (but not relative) brain weight (along with lower body weight). This finding was statistically significant in the DNT study (Jiang et al., 2014). To contextualise the effects on brain weight observed by Jiang et al. 2014 additional regulatory GLP studies were reviewed. These studies were not captured in the systematic and targeted literature searches conducted in the context of this assessment but were identified by experts of the EFSA Working Group on fluoride as a result of an interagency exchange. Two subchronic and chronic toxicity studies on fluoride were conducted in rats and mice by the NTP (NTP TR 393, 1990) and one multigenerational developmental toxicity study was conducted in rats by the US FDA (Collins et al., 2001). None of these studies found evidence of brain weight changes. As these are regulatory studies conducted according to GLP criteria, they were not appraised for risk of bias.

Several papers indicate that histological or ultrastructural changes occur following fluoride exposure. The assessment of these changes presented significant methodological limitations such that the evidence is not considered reliable. For more details see Annex E.2. No injury to the brain in terms of neuronal cell death or microglial activation, up to 2.1 mg/kg bw per day, the highest concentration tested, was reported in the study that tested a low range of doses during development (McPherson et al., 2018).

Several papers described evidence for oxidative stress as a result of exposure to fluoride through drinking water. These are summarised in Annex E.3. The observations were mainly changes in biomarkers such as enzyme activities that are indicative of oxidative stress and occurred in parallel with impaired learning and memory in animal studies (Bartos et al., 2019; Bartos et al., 2018; Cao et al., 2019; Ran et al., 2021). Typically, decreased activity of catalase, superoxide dismutase, glutathione peroxidase and increased malondialdehyde and measurement of ROS were reported.

Apoptosis in brain regions was reported in several studies, inducing microglial activation which was associated with the release of inflammatory mediators, such as IL-1b and IL-6 (Yan et al., 2016) and IL-1b and activation of the JNK-pathway in both male and female Wistar rats and in the microglial cell line, BV-2 (Zhang et al., 2022). Evidence of neuronal autophagy was noted in the hippocampus CA1 and DG region at the higher doses of 4.50 and 9.00 mg/kg/bw per day (corresponding to 6 and 10.5 mg/kg bw per day total dose) (Zhang et al., 2020).

In the study where fluoride was tested at a lower dose range (up to 2.1 mg/kg bw per day), no evidence of neuronal death or microglial activation, typically associated with brain injury, was observed (McPherson et al., 2018). No other evidence of toxicity was observed, such as histological changes in multiple organs examined.

Potential effects of fluoride on synaptogenesis were assessed in vivo in male and female Sprague–Dawley rats (0, 0.4, 2 and 4 corresponding to 1.5, 1.9, 3.5 and 5.6 mg/kg bw per day of total dose) and in vitro in SH–SY5Y cells by the same group (Chen et al., 2018). In both models, impairment of synaptogenesis was observed with decreased expression of SYN, PSD95 and TrKB and increased BDNF. In addition, increases in expression of the ERK1/2 signalling pathway were observed (Chen et al., 2018).

A proteomic and transcriptomic study in male and female Sprague–Dawley rats, which aimed to associate exposure to NaF with dental fluorosis, observed a number of alterations in differentially expressed genes (DEGs) and differentially abundant proteins (DAPs) in the central nervous system (CNS). At the highest dose (9 mg/kg bw per day[59] corresponding to 10.5 mg/kg bw per day of total dose) there were alterations in DEGs associated with neuron generation and differentiation, synaptic membranes and with neurotransmitter receptor activity (Ran et al., 2021). Dental fluorosis was only seen at mild or moderate severity at the highest dose.

[59]Authors reported a total dose of 3.78 mg/kg bw per day. Since no specific measurements or calculations were provided, total dose calculations based on the EFSA Guidance on Default Values (2012) were used for consistency.

In summary, several biochemical changes have been reported that support the (D)NT behavioural toxicity discussed above with these effects generally occurring at or above the NOAEL-LOAEL range determined for (D)NT. Histological and morphometric evidence further supports these findings (see Section 3.3.4.1), but due the complexity in the methods the results cannot be interpreted in quantitative terms for supporting any point of departure.

3.3.1.5 | *Evidence on (D)NT from literature published before 2005*

Among the studies identified through the targeted search on (D)NT prior 2005 that reported on (D)NT, in experimental animals, 12 studies were preliminary appraised for internal validity (RoB), to determine publications that may require more detailed evaluation. Of these, six (Tier 3) studies reporting on behavioural endpoints and nine (five Tier 3 and four higher Tiers) studies reporting on molecular, cellular or organ endpoints were identified (see Annex E.8. Overall, the studies presented similar types of limitations and comparable levels of RoB as the studies published after 2005 considered in this assessment. No additional behavioural studies were identified which met the tier 2 or tier 1 RoB criteria of the systematic literature review protocol applied. Some of the studies reported data on biochemical key events, such as changes in AChE or AChR in brain or plasma without direct measures of neuronal function and behaviour. Similar parameters were also analysed in studies published after 2005 at similar concentration ranges. Overall, the Scientific Committee concludes that a deeper analysis of these older publications and their inclusion in the weight of evidence would not change the outcome of assessment based on the more recent literature.

3.3.2 | Thyroid effects in experimental animals

There are recognised species differences in thyroid homeostasis that are important in interpretation of results from animal studies (see Appendix B.5). Generally, the rat is considered a sensitive and reliable model of thyroid responses to chemical stressors and therefore relevant to humans.

3.3.2.1 | *Appraisal of studies reporting on the thyroid*

Six studies were identified that examined the effects of fluoride on the structure and function of the thyroid. One study in castrated young pigs was excluded as a non-relevant model. The appraisal of study quality for the five relevant studies is shown in Table 21.

Three of the five studies assessed the effect of fluoride on thyroid hormones and/or pathology in adult rats (Dhurvey et al., 2017; Jiang et al., 2016; Liu et al., 2012). These studies lacked reporting for at least two key quality criteria and were therefore considered to be of high risk of bias. The two other studies assessed thyroid effects of fluoride in developing animals (Basha et al., 2011; McPherson et al., 2018). One of these (Basha et al., 2011) lacked reporting for three key quality criteria and judged to be of high risk of bias. An overview of the limitations, especially related to Q7 is provided in Annex E.4.

Only one of the five studies was judged to be of low risk of bias (McPherson et al., 2018).

TABLE 21 Heat map for the risk of bias performed for studies reporting effects on thyroid.[a]

Refid	Author	Year	Tier	Q1* Randomisation	Q2 allocation	Q3* conditions	Q4* blinding	Q5 attrition	Q6* exposure	Q7* outcome	Q8 reporting	Q9 statistics
1581	McPherson, C. A.	2018	Tier 1	+	+	+	+	++	++	+	++	++
178	Basha, P. M.	2011	Tier 3	-	+	+	+	-	+	-	+	+
563	Dhurvey, V.	2017	Tier 3	+	+	-	+	+	+	-	++	--
1046	Jiang, Y. Q.	2016	Tier 3	-	+	-	+	-	-	-	++	+
1401	Liu, G.	2012	Tier 3	-	+	-	+	+	+	+	++	+

[a]The heatmap indicates whether the criteria stated in questions 1–9 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

3.3.2.2 | *Study descriptions and main findings*

In the developmental study of McPherson et al. (2018), fluoride was administered through the drinking water to Long Evans hooded rats, no effect was observed on serum thyroid hormones (T3, T4 and TSH), over the low dose range (1.2–2.1 mg/kg bw per day). Some uncertainty within this study derives from the observation that TSH in standard feed control group (G1) was six-fold higher compared to low fluoride feed control group (G2). However, TSH was not different between drinking water-exposed and control groups maintained on the same low fluoride diet. It is noted that the F content in adult plasma and femur seems lower or similar in the standard feed control group (G1) versus the low dose treated group on low F diet (G3). Moreover, only in this study was fluoride from dietary sources directly controlled and minimised to isolate the contribution

from drinking water exposure. As such the administered dose cannot be directly compared with those of other studies, where the feed content of fluoride, was not characterised. However, this study can support the absence of an effect of Fluoride on thyroid hormone levels at doses 2x of the normal rodent diet which resulted from an exposure of 2.1 mg/kg bw per day.

In summary, a single study of low risk of bias was identified, which indicated no adverse effects on the thyroid at the relatively low total doses of 1.2 and 2.1 mg/kg bw per day. Other studies reported effects of fluoride on thyroid hormones and histopathological changes at total doses of 1.35 mg/kg bw per day or greater, but the quality limitations of these findings introduced high uncertainty on the reliability of the findings. Overall, the available animal data provide insufficient evidence to support thyroid effects from fluoride exposure.

3.3.2.3 | *Evidence on thyroid effects from literature published before 2005*

Among the studies identified through the targeted search on thyroid prior 2005 that reported on thyroid effects in experimental animals, eight studies were preliminary appraised for internal validity (RoB), to determine publication that may require more detailed evaluation. Overall, these studies presented similar types of limitations and comparable levels of RoB (Tier 3) as the studies published after 2005 considered in this assessment (see Annex E.8). No additional studies reporting on thyroid effects were identified which met the tier 2 or tier 1 RoB criteria of the systematic literature review protocol applied. Similar parameters were also analysed in studies published after 2005 at similar concentration ranges. None of the studies showed adverse effects on the thyroid system in terms of organ weight, histology, Iodine uptake or thyroid hormones measurements. One study showed mitochondrial toxicity in several organs including thyroid (Zhan et al., 1988). For an interpretation of the latter, the mechanistic link to later key events and adverse outcomes would require characterisation. Overall, the Scientific Committee concludes that a deeper analysis of these older publications and their inclusion in the weight of evidence would not change the outcome of assessment based on the more recent literature.

3.3.3 | Bone health in experimental animals

A total of 15 studies were identified from the systematic literature search. All studies were assessed for relevance and appraised for Risk of Bias.

3.3.3.1 | *Appraisal of studies reporting on bone effects*

Of the 15 studies identified, 7 were considered to be of high risk of bias (tier 3) (Table 22). The remaining studies are described below.

TABLE 22 Heat map for the risk of bias performed for studies reporting effects on bone.[a]

Refid	Author	Year	Tier	Q1* Randomisation	Q2 allocation	Q3* conditions	Q4* blinding	Q5 attrition	Q6* exposure	Q7* outcome	Q8 reporting	Q9 statistics
451	Chu, Y.	2020	1	+	+	+	+	+	+	+	++	++
687	Fina, B.	2018	1	+	+	+	+	+	+	+	++	++
1455	Lombarte, M.	2021	1	+	+	+	+	+	+	+	++	++
2144	Sharma, P.	2022	1	+	+	+	+	++	+	+	++	+
3092	Li, H.	2023	2	+	+	+	+	-	+	-	+	+
3103	Linghu, Y.	2023	2	+	+	-	+	+	+	+	++	-
3151	Ranjan, R.	2023	2	+	+	+	+	+	+	-	++	-
2725	Yao, Y.	2019	2	+	+	-	+	+	+	+	++	++
3032	Ferreira, M. K. M.	2022	3	-	+	+	+	+	-	-	++	+
241	Bondu, J.	2019	3	-	+	-	+	++	-	-	++	+
2416	Turkekul, R.	2020	3	-	+	-	+	+	+	+	++	+
2680	Yan, D.	2007	3	-	+	+	+	++	-	+	++	+
3072	Jin, Y.	2023	3	--	+	+	+	+	-	-	++	+
3129	Nie	2023	3	+	+	+	+	+	-	-	+	+
3258	Zhu, S.	2022	3	-	+	+	+	-	-	+	++	+

[a]The heatmap indicates whether the criteria stated in questions 1–9 are met (+) or not (–), with additional respective colour coding for visual mapping, where darker and light green shades with '++' and '+', respectively, indicate 'definitely' and 'probably' low risk of bias, respectively, and yellow and red shades with '-' and '--', respectively, indicate the 'probably' and 'definitely' high risk of bias, respectively (see Appendix E, for details).

3.3.3.2 | *Study descriptions and main findings*

Among the 8 tier 1 and 2 studies the effects of fluoride on bone health were assessed in young and adult rodents in 7 studies and in young rabbits in one study exposed either via drinking water or gavage. Alterations were observed on bone deposition, bone remodelling, bone mineral density, bone histology and bone fracture load. In addition, effects were seen on markers of bone health, oxidative stress, autophagy, apoptosis and on signalling pathways such as the Wnt/β-catenin and PI3K/AKT/mTor.

Seven separate studies reported dose-dependent increasing fluoride concentrations in bone, consistent with the bone accumulation of fluoride, which ranged from 5 to 24-times greater than the control values across the studies (Chu et al., 2020; Fina et al., 2018; Linghu et al., 2023; Lombarte et al., 2021; Ranjan et al., 2023; Sharma et al., 2022; Yao et al., 2019). Urinary concentration of fluoride was also increased with increasing dose.

Changes to bone disposition, resorption, remodelling and differentiation were reported in multiple studies. Common changes observed included increased bone anabolism as measured by increases in trabecular bone thickness (Tb.Th), increases in bone volume (BV/TV); increases in trabecular bone area (Tb.Ar); and decreases in bone strength. One study assessed bone mineral density and observed no significant changes. Biomarkers of effects on bone such as ALP and osteocalcin were often measured as were cellular signalling systems such as Wnt/β-catenin (Zhu et al., 2022) and PI3K/AKT/mTor (Linghu et al., 2023).

Fina et al. (2018) evaluated the effect of oral fluoride (2 (CTR), 6, 10 or 18 mg/kg bw per day for 30 days on fracture load of the trabecular and cortical bones in growing 21 day old Sprague–Dawley rats. Bone mineral density of the tibia was measured by X-ray absorptiometry and quantification on digital images. While no change was observed in body weight, consistent dose-dependent decreases were seen at all doses in fracture load, stiffness and Young's Modulus of trabecular bone after fluoride treatment but there were no changes in trabecular bone mineral density. Decreased stiffness was seen in cortical bone only at the highest dose. No changes were seen in cortical bone fracture load, Young's Modulus or bone mineral density. A significant correlation between trabecular fracture load and Young's Modulus was reported but no correlation between bone volume and bone mineral density. The authors concluded that significant decreases in trabecular bone strength resulted from decreased elasticity and not decreased volume of the trabecular bone. The same group later examined bone growth and ossification in growing female 21 day old Sprague–Dawley rats treated with fluoride at 6, 10 or 18 mg/kg bw per day for 30 days, by gavage. Fluoride reduced endochondral ossification and increased chondrocyte proliferation at 10 mg/kg bw per day delaying maturation of new bone. Statistically significant decrease in bone volume was reported at 10 and 18 mg/kg bw per day. In addition, inflammatory damage, oedema and increased apoptosis of bone cells were reported in association with the decreased bone volume (Lombarte et al., 2021).

Chu et al. (2020) found increased cancellous bone formation in male BALB/c mice treated with 3.3 (CTR), 7.05, 10.8 and 18.3 mg fluoride/kg bw per day for 3 months in deionised drinking water. Histological evidence of increased cancellous bone formation was found starting at 10.8 mg/kg bw per day, while the relative area of tibia trabecula (Tb.Ar) was increased only at the highest dose. Serum ALP and osteocalcin were also increased at 10.8 and 18.3 mg/kg bw per day. Thickness of trabecular and cortical bones was observed particularly at the highest dose. Activation of the Wnt/β-catenin pathway was investigated and increased expression of Wnt3a, phospho-GSK3b (ser 9) and Runx2 proteins were noted. Partial correlation analysis indicated that there was no significant correlation between fluoride exposure and Runx2 protein levels but suggested that β-catenin might play a crucial role in fluoride-induced aberrant osteogenesis.

In adult male Wistar rats exposed to 0.2 (CTR), 4.7 and 9.2 mg/kg bw per day fluoride in drinking water for 180 days, radiological examination of bone showed evidence of increased thickness of femoral medulla and total femoral thickness, without significant cortex thinning, decreased bone density, irregular ossification, including ossification of soft tissues, at the top dose. The changes were interpreted as aberrant bone remodelling and reduced bone strength. Plasma Ca, P and Ca:P ratio all decreased in fluoride-treated animals indicating bone matrix alteration (Sharma et al., 2022). Body weight decreased by ~16% in the low dose group after 4 months with no further decrease, while it decreased throughout the study in the high dose group up to ~ 22% after 6 months.

In a study in male and female Sprague–Dawley rats exposed to 1.5 (CTR), 1.95, 6 and 10.5 mg/kg bw per day of fluoride in drinking water for 6 months, Linghu et al. (2023) observed increased trabecular bone thickness (Tb.Th) and area (Tb.Ar) indicating osteosclerosis from 6 mg/kg bw per day. Dental fluorosis was also increased with increasing fluoride dose. In osteoblasts in bone increases in markers of bone autophagy (Beclin1, Atg7 and LLCII/I); decreased p62 and the presence of autophagosomes and autolysosomes by TEM indicated active autophagy. This was replicated in primary osteoblasts isolated from neonatal calvaria within 24 h of birth. Further biochemical analysis implicated the PI3K/AKT/ mTOR signalling pathway in regulating fluoride-induced autophagy in osteoblasts.

In male C57BL/6 mice exposed to 3.3 (CTR), 12.3 and 21.3 mg/kg bw per day fluoride in drinking water for 3 months increased bone formation was observed, as increases in osteoblast biomarkers ALP at the top dose and BGP, with no changes in body weight (Yao et al., 2019). Instead, an inverted U-curve association was found for osteoclast count that was significantly higher at 12.3 mg/kg bw per day compared to control and the top dose group, indicating a dynamic effect between bone formation and degradation with increasing fluoride concentrations. Mild to moderate dental fluorosis was also observed at 12.3 mg/kg bw per day and mild to severe fluorosis at 21.3 mg/kg bw per day.

Li, et al. (2023) carried out a microstructural analysis of cancellous bone in male Wistar rats exposed to 1.5 (CTR), 11.5 and 21.5 mg/kg bw per day over 1, 2 and 3 months. Poorer connectivity and reduced trabecular bone network were observed in cancellous bone. Fluoride in bone was not measured in this study. Poorer connectivity and lower trabecular bone network

were observed in cancellous bone. Decreased serum GPX and increased MDA indicated oxidative stress. Photograph evidence of dental fluorosis showed increasing severity with dose and time of exposure.

Effects of fluoride on the long bones in young New Zealand White rabbits (Oryctolagus cuniculus) exposed to 0, 50, 100, 200 and 400 mg/L[60] for 90 days in drinking water (Ranjan et al., 2023) were consistent with the observations in rodents. Changes observed included thickening of the epiphyseal plate and lack of mineralisation of chondrocytes at 100 mg/L and thickening of cortical region and widening of metaphysis at higher doses. Biochemical analysis from day 45 showed increases in plasma ALT from 50 mg/L and in ALP and AST from 100 mg/L. The authors suggested induction of both osteogenesis and osteoporosis by fluoride exposure.

In summary, increased concentrations of fluoride were found in bone of rodents with increased intake of fluoride, accompanied by changes in bone remodelling and decreased bone strength, while changes of bone density were inconsistent. Changes in bone deposition and remodelling occurred in young and adult animals.

3.3.4 | Consistency within the toxicodynamic animal test data

The consistency within the toxicodynamic data is assessed in Table 23 for developmental neurotoxicity, in Table 24 for neurotoxicity, and in Table 25 for bone health. These tables include the following: a) studies that were appraised as Tier 1 and Tier 2 for neurobehavioural endpoints; b) studies that were appraised as Tier 3 for neurobehavioural endpoints but as Tier 1 or 2 for molecular, cellular and organ endpoints; and c) studies that were appraised as Tier 1 and 2 and only reported molecular, cellular and organ endpoints. Consistency of evidence is not presented for thyroid effects due to limited number of studies available for this purpose.

3.3.4.1 | *Integration of the evidence on developmental neurotoxicity (DNT)*

Following in utero exposure (see Table 23) effects at molecular level were reported at the lowest doses tested, i.e. from 1.6 mg/kg bw per day onwards, for markers for oxidative stress (CAT, Bartos et al. (2019); Bartos et al. (2018), GOT, GPT, Bartos et al. (2018)), astrocyte function (GFAP, Jiang et al. (2014)), glucose placental transport (GLUT1, Jiang et al. (2014)), energy metabolism (GPT, Jiang et al. (2014)), lysosomal function (Lamp2, Qiuyi Zhao et al. (2022)), autophagy (Beclin1, LC3B, p62, Qiuyi Zhao et al. (2022)), apoptosis (cleaved caspase, Zhou et al. (2021)) and other neuronal function (BDNF, Jiang et al. (2014)), (SIK2-CRTC1-CREB-BDNF-VGF, Zhou et al., 2021, SYN & JIP1 Qiuyi Zhao et al. (2022)).

At cellular and organ level DNT relevant effects were reported in rodent models with an overall LOAEL of 3.5 mg/kg bw for body weight, brain weight,[61] glucose utilisation in brain (Jiang et al., 2014). However, the observed brain weight effects were considered unreliable since this was not observed within a series of other rodent studies[62] (NTP TR 393, 1990) (not reproducible effect). The other measurements were considered equally uncertain due to reporting deficiencies regarding the morphometric protocols and blinding: morphology and arrangement of neurons in hippocampus and medial prefrontal cortex (Zhao et al., 2021), neuronal cell count and cellular microstructure of mitochondria and in higher doses mitochondrial fission/fusion balance (Xu et al., 2023; Zhao et al., 2019).

In vitro cellular responses to fluoride were reported as effects on mitochondrial ultrastructure and membrane potential as well as mitochondrial fission/fusion balance using an undifferentiated human neuroblastoma SH-SY5Y cell line (non-neuronal cells) at the lowest tested concentration of 20 µg/mL cell culture medium (Zhao et al., 2019). Reduced proliferation and viability of neuronal precursor cells was shown in a specific in vitro cellular DNT test (Masjoshusmann et al., 2020) with a BMD of 20 µg/mL culture medium. Kinetic data and models for comparing this in vitro concentration to an in vivo tissue concentration are not available. Despite this uncertainty, the concentration required to induce these effects in vitro appears high relative to the concentration of 0.08 µg/g attained in brain (McPherson et al., 2018). This in vivo brain concentration from developmental exposure to fluoride in rat did not induce any cellular or organism level effects.

With the exception of the McPherson et al. (2018) study, a critical uncertainty of the in vivo DNT studies is the lack of internal exposure measurements that obscure direct comparisons across the dose levels examined. Therefore, comparing effective doses in terms of NOAEL-LOAELs ranges contains considerable uncertainties if done between the different in vivo exposure regimes as well as with the in vitro test data. Behavioural effects were reported at 2.5 mg/kg bw per day (Jiang et al., 2014). No adverse behavioural effects were observed within McPherson et al. 2018, up to the highest dose of 2.1 mg/kg bw per day. The conduct of the two studies differs due to additional sources of risk of bias in Jiang et al. 2014 (limited reporting of control for confounders in behavioural tests and limited statistics reporting) and by the different study design (for details see left column in Table 23 above). Moreover, only in McPherson et al. 2018 fluoride content in feed was reported and controlled using a low fluoride diet, whereas the feed content of fluoride in the other studies, which contributes to the

[60]Conversion to units of dose was not reported; no default factors for this species are available in the EFSA guidance (2012). Intake may be estimated approximately at about 0, 10, 20, 40, 80 mg/kg bw per day based on literature (Bodnar, 2023). The fluoride content in feed was not reported.

[61]'In adult animals, brain weight is highly conserved in the presence of changes in body weight, and the convention of using organ to body weight ratios is less useful when studying brain weight changes (Sellers et al., 2007). Studies of malnutrition during development suggest that severe reductions in body weight (e.g. < 50% of controls) will result in lower brain weight (Fernandez et al., 1985), but this has been less studied with regards to moderate or slight (e.g. < 10% of control) body weight differences. In general, effects on brain weight cannot be dismissed even in the presence of body weight differences and should be considered treatment-related and adverse' (NAFTA, 2016).

[62]One rat multigeneration study, one rat 6-month study, one rat 2-year study, one mouse 2-year study.

total exposure, is usually not reported and may vary between vendors. Where no specific data were provided within the publications, we have assumed a contribution of about 1.5 mg F/kg bw per day from chow for rats (16–18 ppm in chow; see footnote to Table 23). However, this limitation does not strongly affect the uncertainty within the animal data derived point of departure (see Appendix D.2 and D.3).

The principle that relevant effects at the molecular level are observed at concentrations below concentrations where relevant effects are observed at the cellular, organ and organism level is in line with established toxicological concepts and as reflected in the guidance for the development of Adverse Outcome Pathways (https://aopwiki.org/). From a scientific perspective, molecular and cellular effects may be considered as adverse and relevant as such since they represent a toxicity that may compromise an organism's capacity to compensate for the multitude of additional real-world stressors.

In contrast to molecular changes, effects at the cellular and organ level are considered to be closer to the organism level effects that are typically used for a regulatory risk assessment. At the cellular/organ levels, a value of about 3.5 mg/kg bw per day may indicate the onset of adverse effects. These effects included alterations in body weight, brain weight, neuronal cellular and behavioural effects observed in the available studies (see Figure 6).

Inclusion of effects reported in the lower tier behavioural studies would not change this conclusion, since the NOAEL/LOAEL values fall within that range (see Figure 5).

TABLE 23 Integration of the evidence on DNT from fluoride exposure in utero along biological hierarchy levels.

References Study design	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) brain (μg/g) or plasma (μg/mL) Summary of effects Major uncertainties in grey rows **Molecular**	**Cellular**	**Organ**	**Rodent behaviour**	**Consistency**
McPherson et al. (2018) Long-Evans Rats (M) Drinking water 0.3 (C), 1.2, 2.1 mg/kg bw per day GD4 - PND60 (behaviour) PND90 (cell, organ) Reduced-F feed (0.3 mg F/kg bw per day)	NA	**NOAEL** **≥2.1 mg/kg bw day** ≥20 mg/L drinking water ≥0.0811 ± 0.04 μg/g brain of weanlings No activation of microglia or astrocytes	**NOAEL** **≥2.1 mg/kg bw day** ≥20 mg/L drinking water ≥0.0811 ± 0.04 μg/g brain of weanlings No ↑ TSH or ↓ T3, T4 or thyroid histopathology	**NOAEL** **≥2.1 mg/kg bw day** ≥20 mg/L drinking water ≥0.0811 ± 0.04 μg/g brain of weanlings No effects in MWM, ASR, MA, EPM, Y-M, RW, PA, L/D	**Yes** No effects at cellular, organ and behavioural level
			6× ↑ TSH in normal versus low F diet control, but not in low F diet control versus F treated drinking water; no effects on T3, T4	no HNCs, HPCs; use of low F diet prohibits NOAEL comparison with all other studies using normal diet	
Jiang et al. (2014) Sprague–Dawley Rats (M, F) Drinking water 1.5 (C), 2.5, 3.5, 5.5 mg F/kg bw per day* 10 days premating till PND60 'standard diet*'	**LOAEL** **≤2.5 mg/kg bw day** ≤10 mg/L Neuronal function altered in cortex & hippocampus (GLUT1, GFAP; BDNF)	NA	**NOAEL–LOAEL** **2.5–3.5 mg/kg bw day** 10–23 mg/L ↓ glucose utilisation in brain; ↓ brain weight (stat.sign, ca. −7% in top dose, −6% in mid dose)	**LOAEL** **≤2.5 mg/kg bw day** ≤10 mg/L MWM	**Partly** Low dose effects are uncertain, since inconsistent between the 4 levels and generic toxicity & RoB
			Brain weight unaffected in other rodent studies[63] Generic toxicity: bw ↓ by 8% (mid dose f) to 19% (high dose m)	No HNCs, HPCs; critical elements of study conduct & statistics	
Zhao et al. (2021) Sprague–Dawley Rats (M) Drinking water 1.5 (C), 1.9, 3.5, 5.5 mg F/kg bw day* 1w premating, gestation till PND90 'standard diet*'	**NOAEL–LOAEL** **1.9–3.5 mg/kg bw day** 4.5–23 mg/L Apoptosis ↑ in hippocampus (HC) and medial prefrontal cortex (mPFC) (cleaved caspase western blot) **LOAEL** **≤1.9 mg/kg bw day** ≤4.5 mg/L Neuronal function altered in HC & mPFC (SIK2-CRTC1-CREB-BDNF-VGF mRNA & protein)	**NOAEC–LOAEC** **9–18 μg/mL** in culture medium, undifferentiated PC12 cells ↓ cell viability, ↑ apoptosis, At these doses cell viability is 75, 62% of control	? ↓ number and irregular morphology & arrangement of neurons in HC and mPFC	NA Behaviour data = tier 3, therefore not listed here	**Yes** Signalling pathways affected also at lowest dose, molecular and organ level effects at mid and high dose In vitro to in vivo concentrations difficult to compare

(Continues)

[63]One rat mutigeneration study, one rat 6-month study, one rat 2-year study, one mouse 2-year study (see text for details).

TABLE 23 (Continued)

References Study design	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) brain (μg/g) or plasma (μg/mL) Summary of effects Major uncertainties in grey rows **Molecular**	**Cellular**	**Organ**	**Rodent behaviour**	**Consistency**
	Body weight or litter size not reported; randomisation not reported		Images appear reliable, but morphometric method description is minimal, and blinding not indicated, thus evidence for dose–response relationship is uncertain; body weight or litter size not reported; randomisation of animal selection not reported		
Bartos et al. (2018) Wistar Rats (F) Drinking water 1.5 (C), 2, 2.4 mg F/kg bw day* Gestation till PND21; test at PND90 'standard diet*'	**NOAEL–LOAEL** **2–2.4 mg/kg bw day** 5–10 mg/L Neuronal function altered in hippocampus (α7-AChR mRNA)	NA	NA	NA Behaviour data = tier 3, therefore not listed here	**Yes** Some molecular level effects also at low and mid dose
	Randomisation not reported; measured only at mRNA, not at protein level				
	LOAEL **≤ 2 mg/kg bw day** ≤ 5 mg/L Oxidative stress ↑ in hippocampus (CAT activity) ↓				
	Randomisation not reported; NMDR with 38% and 60% of ctrl. At 0.45 and 0.9 mg/kg bw day; no effect on GPx; no sign. effect on CAT, GPx, MDA in total brain homogenate				
Bartos et al. (2019) Wistar Rats (M, F) Drinking water 1.5 (C), 2, 2.4 mg F/kg bw day: Gestation till PND21; test at PND45 'standard diet*'	**LOAEL** **≤ 2 mg/kg bw day** ≤ 5 mg/L) Oxidatives stress ↑ in prefrontal cortex, striatum, hippocampus (CAT, GPT, GOT activity) ↓ in prefrontal cortex, striatum, hippocampus	NA	NA	NA Behaviour data = tier 3, therefore not listed here	NA Several molecular level effects also at low dose
	Randomisation not reported				

TABLE 23 (Continued)

References Study design	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) brain (μg/g) or plasma (μg/mL) Summary of effects Major uncertainties in grey rows **Molecular**	**Cellular**	**Organ**	**Rodent behaviour**	**Consistency**
Zhao et al. (2019) Sprague–Dawley Rats Drinking water 1.5 (C), 1.9, 3.5, 5.5 mg F/kg bw day: 2 months premating, till PND60 'standard diet*'	NA	**LOAEC** **≤ 20 μg/mL** in culture medium Human neuroblastoma SH-SY5Y (24 h exposure) mitochondrial ultrastructure and membrane potential, fission/fusion balance affected	? In hippocampus CA1 region ↓ Neuronal Cell Count, effect on microstructure of mitochondria; fission/fusion balance (in higher doses)	NA Behavioural data = tier 3, therefore not listed here	NA Organ level effects also at low and mid dose In vitro to in vivo concentrations difficult to compare
		Cells were not differentiated, thus do not present a neuron specific model	Images appear reliable, but morphometric method description is minimal, and blinding not indicated, thus evidence for dose–response relationship is uncertain; No information on bw/general tox, no information on randomisation of animals		
Zhao et al. (2022) Sprague–Dawley Rats Drinking water 1.5 (C), 3.5, 5.5 mg F/kg bw day 1 week premating, till PND90 'standard diet'*	**LOAEL** **≤ 3.5 mg/kg bw day** ≤ 22.6 mg/L in hippocampus lysosomal function altered (Lamp2↑), autophagy↑ (Beclin1, LC3B, P62), neuronal function altered (SYN, JIP1) (all western blot)	NA	? Cortex & hippocampus histopath↑; neuron microstructure (TEM) deteriorated	NA	**Yes**
			Method description not detailed, no incidence table, one dose only		

(Continues)

TABLE 23 (Continued)

References Study design	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) brain (μg/g) or plasma (μg/mL) Summary of effects Major uncertainties in grey rows **Molecular**	**Cellular**	**Organ**	**Rodent behaviour**	**Consistency**
Xu et al. (2023) Sprague–Dawley Rats Drinking water 1.5 (C), 3.5, 5.5 Gestation till PND60 'standard diet'*	NA	? Hippocampus immunhistochem. stain: ↑ number of positive cells for LC3-II & p62 (autophagy↑) and TUNEL, PARA1, cleaved-caspase 3 (apoptosis↑) TEM ultrastructure: ↑number of lysosomes	? Hippocampus histopath: ↓number of Nissl bodies, ↑ lighter & blurred	NA	**Yes**
		No morphometric protocol, no blinding	No morphometric protocol, no blinding		
Masjosthusmann et al. (2020) 7–7000 uM;	NA	**BMD_{30} = 19.435 μg/mL** cell culture medium 1023 μM [CI: 820–1840] ↓ Neuronal Precursor Cell proliferation & cell viability	NA	NA	NA In vitro effects at relatively high concentrations
		Uncertain if neuronal cells are more sensitive than others; effect size and duration necessary to trigger organism level effect; concentration concordance with organism level effect			
Consistency across studies	Partly, can be explained by differences in uncertainty, exposure regime, strain & sex and specific endpoint analysed	Partly, can be explained by differences in uncertainty, exposure regime, strain & sex and specific endpoint analysed Yes for data available in McPherson et al.: BMC 30 = 19.435 μg/mL cell culture medium versus no effect in McPherson at 0.08 ug F/g brain in top dose.	Partly, can be explained by differences in uncertainty, exposure regime, strain & sex and specific endpoint analysed	No, but can be explained by differences in uncertainty, exposure regime, strain & sex and specific endpoint analysed	A NOAEL is not available from these studies. At molecular level relevant effects are apparent from 2 mg/kg bw day onwards (Bartos et al., 2018). At cellular, organ and organism level from 3.5 mg/kg bw per day onwards (Zhou 2021; Zhao et al., 2019; Zhao et al., 2021), but the behavioural data are inconsistent and uncertain.

↑ = increased

↓ = reduced

(C) indicates the control group, a non-zero exposure based on fluoride content in feed

NOAEL ≥ [… mg/kg bw per day]: the NOAEL is equal or above the highest dose tested (i.e. highest dose tested is a NOAEL)

LOAEL ≤ [… mg/kg bw per day]: the LOAEL is equal or below the lowest dose tested (i.e. lowest dose tested is a LOAEL)

ASR = Acute Startle Response,

CI = Confidence Interval

EPM = Elevated Plus Maze,

HNCs = historical negative controls,

TABLE 23 (Continued)

? indicates no reliable quantification (see related uncertainties)

BDNF = Brain-Derived Neurotrophic Factor: survival, growth, differentiation, synaptic plasticity

Ca = Calcium

CaMKIIα subunit: Calcium/Calmodulin-dependent protein kinase II alpha: neuronal signalling, synaptic plasticity, learning & memory

CAT = **Catalase:** decomposition of hydrogen peroxide, antioxidant defence

c-fos = transcription factor: neuronal activity, stress response

CREB = cAMP Response Element-Binding Protein

CRTC1 = CREB-Regulated Transcription Coactivator 1

GFAP = Glial Fibrillary Acidic Protein: Astrocyte activation mark

GLUT1 = Glucose Transporter 1: Glucose transport across blood–brain barrier and energy metabolism

GOT = **Glutamate Oxaloacetate Transaminase: synthesis of neurotransmitter glutamate**

GPT = Glutamate Pyruvate Transaminase: synthesis of glutamate and energy metabolism

GSH-Px = glutathione peroxidase

IR = Insulin Receptor: regulating synaptic plasticity and neuroprotection pathways

NMDR = Non-Monotonic Dose–Response

MDA = malondialdehyde: lipid peroxidation marker

ROS = Reactive Oxidant Species

TSH = Thyroid-stimulating hormone

T3 = Triiodothyronine

T4 = tetraiodothyronine

SIK2 = Salt-Inducible Kinase 2:

SIK2-CRTC1-CREB-BDNF-VGF signalling pathway: for neuronal survival & plasticity

SNAP25 = Synaptosomal-Associated Protein 25: synaptosomal function marker

SOD = superoxide dismutase

SYP = Synaptophysin: synaptosomal function marker

VAMP-2 = Vesicle-Associated Membrane Protein 2: neurotransmitter release, synaptic vesicle trafficking, synaptic function

VGF = neuropeptide precursor

L/D = Light–Dark Transition Test;

MA = Motor Activity

MWM = Morris Water Maze,

PA = Passive Avoidance;

PNCs = historical positive control data,

RW = Running Wheel;

Y-M = Y-Maze

*Standard diet assumption 16–20 ppm F ~ 1.5 mg/kg bw day (EFSA default factors)

Uncertainties relevant for all methods and data

Modulating factors: (epi)genetic background, diet, lifestyle, stress, infections, co-exposure

Methodical uncertainties: selection/combination of test systems/endpoints; data-assessment pipelines; mechanistic understanding & bridging to human relevance; including coverage of higher cerebral functions; metabolism and kinetics; extrapolation to human limit values; methods covering a sufficient number of relevant DNT processes; methods covering interactions of relevant DNT processes; specific versus unspecific effects

3.3.4.2 | *Integration of the evidence on neurotoxicity (NT)*

LOAEL identified in the juvenile/adult animals for relevant molecular level effects are apparent at the lowest doses tested (between 2 and 11 mg/kg bw per day and up to the highest dose levels) are summarised in Table 24. These effects include alterations in s, i.e. different markers for oxidative stress and neuroinflammation in neurons and glia (Cao et al., 2019; Ran et al., 2021; Wang et al., 2023; Xiang et al., 2024; Yan et al., 2016), activation of microglia (Cao et al., 2019; Zhang et al., 2022), various measures of apoptosis (Wang et al., 2023; Xiang et al., 2024; Yan et al., 2016; Zhang et al., 2022, 2023a), and markers of autophagy (Ran et al., 2023; Zhang et al., 2023b). Disruption of brain cholinergic function (Wang et al., 2023) and several key signalling pathways involved in neuronal function were identified using biochemical, transcriptomic and proteomic approaches (Bittencourt et al., 2023; Ran et al., 2021; Teng et al., 2018; Wang et al., 2023).

NOAEL for molecular level effects ranged between 1.7 and 3.3 mg/kg bw per day based on of markers for neuronal signalling, synaptic plasticity, learning & memory (Teng et al., 2018) or synaptosomal protein expression (Cao et al., 2019) or neurotransmitter release/uptake pathways (Han et al., 2014).

At cellular and organ level NOAELs were identified within all reliable studies available. Respective NOAEL-LOAEL ranges were at 4.2–6.5 mg/kg bw per day for number of Purkinje cells in cerebellum sections and cerebellum weight (Agustina et al., 2019) or 5–6.6 mg/kg bw day for microtubule ultrastructure (Tubα1a and Tubβ2a in Niu et al. (2015)). No effect on number of Purkinje cells in prefrontal cortex and no increase in volume of medial prefrontal cortex was observed up the highest dose of 11 mg/kg bw day within Pulungan et al. (2018).

Effects on brain histological markers were reported in two other studies and included altered neuron number and blood–brain-barrier function, but methodological concerns question the reliability of the reported LOAELs of 3–9 mg/kg per day of the various endpoints (Bittencourt et al., 2023; Xiang et al., 2024).

For rodent behavioural Morris Water Maze tests a NOAEL–LOAEL range of 2–11 mg/kg bw per day was observed in Cao et al. (2019) and this also covers the NOAEL–LOAEL range of 6 to 10.5 mg/kg bw per day of Ran et al. (2021) as well as the LOAEL of 3.5 mg/kg bw per day in Wang et al. (2023).

For some in vivo studies internal exposure measurements are available (Ran et al., 2021; Yan et al., 2016) which support that within these different in vivo studies with similar exposure doses the fluoride concentrations reported in brain were within one order of magnitude (0.15–1.7 µg/g brain). Yet in one study (Han et al., 2014) brain concentrations were much higher (ca 40–50 ug/g in hippocampus) in spite of lower or similar drinking water concentrations. This introduces additional uncertainty for the comparative assessment of these studies in addition to the extrapolation from rodents to humans.

As previously discussed for DNT, the principle that relevant effects at the molecular and cellular and organ level are apparent at concentrations below concentrations where relevant effects are observed at the organism level is in line with established toxicological concepts and it is reflected in the guidance for the development of Adverse Outcome Pathways (https://aopwiki.org/). Molecular and cellular effects may be considered as adverse and relevant as such, since they represent a toxicity that compromises an organism's capacity to compensate for the multitude of additional real-world stressors.

However, given that the effects at cellular and organ level are proximal to the organism level effects used for establishing regulatory HBGVs. As such, from the data available from juvenile/adult animal studies, a value of 3.5 mg/kg bw per day is indicative of the onset of adversity as defined by reductions in body weight, brain weight, neuronal cellular, molecular level changes and impairments in behavioural measures. This value is similar to that derived for our evaluation of studies in developing animals (see Figure 6).

Including the lower tier behavioural data would not change this conclusion, since the NOAEL/LOAEL values are within range (see Figure 5 in section on behavioural (D)NT summary) above.

TABLE 24 Integration of the evidence on NT from fluoride exposure in juvenile/adult animals.

References Study design	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) brain (μg/g) or plasma (μg/mL) Summary of effects Major uncertainties in grey rows Molecular	Cellular	Organ	Rodent behaviour	Consistency
Ran et al. (2021) Sprague–Dawley Rats (M, F) Drinking water 1.5 (C), 2, 6, 10.5 mg F/kg bw day 5-week-old for 3 months No info on F content in feed*	**LOAEL** **≤ 2 mg/kg bw day** ≤ 5 mg/L drinking water ≤ 0.7 μg/g brain Oxidative stress in brain ↑ (MDA, ROS, SOD)	NA	NA	**NOAEL–LOAEL** **6–10.5 mg/kg bw day** 5–50 mg/L drinking water 0.7–1.1 μg/g brain MWM	**Yes** Molecular level effects at concentrations below cellular and organism level effects.
	F concentration identical in ctr. and low dose (both 0.7 μg/g brain)			HNCs, HPCs; critical elements of study conduct & statistics	
	LOAEL **≤ 10.5 mg/kg bw day** ≤ 100 mg/L Negative regulation of defence response, CNS neuron differentiation, Melanogenesis (GO & KEGG analysis, i.e. gene & protein level)				
	Analysis only for high dose animals				
Cao et al. (2019) Mice, strain not specified, (M, F) Gavage[64] 1 (C), 2, 11 mg/kg bw day (M) 1 (C), 2.5, 16.5 mg/kg bw day (F) 3 months old for 3 months ≤ 1 mg F/kg bw day contributed via F content in feed (≤ 5 mg/kg)	**LOAEL** **≤ 2 mg/kg bw day** Oxidative stress in brain↑ (MDA, SOD + GSH-Px activity) Inflammation in brain ↑ (microglia activation maker (Iba-1), complement activation marker(C3))	NA	NA	**NOAEL–LOAEL** **2–11 mg/kg bw day** MWM	**Yes** Molecular level effects at concentrations below or similar to organism level effects.
	No info on bw/generic tox			HNCs, HPCs; critical elements of study conduct & statistics	
	NOAEL–LOAEL **2–11 mg/kg bw day** ↓ synaptosomal proteins (SNAP25 + SYP) in brain				
	No info on bw/generic tox				

(Continues)

[64]Gavage with 0.3 mL/mouse with 0.1 and 1 mg/L; (0, 0.03 and 0.3 mg/day) Extrapolation to mg/kg bw day is based on reported BW (30 g M; 20 g F).

TABLE 24 (Continued)

References Study design	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) brain (μg/g) or plasma (μg/mL) Summary of effects Major uncertainties in grey rows **Molecular**	**Cellular**	**Organ**	**Rodent behaviour**	**Consistency**
Wang et al. (2023) Sprague–Dawley Rats Drinking water 1.5 (C), 3.5, 5.5, 7.5 mg F/kg bw day Adults for 82 days No info on F content in feed*	**LOAEL** **≤ 3.5 mg/kg bw day** ≤ 23 ug/mL in blood Inflammation ↑(IL-1β and IL-6); ↑AChE, ↓ChAT; Apoptosis ↑(Bcl-2, BAX, Caspase-3, P53, FOXO1); neuronal function altered (↑AChE, ↓ChA, ↓SIRT1,- BDNF–TrkB-PI3K- Akt- MAPK-, NF-κB) Oxidative stress in serum ↑(GSH-Px, T-AOC, SOD; MDA)	NA	NA (tier 3)	**LOAEL** **≤ 3.5 mg/kg bw day** ≤ 23 mg/L MWM	**Yes** Molecular level effects at concentrations below or similar to organism level effects
				HNCs, HPCs; critical elements of study conduct & statistics	
Whitford et al. (2009) Sprague–Dawley Rats Drinking water 0.04 (C), 2.9, 5.7, 11.5 mg F/kg bw day Adults for 8 months Chemically defined low F diet (0.4 mg/kg = 0.02 mg/kg bw day)	NA	NA	NA	**NOAEL** ≥ 11.5 mg/kg bw day ≥ 50 mg/L OP	NA
				HNCs, HPCs; critical elements of study conduct	
Yan et al. (2016) Wistar Rats Drinking water 1.5 (C), 6.9, 12.3 mg F/kg bw day Adults for 10 weeks No info on F content in feed*	**LOAEL** **≤ 6.9 mg/kg bw day** ≤ 60 mg/L drinking water ≤ 0.655 μg/g in brain ↑ apoptosis in cortex and hippocampus (TUNEL, Bcl/Bax)	Brain (intracellular) ultrastructural degeneration effects (TEM analysis)	NA	NA	**Yes** Molecular level effects at concentrations of cellular level effects.
	TUNEL stain used without additional marker, i.e. not very specific for apoptosis; needs to be interpreted in combination with TEM analysis	Images appear reliable, but morphometric method description is minimal, thus evidence for dose–response relationship is uncertain			
	Inflammatory markers in glia cells ↑ (all protein level: OX-42; IL-1ß, IL-6, TNF-alpha)				

TABLE 24 (Continued)

References Study design	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) brain (μg/g) or plasma (μg/mL) Summary of effects Major uncertainties in grey rows **Molecular**	**Cellular**	**Organ**	**Rodent behaviour**	**Consistency**
Agustina et al. (2019) Wistar Rats Gavage 2 (C), 4.25, 6.5, 11 mg F/kg bw day:, Adults for 30 days No info on F content in feed*	NA	**NOAEL–LOAEL** **4.25–6.5 mg/kg bw day** ↓ number of purkinje cells in cerebellum sections	**NOAEL–LOAEL** **4.25–6.5 mg/kg bw day** ↓ cerebellum weight with relevant effect size (87%–90% of ctrl.)	NA Behavioural data = tier 3, therefore not listed here	**Yes** Cellular level effects at concentrations of organ level effects.
		Morphometric analysis well described, blinded, but very complex, which is an uncertainty.	Not statistically significant, no clear dose–response		
Pulungan et al. (2018) Wistar Rats Gavage 2 (C), 4.25, 6.5, 11 mg F/kg bw day Adults for 30 days No info on F content in diet*	NA	**NOAEL** **≥ 11 mg/kg bw day** Number of purkinje cells in prefrontal cortex sections not statistically significantly affected	**NOAEL** **≥ 11 mg/kg bw day** Volume of medial prefrontal cortex not statistically significantly affected	NA Behavioural data = tier 3, therefore not listed here	NA No effects at cellular level.
		Morphometric analysis well described, blinded, but very complex, which is an uncertainty; a non-significant trend in the reduction is observed; bw not reported	Well described but complex procedure; U-shaped dose–response with top dose volume higher than control		
Zhang et al. (2022) Wistar Rats Drinking water 1.5 (C), 3.75, 6, 10.5 mg F/kg bw day Adults for 3 months No info on F content in feed*	**LOAEL** **≤ 3.75 mg/kg bw day** ≤ 25 mg/L Hippocampal neurons: apoptosis markers ↑ (TUNEL, Bcl-2, Bax and caspase3 (western blot); JNK-pathway) Hippocampal Microglia activation (Iba-1, IL-1ß protein) **2.5–5 mg/L** BV-2 microglia cell line activation: IL-1ß release	? Hippocampal neurons: disturbed ultrastructure	NA	NA Behavioural data = tier 3, therefore not listed here	**Yes** Molecular level effects at concentrations of cellular level effects.

(Continues)

TABLE 24 (Continued)

References Study design	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) brain (μg/g) or plasma (μg/mL) Summary of effects Major uncertainties in grey rows **Molecular**	**Cellular**	**Organ**	**Rodent behaviour**	**Consistency**
		Images appear reliable, but morphometric method description is minimal, no info on blinding; thus, evidence for dose–response relationship is uncertain; no info on randomisation of animals, no info on generic tox (bw)			
Teng et al. (2018) Sprague–Dawley Rat Drinking water 0.8 (C), 1.3, 1.7, 2.6 mg F/kg bw day Adults for 18 months No info on F content in feed*	**NOAEL–LOAEL** **1.3–1.7 mg/kg bw day** 3.9–7.4 mg/L Neuronal function altered (↑ Ca concentration in hippocampal CA3 synaptosome; ↑ CaMKIIα subunit and ↑ c-fos)	NA	NA	NA	NA Effects at molecular level
	NMDR in Ca concentration				
Niu et al. (2015) Kunming mice (M) Drinking water 3.3 (C), 5, 6.6, 10 mg F/kg bw day Adults for 60 days No info on F content in feed* Rob tier 2	NA	**NOAEL–LOAEL** **5–6.6 mg/kg bw day** 11.3–22.6 mg/L ↓Tubα1a and Tubβ2a mRNA and protein level (western & immunoblot)	NA	NA	**NA** Effects at cellular level
		Protein not significant, but ca 20% reduction; microtubule ultrastructure (TEM) reported as affected at all doses, but no blinding and image not clear			
Zhang et al. (2023a) Sprague–Dawley Rat (M, F) Drinking water 1.5 (C), 2, 3.5, 5.5 mg F/kg bw day Adults for 2 months, No info on F in feed*	**LOAEL** **≤2 mg/kg bw day** ≤4.5 mg/L Apoptosis ↑ in cortex IRE1α, cleaved caspase-12, cleaved caspase-3, GRP78 western blots: ↑); autophagy↑ (Beclin1, p62, LC3-II)	? Cortex immunhistochemistry: ↑number of cells with stain for caspase-12 & GRP78 (apoptosis ↑), LC3 (autophagy↑)	NA	NA	**Yes**
		No morphometric protocol, no blinding			

TABLE 24 (Continued)

References Study design	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) brain (μg/g) or plasma (μg/mL) Summary of effects Major uncertainties in grey rows **Molecular**	**Cellular**	**Organ**	**Rodent behaviour**	**Consistency**
Ran et al. (2023) Sprague–Dawley Rat (M, F) Drinking water 1.5 (C), 2, 6, 10.5 mg F/kg bw day Adults for 3 & 6 months No info on F in feed*	**LOAEL** **≤ 2 mg/kg bw day** ≤ 5 mg/L Autophagy ↑ (p-mTOR↓, ATG5↑, LC3II↑ and p62↑ (western blot) Dose-dependent enrichment of gene-encoding components of the autophagy signalling pathway (RNO04140)	? Ultrastructure of neurons by TEM ↑ deteriorated (nuclear membrane & chromatin, autophagosomes ↑)	NA	NA	**Yes**
		No morphometric protocol & no info on blinding; no info on housing conditions			
Xiang et al. (2024) Sprague–Dawley Rat brain Drinking water 1.5 (C), 3, 6 mg/kg bw day Adults for 4 months No info on F in feed*	**NOAEL–LOAEL** **3–6 mg/kg bw day** 5–50 mg/L Oxidative stress ↑ (ROS, 8-OHdG (, p-histone H2A.X (ser139)) Apoptosis ↑ (PARP, PAR, AIF, p53 (protein)	NA	↓ Neuronal count (Nissel stain) & BBB integrity (Evans blue)	NA	**Yes**
	Unclear which brain region was sampled		No morphometric protocol, no blinding, unclear which brain region was sampled		
Bittencourt et al. (2023) Mice (M) Drinking water 1.5 (C), 3, 9 mg/kg bw day PND21 for 60 days No info on F in feed*	? Changed proteomics profile, interpreted via Gene Ontology (GO): cellular component organisation, nervous system development, response to stimulus, metabolic process, nervous system process and synaptic signalling	NA	? ↓Number of mature neurons (NeuN positive) in hippocampus CA3 & dentate gyrus	NA	**Yes**
			No info on blinding for morphometric protocol		

(Continues)

TABLE 24 (Continued)

References Study design	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) brain (μg/g) or plasma (μg/mL) Summary of effects Major uncertainties in grey rows **Molecular**	**Cellular**	**Organ**	**Rodent behaviour**	**Consistency**
Han et al. (2014) Kumming Mice (M) Drinking water 1.68 (C), 3.33, 4.98, 8.43 mg F/kg bw day Adult for 180 days 1.16 mg F/kg bw day via feed content	**NOAEL–LOAEL** **3.3–5 mg/kg bw day** 11–22 mg/L drinking water 42–48 μg/g brain in hippocampus Neuronal function altered (Neurotransmitter release/uptake pathway affected: VAMP-2 mRNA ↑)	NA	NA	NA Behavioural data = tier 3, therefore not listed here	NA
	Measured only at mRNA, not at protein level; generic toxicity (body weight) not reported;				
Consistency	Yes	Partly and can be explained by differences in uncertainty, exposure regime, strain & sex and specific endpoint analysed	Partly and can be explained by differences in uncertainty, exposure regime, strain & sex and specific endpoint analysed	Yes	

↑ = increased

↓ = reduced

(C) indicates the control group, a non-zero exposure based on fluoride content in feed

NOAEL ≥ [… mg/kg bw per day]: the NOAEL is equal or above the highest dose tested (i.e. highest dose tested is a NOAEL)

LOAEL ≤ [… mg/kg bw per day]: the LOAEL is equal or below the lowest dose tested (i.e. lowest dose tested is a LOAEL)

? indicates no reliable quantification (see related uncertainties)

BDNF = Brain-Derived Neurotrophic Factor: survival, growth, differentiation, synaptic plasticity

Ca = Calcium

CaMKIIα subunit: Calcium/Calmodulin-dependent protein kinase II alpha: neuronal signalling, synaptic plasticity, learning & memory

CAT = **Catalase:** decomposition of hydrogen peroxide, antioxidant defence

c-fos = transcription factor: neuronal activity, stress response

ASR = Acute Startle Response,

CI = Confidence Interval

EPM = Elevated Plus Maze,

HNCs = historical negative controls,

L/D = Light–Dark Transition Test;

MA = Motor Activity

MWM = Morris Water Maze,
OP = Operand Procedure

PA = Passive Avoidance;

PNCs = historical positive control data,

TABLE 24 (Continued)

CREB = cAMP Response Element-Binding Protein

CRTC1 = CREB-Regulated Transcription Coactivator 1

GFAP = Glial Fibrillary Acidic Protein: Astrocyte activation marker

GLUT1 = Glucose Transporter 1: Glucose transport across blood–brain barrier and energy metabolism

GOT = **Glutamate Oxaloacetate Transaminase: synthesis of neurotransmitter glutamate**

GPT = Glutamate Pyruvate Transaminase: synthesis of glutamate and energy metabolism

GSH-Px = glutathione peroxidase

IR = Insulin Receptor: regulating synaptic plasticity and neuroprotection pathways

NMDR = Non-Monotonic Dose–Response

MDA = malondialdehyde: lipid peroxidation marker

ROS = Reactive Oxidant Species

TSH = Thyroid-stimulating hormone

T3 = Triiodothyronine

T4 = tetraiodothyronine

SIK2 = Salt-Inducible Kinase 2:

SIK2-CRTC1-CREB-BDNF-VGF signalling pathway: for neuronal survival & plasticity

SNAP25 = Synaptosomal-Associated Protein 25: synaptosomal function marker

SOD = superoxide dismutase

SYP = Synaptophysin: synaptosomal function marker

VAMP-2 = Vesicle-Associated Membrane Protein 2: neurotransmitter release, synaptic vesicle trafficking, synaptic function

VGF = neuropeptide precursor

RW = Running Wheel;

Y-M = Y-Maze

*Standard diet assumption 16–20 ppm F ~ 0.8 or 1.5 or 2 mg/kg bw day for chronic or subchronic or subacute exposure in rats respectively and 3.3 mg/kg bw day in subchronic exposure in mouse (see Annex E.7)

Uncertainties relevant for all methods and data

modulating factors: (epi)genetic background, diet, lifestyle, stress, infections, co-exposure

methodical uncertainties: selection/combination of test systems/endpoints; data-assessment pipelines; mechanistic understanding & bridging to human relevance; including coverage of higher cerebral functions; metabolism and kinetics; extrapolation to human limit values; methods covering a sufficient number of relevant DNT processes; methods covering interactions of relevant DNT processes; specific versus unspecific effects

3.3.4.3 | *Integration of the evidence on bone effects in animals*

In rats, mice and rabbits there were effects observed on bone histology and metabolism. However, these effects were not consistent across or within species and occurred at higher doses. At the organismal level, changes in body weight and dental fluorosis were noted. Again, these were inconsistent with three studies showing no change in body weight (Fina et al., 2018; Lombarte et al., 2021; Yao et al., 2019), one study reported decreased body weight after 3 or 4 months (Sharma et al., 2022) and three studies did not measure body weight (Chu et al., 2020; Li et al., 2023; Ranjan et al., 2023) after exposure to Fluoride. Dental fluorosis was observed in four studies at exposures above 6 mg/kg bw day with the severity increasing with dose and time of exposure (Chu et al., 2020; Li et al., 2023; Linghu et al., 2023; Yao et al., 2019).

At the organ level, bone mineral density was either not changed (Fina et al., 2018) or was decreased (Ranjan et al., 2023; Sharma et al., 2022). Trabecular bone was generally more affected than cortical bone, with changes in trabecular bone observed in several studies while changes in cortical bone were inconsistent. There were increases observed in bone thickness (Chu et al., 2020; Sharma et al., 2022) and decreases in trabecular bone fracture load, stiffness, volume and connectivity (Fina et al., 2018; Li et al., 2023).

Increases in molecular signalling pathways such as Wnt/b-catenin were observed in mice at 10.8 mg/kg bw day and above and increases in mTOR/AKT pathway in rats above 6 mg/kg bw day. Increased markers of autophagy were seen at 6 mg/kg bw day and above. Markers of oxidative stress gave inconsistent results with one study in Wistar rats showing decreased enzyme activities (SOD, Cat, GR) and increased lipid peroxidation (MDA, AOPP) above 4.7 mg/kg bw day (Sharma et al., 2022) while a separate study also in Wistar rats observed no changes in GPx, MDA or SOD up to 21.5 mg/kg bw day (Li et al., 2023).

Studies in isolated cells or cell lines showed some consistency at the level of cellular and molecular markers EG increases in Wnt/b-catenin pathway and increased autophagosomes, autolysosomes and markers of autophagy (Linghu et al., 2023).

Overall, adverse effects on bone were observed but at higher doses and were not consistent (Table 25).

TABLE 25 Integration of the evidence on bone effects from fluoride exposure in animals.

Reference Study design Tier	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) Summary of effects Molecular	Cellular	Organ	Organism	Consistency
Chu et al. (2020) BALB/C mice (M) (4 weeks old) Drinking water 3.3 (C), 7.05, 10.8, 18.3 mg/kg bw per day 3 months No info on F content in feed RoB Tier 1	**NOAEL–LOAEL** 7.05–10.8 mg/kg bw day (25 mg/L - 50 mg/L) ↑ Wnt/b-catenin signalling; ↑ Wnt3a; ↑ b-catenin; ↑ Runx2; ↑ P-Gsk3b	**LOAEL** ≥ 10.8 mg/kg bw day (50 mg/L) ↑ serum ALP, ↑ ALP and osteocalcin SaoS2 cells ↑ viability and differentiation ↑ Wnt/b-catenin	**LOAEL** ≥ 10.8 mg/kg bw day (50 mg/L) ↑ formation cancellous bone ↑ thickness trabecular bone ↑ thickness cortical bone **NOAEL – LOAEL** 10.8–18.3 mg/kg bw day (50 mg/L - 100 mg/L) ↑ in Tb.Ar; ↑ Bone anabolism (histhologic observations)	**LOAEL** ≥ 7.05 mg/kg bw day (25 mg/L) ↑ dental fluorosis BW was not reported	Yes, among bone markers and in mice and cell results
Fina et al. (2018) Sprague–Dawley rats (F) (21 days old) Gavage 2 (C), 6, 10, 18 mg/kg bw per day 30 days No info on F content in feed RoB Tier 1			**LOAEL** ≥ 6 mg/kg bw day ↓ trabecular bone fracture load, stiffness, bone volume and connectivity. No effects on cortical bone. No effects on BMD	No change in BW	Yes, among endpoints of effects on bone
Lombarte et al. (2021) (1455) Sprague–Dawley rats (F) (21 days old) Gavage 0.2 (C), 6, 10 and 18 mg/kg bw per day) 30 days No info on F content in feed RoB Tier 1		**NOAEL–LOAEL** 10–18 mg/kg bw day Significant apoptosis in subchondral bone	**LOAEL** ≥ 10 mg/kg bw day ↓ Bone volume Cartilage: hyperplasia of chondrocytes in the proliferating zone only ↓Tb.Th No change in tibia length; no difference in total growth plate thickness No change Tb.N or Tb.Sp Histopathology: histopathological immature trabeculae and some inflammatory foci observed in all treated groups.	No change in BW	Effects on bone
Sharma et al. (2022) (2144) Wistar rats (M) (12–14 weeks) Drinking water 0.2 (C), 4.7, 9.2 mg/kg bw per day 180 days F- in feed: 1.93 ± 0.65 mg/kg (% dry matter basis) F-in tap water: 1.43 ± 0.17 mg/L fluoride. RoB Tier 1	**LOAEL** ≥ 4.7 mg/kg bw day (50 mg/L) ↓ Ca and P ↓ Ca:P ratio ↓ GR, CAT, SOD, thiols **LOAEL** ≥ 9.2 mg/kg bw day (100 mg/L) ↑MDA and AOPP ↓ AChE and GSH		**LOAEL** ≥ 4.7 mg/kg bw day (50 mg/L) ↓ overall bone density **NOAEL – LOAEL** 4.7–9.2 mg/kg bw day (100 mg/L) ↑ thickness of medulla	**LOAEL** ≥ 9.2 mg/kg bw day (100 mg/L) ↓ BW up to 22% throughout 6 months **LOAEL** ≥ 4.7 mg/kg bw day (50 mg/L) ↓ BW up to 16% by 4 months	

(Continues)

TABLE 25 (Continued)

Reference Study design Tier	NOAEL, LOAEL or ranges (mg/kg bw per day) NOAEC, LOAEC or ranges in drinking water (mg/L) Summary of effects Molecular	Cellular	Organ	Organism	Consistency
Yao et al. (2019) (2725) C57BL/6 mice (M) (3 weeks old) Drinking water 3.3 (C), 12.3, 21.3 mg/kg bw day) 3 months RoB Tier 2	**NOAEL–LOAEL** 12.3–21.3 mg/kg bw day (100 mg/L) ↑ ALP (osteoblast marker) **LOAEL** > 12.3 mg/kg bw day ↑ TRAP	**LOAEL** 12.3 mg/kg bw day (50 mg/L) Number of osteoclasts highest; number lower at 21.3 mg/kg bw day (100 mg/L) Non-statistically significant increase in osteoblast marker BGP	**LOAEL** ≥ 12.3 mg/kg bw day (50 mg/L) disordered chondrocytes ↑ bone mass	**LOAEL** ≥ 12.3 mg/kg bw day Increasing dental fluorosis with dose No change in BW (50 mg/L) dental fluorosis	Some consistency between osteoclasts and organ effects
Li et al. 2023 (3092) Wistar rats (M) (6 weeks) Gavage 1.5 (C), 11.5 and 21.5 mg/kg bw/day 3 months RoB Tier 2	Non dose related changes in GPX, MDA or SOD		**LOAEL** ≥ 11.5 mg/kg bw day ↑ in trabecular bone separation (Tb.Sp) ↓ bone volume; ↓ bone connectivity Histological changes in bone	**LOAEL** ≥ 11.5 mg F/kg bw day dental fluorosis from 6 weeks BW as not reported	Limited data to assess consistency
Linghu et al., 2023 (3103) Sprague–Dawley rats (M, F) Drinking water 1.5 (C), 1.95, 6 and 10.5 mg/kg bw per day 6 months; Rob Tier 2	**LOAEL** ≥ 1.95 mg/kg bw day ↓ p62 (5 mg/L) **NOAEL–LOAEL** 1.95–6 mg/kg bw day (50 mg/L) ↑ Beclin1, Atg7 and LC3II/I ↑ p-mTOR/m-TOR and p-AKT/total AKT	Primary osteoblasts in vitro: **LOAEL** ≥ 0.5 mM ↑ Beclin1, Atg7 and LC3II/I ↓ p62 **LOAEL** ≥ 1.0 mM ↓ p-mTOR/m-TOR and p-AKT/total AKT ↑ autophagosomes and autolysosomes	**NOAEL–LOAEL** 1.95–6 mg/kg bw day (50 mg/L) ↑ Tb.Ar and Tb.Wd **LOAEL** ≥ 1.95 mg/kg bw day (5 mg/L) Autophagosomes and autolysosomes in bone tissue	Increasing dental fluorosis with dose (I^o–III^o); At 1.95 mg/kg bw day (5 mg/L) 25% normal; 66% I^o; 9% II^o	Yes, between molecular markers and organ effects
Ranjan et al. (2023) (3151) New Zealand rabbits (Oryctolagus cuniculus) 4–6 weeks Drinking water 0, 50, 100, 200, 400 mg/mL in 45 and 90 days RoB Tier 2	**NOAEC–LOAEC** 50–100 mg/mL ↑ ALP, ALT and urea nitrogen from day 45 **NOAEC–LOAEC** 100–200 mg/mL ↑ AST from day 45 ↑ creatinine from day 90		**LOAEC** ≥ 50 mg/mL Tibia: ↓ cortical index ↑ metaphyseal width **NOAEC–LOAEC** 50–100 mg/mL ↓ bone density in flat bones 100–200 mg/mL Thickening of epithelial plate and disorganised chondrocytes	Induced osteogenesis and osteoporosis	

3.3.4.4 | *Visual overview of NOAEL–LOAEL ranges for adverse effects in DNT, NT and bone in animal studies*

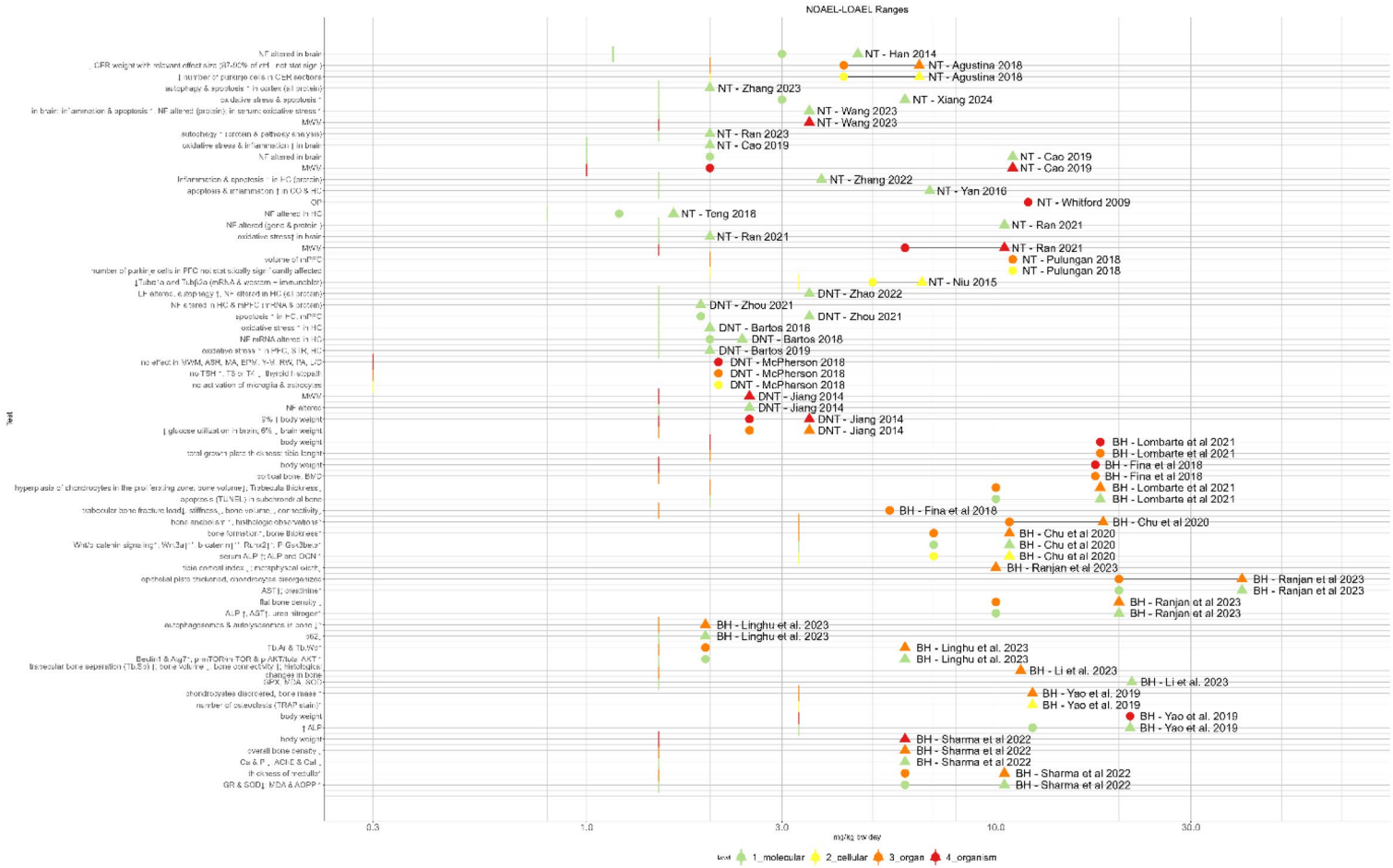

FIGURE 6 Visual integration of all available data for Developmental Neurotoxicity (DNT) and adult Neurotoxicity (NT) and Bone Health (BH) at the molecular, cellular, organ and organism level. Vertical bars = baseline F exposure of controls via feed, Points = NOAELs, Triangles = LOAELs; LOAEL-LOAEL ranges are grouped by study type (BH, DNT vs. NT) and study reference; observed effects are indicated at the y-axis. Details are available in Tables 16–18 for bone health (above, before this WoE text). Note that no yellow, orange or red triangles or circles (cellular, organ, organisms LOAELs or NOAELs) are apparent below 2 mg/kg bw per day. Note that only McPherson et al. (2018) used low F diet and total F intake was corrected in the other studies based on a default assumption of 16–20 ppm F in feed (i.e. 1.5 mg/kg bw and day from feed content in subchronic rat studies) or specific information available in the publications. Note that BH effects were reported at doses higher than DNT or NT effects. Including the lower tier behavioural data would not change this conclusion, since the NOAEL/LOAEL values are within ranges do not differ (see Figure 5). CER, cerebellum; CO, cortex; HC, hippocampus; mPFC, medial prefrontal cortex; NF, neuronal function markers; LS, lysosomal function; PFC, prefrontal cortex.

3.4 | Evidence from in vitro studies

3.4.1 | Evidence on neuronal cells from in vitro battery results

Within the US EPA CompTox Database, an in vitro battery (IVB) of assays was assembled focused on key DNT processes, rather than along AOP-associated processes (DNTIVB). Although fluoride has not been tested in the complete (DNTIVB), most assays were negative for neurotoxicity. One positive response was returned for fluoride inhibition in a neural progenitor cell proliferation assay but at a dose that overlapped with cytotoxicity and suggestive of a non-specific effect on cell viability (see Appendix C, Table C.1).

The BMC30 of 1023 μM F (1.023 mM) corresponds to 19.44 μg/mL cell culture fluid. For comparison, in a single in vivo rodent DNT study, brain concentration data were measured as 0.08 μg F/g brain in top dose (McPherson et al., 2018). In this study no adverse effect was observed.

In animal studies using adult animals brain concentrations were measured and ranged up to ca. 1 μg/g (Ran et al., 2021; Yan et al., 2016), where molecular/cellular and behavioural effects were observed; and up to 50 μg/g (Han et al., 2014) where some molecular effects were observed.

These relationships may be considered within the final weight of evidence approach.

3.4.2 | In vitro evidence on neuronal cells in the peer reviewed literature

Fluoride has been tested in vitro at concentrations between 0.12 and 6 mM as NaF (0.05–2.7 mM fluoride), in human neuroblastoma SH-SY5Y cells with LOAELs ranging from 0.12 to 0.95 mM NaF (0.05–0.43 mM fluoride) (Chen et al., 2018; Chen, Jia, et al., 2023; Chen, Ning, et al., 2023; Han et al., 2022; Lai et al., 2020; Liu et al., 2011; Niu et al., 2018; Tang et al., 2023; Tu et al., 2018; Zhang, Lou, & Guan, 2015; Zhao et al., 2019; Zhao et al., 2020). Molecular changes at a similar range of LOAELs were also reported in Neuro-2A cells (Chen, Ning, et al., 2017; Chen, Ning, et al., 2023), HT-22 cells (Chen, Jia, et al., 2023), BV-2 microglia cells (Chen, Zhao, et al., 2017), Muller Glial cells (García-López et al., 2020), PC12 cells (pheochromocytoma cells) (Hoorang et al., 2020; Ke et al., 2016; Li et al., 2023). Changes in mRNA and protein of NMDAR subunits were reported in primary hippocampal neurons of rats at 0.26 mM fluoride. Molecular changes such as increased nAChRa7 protein but not mRNA and decreased binding sites for α-bungarotoxin were reported at concentrations as low as 0.5–5 μM in one study (Gao et al., 2008). Cytotoxicity at 24 h was observed in SH-SY5Y cells from 0.12 mM (Liu 2011) to 1 mM NaF (Tu et al., 2018; Zhao et al., 2019), 1 mM in BV-2 microglial cells (Chen, Zhao, et al., 2017) and 2.0 mM NaF in Neuro-A (Chen, Ning, et al., 2017; Chen, Ning, et al., 2023). Similar evidence has been presented in an overview of 26 in vitro studies (Guth et al., 2020), with reported LOAELs at concentrations overlapping with cytotoxicity (0.1–4 mM NaF).

3.4.3 | Evidence on thyroid effects in vitro

Based on structural similarity, it has been postulated that fluoride may compete with iodide for transfer across the sodium iodine symporter (NIS). In a study examining substrate specificity of NIS, (Eskandari et al., 1997) compared membrane currents generated in NIS cRNA-injected oocytes by iodine and a number of anions including fluoride. Fluoride did generate a minor current but much less than iodine or any other anions tested and was not examined further. No experimental data have since been identified in the published literature to support a competitive interaction between these two ions. The assertion of fluoride inhibition of NIS by Waugh (2019) is based on reduced gene expression of NIS and Na^+/K^+ ATPase. Rather than competition with iodide on the transporter active site for uptake in the thyroid, an indirect mode of action was postulated, involving upregulated expression and activity of other factors that are reported to inhibit NIS expression and function (Waugh, 2019). Alternatively, fluoride may gain access to tissue (gland, brain) through a specific fluoride transporter channel, recently described in vitro, but for which no in vivo information on distribution or functionality has been reported (McIlwain, Martin, Hayter, & Stockbridge, 2020).

An increase in intracellular calcium concentration (Raspé et al., 1986), increased iodine binding and formation of thyroxine in preincubated thyroid slices in vitro (Ahn & Rosenberg, 1970; Willems et al., 1972), increase of iodide binding to proteins but inhibition of accumulation of intracellular colloid droplets and secretion (Willems et al., 1972; Williams & Wolff, 1971) have also been reported in response to fluoride exposure in in vitro studies. However, in vivo, no effect on iodine uptake into the thyroid, thyroid hormone biosynthesis, thyroglobulin content of the thyroid gland or degree of thyroglobulin iodination was seen in rats given 60 or 200 mg/L fluoride in the drinking water (Siebenhüner et al., 1984). Others have postulated that fluoride may decrease circulating T4 and T3 levels indirectly through modified thyroid hormone transport in blood (Bobek et al., 1976) but no evidence to support this claim has been documented.

3.4.4 | Other in vitro effects and other test systems

Activation of adenyl cyclase by fluoride has been reported since the 1960's, in canine thyroid homogenate, rat thyrocytes in vitro and similar assays (Ahn & Rosenberg, 1970; Cochaux et al., 1987; Friedman et al., 1983; Goldhammer & Wolff, 1982;

Kalderon & Sheth, 1978; Kaneko et al., 1969; Segal et al., 1985). Although increased cAMP is implied after adenyl cyclase stimulation, either no increase (Kaneko et al., 1969) or a suppression (Willems et al., 1972) of cAMP have been reported. A biphasic effect on adenyl cyclase was reported in one study, where activity increased at fluoride concentrations between 0.01 and 1 mM and decreased at concentrations between 1 mM and 100 mM (Jenq et al., 1993).

Other effects reported include inhibited metamorphosis, thyroid and skeletal development in tadpoles of Chinese Toad at 50 mg/L NaF (1.2 mM) (Zhao et al., 2013), histological, T3, T4 and gene expression changes in zebrafish at molar concentrations (Jianjie et al., 2016; Lu et al., 2022).

3.5 | Weight of evidence assessment

Based on the hazard assessment in Sections 3.2 and 3.3 whose underlying data are presented in Appendices C, D and E, the weight of evidence assessment for effects on the CNS (neurodevelopment, (D)NT; and NT), thyroid and bone health was evaluated following the EFSA Guidance on the use of the weight of evidence (EFSA Scientific Committee, 2017a, 2017b, 2017c). First each line of evidence is briefly defined after which summary and conclusion for each line of evidence is provided prior to their integration for the overall weight of evidence.

3.5.1 | Weight of evidence for lines of evidence (LOEs) of neurodevelopment and neurotoxicity from human and animal studies

Neurodevelopment or neurotoxicity; (D)NT

Assemble the evidence

Select evidence

Systematic review of human, animal and in vitro *studies assessing the association between fluoride exposure and neurodevelopmental effects*.

Lines of evidence

$LOE1_{(D)NT}$: Prospective studies in humans
- Results reported from 7 pregnancy cohort studies and one cohort examining neurotoxicity in adults in 15 different publications. Six publications were judged to be of moderate (Tier 2) and nine of low (Tier 1) risk of bias.

$LOE2_{(D)NT}$: Cross-sectional studies in humans
- A total of 49 studies reported in 53 publications. The majority of studies were conducted in areas with fluoride water concentrations > 1.5 mg/L. Half of the publications (27) were of high risk of bias (Tier 3) mostly due to no or limited confounder control, 11 were of moderate (Tier 2), 15 were of low (Tier 1) risk of bias.

$LOE3_{(D)NT}$: Animal studies, (D)NT behavioural effects
- A total of 21 rodent studies reported neurobehavioural tests following developmental exposure and 20 following adult exposure; of those, 2 developmental and 4 adult studies were of moderate (tier 2) risk of bias. No low RoB (tier 1) data were available.

$LOE4_{(D)NT}$: Animal studies, organ level effects (histopathology and organ weight)
- A total of six rodent studies reported on absolute brain weight, neuronal cell counts, morphology and/or microstructure of some brain regions. One study was of low (tier 1) and five were of moderate (tier 2) risk of bias.

$LOE5_{(D)NT}$: Animal studies molecular and cellular level effects
- A total of 14 rodent studies reported molecular and cellular level data, such as markers in brain for apoptosis, oxidative stress and neuronal function; two studies were of low (tier 1) and 12 were of moderate (tier 2) risk of bias.

$LOE6_{(D)NT}$: Mechanistic in vitro studies
- One assay was identified in the ToxCast database, on neuronal precursor cell proliferation, a potential mechanism leading to DNT.
- Published literature reported on neuronal cell toxicity, Na+/K+ ATPase and adenyl cyclase activity

$LOE7_{(ADME)}$: Total dose, Kinetics, Target Tissue Concentrations, Other Considerations
- Evidence on placenta crossing
- Evidence on blood–brain-barrier crossing

Weigh the evidence

Methods

Reliability[65] and relevance[66] were assessed for each study, as described in the pertinent EFSA guidance. This was done by taking internal validity (RoB) together with other study characteristics into consideration, for each line of evidence, including consistency[67] across studies and the strength of the associations/effects observed across studies (i.e. effect size direction, magnitude and precision thereof, and dose–response). Across different lines of evidence for each health outcome category, biological plausibility was evaluated. For the animal studies reliability, relevance and consistency were considered as summarised in Tables 22 and 23, Figure 6 and Annex E.2.

[65] Reliability is the extent to which the information comprising a piece or line of evidence is correct, i.e. how closely it represents the quantity, characteristic or event that it refers to. This includes both accuracy (degree of systematic error or bias) and precision (degree of random error).

[66] Relevance is the contribution a piece or line of evidence would make to answer a specified question, if the information comprising the evidence was fully reliable. In other words, how close is the quantity, characteristic or event that the evidence represents to the quantity, characteristic or event that is required in the assessment. This includes biological relevance (EFSA Scientific Committee, 2017a, 2017b, 2017c) as well as relevance based on other considerations, e.g. temporal, spatial, chemical, etc.

[67] Consistency is the extent to which the contributions of different pieces or lines of evidence to answering the specified question are compatible.

Results

$LOE1_{DNT}$: Prospective studies in humans:

For this line of evidence, intelligence quotient (IQ) was considered the endpoint of primary interest due to within- and between-population comparability and established adversity (EFSA CONTAM Panel, 2010; EFSA CONTAM Panel, 2012). Results on children's IQ, eight publications in total, were reported in four pregnancy cohorts and one cohort in children:

- In the MIREC cohort (Canada, *n* ~ 400, three publications), a sub-set of participants had been exposed during pregnancy to fluoridated water, up to 1.0 mg/L (mean + 2 SD). In these studies, a significant decrease in offspring IQ at ages 3–4 years with higher fluoride exposure was consistently observed. The effect estimates corresponded to a loss of around 4 IQ points over the effective exposure range.
- In the ELEMENT cohort (Mexico, n ~ 300, two publications) the pregnant mothers had been exposed to naturally occurring fluoride in drinking water with concentrations up to 1.4 mg/L. A statistically significant decrease in offspring IQ at 6–12 years was observed. The observed effect size corresponded to a loss of around 2 IQ points per 0.5 mg/L increase in fluoride concentrations in drinking water which appears comparable to what was reported in the MIREC cohort.
- In the Odense Child Cohort study (Denmark, n ~ 800, one publication), women had been exposed to non-fluoridated drinking water (< 0.3 mg/L). Therefore, the urinary fluoride concentrations [mean (SD): 0.58 mg/L (0.32)] (spot or 24 h urine) should largely reflect exposures from the diet and dental care products. No association was observed between maternal urinary fluoride concentrations and offspring IQ at age 7 years.
- In the INMA study (Spain, *n* ~ 250), a sub-set of participants had been exposed to either non- or low fluoridated water (0.7 mg/L). Offspring IQ was assessed at age 4 years. A significant association with higher IQ was observed between increasing maternal urinary concentrations and higher IQ in boys, while no association was observed for girls. Although not reported, these results suggest that a non-significant association would have been observed for both sexes combined.
- Finally, a study from New Zealand (*n* ~ 1000) found no significant association between 5 year old children living in the fluoridated water (0.7–1.0 mg/L) area when compared to children in the non-fluoridated water area and their IQ at age 7–17 and 38 years (Broadbent et al., 2015). No significant difference in IQ was observed in relation to the use of fluoridated toothpaste at 5 years.

Five other studies examined associations between fluoride exposures in pregnancy and early childhood with different neurodevelopmental outcomes in children. Given the varying outcome assessment and divergent findings, the contribution of these studies was assigned low weight in the weight of evidence assessment.

Reliability and Relevance

The studies from MIREC, Odense Child Cohort and INMA were all assessed as low risk of bias (Tier 1). For the ELEMENT cohort, one study was assessed as Tier 1 and one as moderate risk of bias (Tier 2). The study from New Zeeland was assessed as Tier 2.

The MIREC, ELEMENT, INMA and Odense Child Cohort were considered to have high relevance and reliability as they addressed possible neurodevelopmental outcomes in relation to in utero exposures when the fetal blood–brain barrier is less developed. In the MIREC cohort the findings for internal (urine) and external (water) exposure were largely concordant which provides better evidence than relying on one source of exposure alone. Compared to the other studies, the study from New Zealand was considered to have slightly less relevance and reliability as external exposure was not validated against more objective biomarkers. The lower reliability also relates to the uncertainty around duration of residency of the children exposed to fluoridated versus non-fluoridated water, which makes it difficult to draw conclusions on possible earlier exposures (in utero and until 5 years old).

Although the eight studies from the five cohorts were assessed to have moderate to low risk of bias, all these studies suffer from limitations such as uncontrolled confounding and selection bias which is, despite use of risk of bias assessment, difficult to assess. The association observed with higher IQ in boys from the INMA cohort does point, in the absence of any plausible biological explanation, toward bias.

Consistency

Overall, the results from the four pregnancy cohorts and one childhood cohort were inconsistent. With no major differences assessed in terms of relevance and reliability for MIREC, ELEMENT, INMA and Odense Child Cohort, and taking into consideration the findings from the New Zealand cohort, there is inconclusive evidence for neurotoxic effects of fluoride exposure at water concentrations < 1.4 mg/L[68] in drinking water. The results from the relatively large (*n* ~ 800) Odense Child Cohort study suggest that in the absence of water fluoridation no such effects are expected from fluoride exposure from normal diet or dental care products in the general population. The overall null findings from the INMA cohort and more circumstantial finding from New Zeeland reduce the confidence in the adverse association with IQ observed at relatively low fluoride concentrations, as reported in the MIREC and ELEMENT cohorts.

Strength

The effect size reported in the MIREC and ELEMENT cohorts suggests an around 4-point loss in IQ for exposures < 1.4 mg fluoride/L in drinking water. This effect size is both adverse and biologically relevant. No inverse association was observed in the other three pregnancy cohorts and the child (New Zeeland) cohort.

[68] This was the highest concentration in drinking water reported in the prospective studies (Bashash et al., 2017).

Conclusion

Based on the available prospective studies, the reliability and relevance of existing studies is high. However, the consistency of the current evidence is insufficient to draw conclusions on a possible adverse association between fluoride exposure in early life and children's neurodevelopment in areas with fluoride content in drinking water below 1.4 mg/L (from 1.14 to 3.94 mg/day; see Section 3.9.3.1). Further studies from areas with low levels of community drinking water fluoridation (< 1.0 mg/L) or low naturally occurring fluoride in drinking water are needed to address uncertainties around possible adverse effect of fluoride on CNS at low fluoride exposures.

$LOE2_{DNT}$: Cross-sectional studies in humans:

For human cross-sectional studies, the intelligence quotient (IQ) was again considered the endpoint of primary interest for the WoE assessment. Results on children's IQ were reported in 37 cross-sectional studies. Most of these studies originated from China and India ($n = 15$ and 11, respectively) and focused on populations exposed to moderate (~ 1–2 mg/L) to high (> 2 mg/L) fluoride concentrations in drinking water.

- Of these 37 studies, 26 (70%) reported lower IQ in children (7 Tier 1, 4 Tier 2 and 15 Tier 3). Nine studies (1 Tier 1, 2 Tier 2 and 6 Tier 3) reported no or unclear association. Two studies (Tier 2 and 3) reported positive association (higher IQ).
- Associations with lower IQ were consistently observed when stratified for risk of bias or when grouped based on duration of residency among study participants and confounder control. If anything, the more reliable studies tended to report associations with lower IQ with higher frequency.
- Among these studies, a significant decrease in IQ was reported in 7 of the 8 cross-sectional studies that provided covariate-adjusted results and reported that participants had been long-term residents (meaning that they had been continuously exposed since birth).
- No major differences in results were noted related to the geographic origin of the included studies.
- The effect sizes observed in these studies are difficult to summarise given the heterogeneity in reporting. The observed effect size ranged between loss of 2 to 10 IQ points with larger effects size generally reported at higher exposures. At moderate exposure (fluoride in drinking water up to 2 mg/L) the effect size appeared to range between loss of 2 and 4 IQ points. In some cases, the larger effect size is likely to be influenced by some (residual) confounding.
- Overall, the inverse associations between fluoride concentrations in drinking water and IQ was consistent at concentrations above ≥ 1.5 mg fluoride/L while at lower concentrations (< 1.5 mg/L) the association was more divergent and there is lower confidence those findings.

Reliability and Relevance

The eight studies (12 publications) that addressed duration of residency and presented covariate-adjusted results were considered having the highest relevance and reliability. These studies, despite their cross-sectional design, were of limited risk for exposure misclassification, typically associated with that design, as participants had most likely been continuously exposed throughout lifetime (that is, exposure preceded the outcome).

Other studies that also addressed duration of residency but did not consider potential confounders were considered similarly relevant but less reliable. Results from studies where duration of residency was not reported were interpreted under the assumption that participants were mainly permanent residents. Studies that neither reported duration of residency nor performed confounder control were considered as having low relevance and reliability but still provided support in the overall weight of evidence. As for the prospective studies, uncontrolled confounding and other biases may play a role. Based on the covariate-adjusted results presented in some studies and information on participants background, confounding by socioeconomic background is unlikely to explain the results observed in most of the studies.

Consistency

The results from the cross-sectional studies consistently reported lower IQ scores in children with higher fluoride exposure from drinking water. That is, living in areas with elevated fluoride concentrations in drinking water (generally above 1.5 mg/L) is associated with children having lower IQ. Consistent results were observed across studies regardless of risk of bias evaluation or other study attributes used to assess reliability. As a result, the role of chance finding is judged to be implausible. Publication bias seems plausible given the varying quality of study conduct and reporting. Role of confounding can, as always, never be excluded but consistency across study findings in the absence of a clear confounding mechanism makes the role of confounding less likely.

Strength

Effect sizes reported in the cross-sectional studies ranged from loss of 4 to 10 IQ points in children living in areas of high versus low fluoride exposure levels in drinking water. This effect size is both adverse and biologically relevant. It seems plausible that partial confounding has played a role in studies reporting the larger effect sizes.

Conclusion

Based on the available cross-sectional studies, the reliability and relevance of existing studies is moderate. However, the relative consistency of findings observed across studies with low to high reliability and across different populations increases the confidence in the study findings. It is therefore concluded that being exposed to levels of fluoride in drinking water above 1.5 mg/L is consistently associated with lower IQ scores in children. There is lower confidence for an association below this value.

LOE3$_{(D)NT:}$ Rodent in vivo (D)NT behavioural data

Neurobehavioural assessments were evaluated in a total of 42 rodent studies and only 6 were found to be of sufficient quality. Five studies included a Morris Water Maze, a test of spatial learning and one of these studies included a passive avoidance test plus seven additional behavioural tests; one study included just a Passive Avoidance test. Spatial learning was the most common endpoint reported. LOAELs ranged between 2.5 and 11 mg/kg bw per day. All dose levels reported here refer to total dose including fluoride feed content (see LOE7).

Reliability & Relevance

The 6 studies that were assessed met most reliability criteria for the complexity and challenges of these assays, but all lacked historical negative and positive control data documenting the proficiency of the laboratories to conduct behavioural tests. In most cases, performance control assessments or sufficient method description on key quality aspects as well as robust statistical analysis were missing.

Consistency

- LOAELs of the behavioural studies ranged from 2.5 to 11 mg/kg bw per day.
- One developmental (LOAEL 2.5 mg/kg bw per day, the lowest dose tested) and 3 adult studies (LOAEL from 3.5 to 11 mg/kg bw per day) reported impairments in water maze learning.
- No impairments in operant conditioning were found in adult rats exposed at higher levels (NOAEL > 12 mg/kg bw per day).
- A low dose developmental study by McPherson et al. (2018) did not observe any neurobehavioural impairments up to a total dose of 2.1 mg/kg bw per day (the highest dose tested). This value is slightly below the LOAEL of 2.5 mg/kg bw per day established by Jiang et al., 2014.
- Differences across studies in route, duration, age, dose range assessed, species, strain and sex, as well as differences in test system examined and its implementation contribute to the five-fold range in effective doses reported in these studies.
- An area of uncertainty for assessing consistency pertains to the fluoride content in diet which was not reported in most studies. In two studies where feed content was reported, it varied from 0.0003 to 20 mg/kg feed and it contributed ~10 to 50% of the total administered dose. Calculating the BMDs from the critical organ level endpoints with and without fluoride background in feed, provides similar results (see LOE7 below and Appendix D.2).

Strength

As the dynamic ranges and coefficients of variation for the effect sizes in neurobehavioural tests can vary widely depending on the test system used and how it is implemented, for this assessment, the statistically significant effect sizes were considered biologically relevant in the six studies meeting tier 2 data quality requirements.

Conclusion

Based on the reliability, relevance and strength of the evidence presented above, it is concluded that adverse effects of fluoride on neurobehaviour in animal studies emerge at dose levels above 2.1 mg/kg bw per day.

LOE4$_{(D)NT:}$ Rodent Organ level effects

Organ level assessments including measures of brain weight, neurohistology and brain morphology were reported in 10 studies with a lowest LOAEL of 3.5 mg/kg bw per day. Reliability, relevance and consistency of these studies are summarised in Tables 22 and 23, Figure 6 and Annex E.2.

Reliability & Relevance

- Lower postnatal absolute (but not relative) brain weight was reported in two studies (along with lower body weight) and was statistically significant in one of them.
- Absolute brain weight changes are generally considered reliable and relevant, albeit a relatively crude indicator of neurotoxicity, even in the absence of changes in relative brain weights.
- GLP studies in rats and mice conducted by the NTP (NTP TR 393, 1990) and a multigenerational developmental toxicity study in rats conducted by the US FDA (Collins et al., 2001) found no evidence of brain weight changes.[69]
- Histological analyses of the brain from 6 studies were deemed uncertain due to the varied nature of the preparations, analyses and report of the results, but generally supported an effect on brain structure in qualitative terms.

[69] NTP TR 393, 1990 and Collins et al., 2001 were not captured in the systematic and targeted literature searches conducted in the context of this assessment. These publications were retrieved by experts of the EFSA Working Group Fluoride.

Consistency

- Six developmental and seven adult studies examined the effects of fluoride exposure on the brain at the organ level, and 5 of the 13 reported no effects at the dose levels assessed.
- Developmental studies
  - Jiang et al. (2014) observed a 6–7% lower postnatal absolute (but not relative) brain weight and decreased glucose utilisation at 3.5 and 5.5 mg/kg bw per day with a NOAEL of 2.5 mg/kg bw per day.
  - One rodent multigeneration study did not observe effects on brain weight in doses up to 10.8 mg/kg bw per day (250 ppm) (Collins et al., 2001). McPherson et al. (2019) tested exposures up to 2.1 mg/kg bw per day and found no evidence of cell death or reactive glia, indices of brain injury.
  - Effects on brain histological markers were reported in four other studies and included mitochondrial changes, altered neuron number, patterning and morphology, but methodological concerns question the reliability of the reported LOAELs of 3.5–6 mg/kg bw per day of the various endpoints (Zhao et al., 2019; Zhou et al., 2021, Zhao et al., 2022, Xu et al., 2023).
- Adult animals studies:
  - High doses of 6.5 and 11 mg/kg bw per day administered by Agustina et al. (2019) resulted in non-significantly lower (10%–13%) cerebellar weight, an effect that was supported by significantly lower (10%–25%) number of Purkinje cells, the most prominent cell type in this region. At doses up to 3.2 mg/kg bw per day, Pulungan et al. (2018) did not observe effects on prefrontal cortex volume or Purkinje cell number in the cerebellum.
  - One rat and one mouse GLP studies (each of 27 and 66 weeks) at doses of ~4 mg/kg bw per day for rat and ~8–9 mg/kg bw per day for mouse did not show effects on brain weight (NTP TR 393, 1990).
  - Effects on brain histological markers were reported in two other studies and included altered neuron number and blood–brain-barrier, but methodological concerns question the reliability of the reported LOAELs of 3–9 mg/kg bw per day of the various endpoints (Bittencourt et al., 2023; Xiang et al., 2024).

Strength

- The evidence of the animals DNT for brain weight effects is not considered to be strong.
- Lower absolute brain weight accompanied by lower body weight was reported in one DNT study in adult rats exposed in utero and postnatally; this was not seen as compromise of the finding, as brain weight is usually highly conserved upon body weight changes (see footnote 33 in opinion).
- However, there is stronger evidence of no brain weight changes reported in three GLP rodent studies at comparable and higher dose levels (NTP TR 393, 1990; Collins, 2001).
- Adverse histological and morphometric endpoints in the brain at higher doses appeared clearly affected, but due to the complexity in these analyses, they cannot be interpreted quantitatively to derive a reliable reference point (RP).

Conclusion

Based on the reliability, relevance and strength of the evidence presented above on adverse effects of fluoride on organ level outcome measures in the brain, it is concluded that (a) the effects on brain weight are not reliable and (b) morphometric and histological findings indicate qualitative evidence and do not provide a robust quantitative point of departure. Overall effects in the brain at the cellular level are unlikely below 3.5 mg/kg bw per day, while organ level histological changes appear at higher doses.

$LOE5_{(D)NT:}$ Rodent molecular and cellular level effects

Molecular and/or cellular level effects in the brain were reported in 20 studies. Numerous endpoints were assessed, summaries of which can be found in Tables 23 and 24 and Figure 6. These included markers of oxidative stress, inflammation, apoptosis, activation of microglia and astrocytes, energy metabolism, neurotransmitter synthesis, release and reuptake, synaptosomal protein expression, synaptic plasticity, neuroprotection pathways, melanogenesis and cell numbers in different brain regions. Given this diversity, detailed evaluations of reliability, relevance and consistency of effects were not possible. LOAELs ranged between 1.3 and 11 mg/kg bw per day.

Reliability & Relevance

Knowledge regarding how toxic effects is progressing from molecular to cell, organ and organism level and can strengthen any conclusion on the reliability and relevance of adverse effects. However, the available evidence does not provide a robust mode of action hypothesis for fluoride. Therefore, studies reporting effects at this level of analysis were considered in this assessment, but not given significant weight to determine dose levels of concern.

Consistency

- Molecular/cellular level effects were available for 7 developmental and 13 adult animal studies, but the nature of the endpoint investigated varied widely across studies.
- In developmental studies, LOAELs for various molecular and cellular changes ranged between 1.9 and 3.5 mg/kg bw per day.
- In adult studies, LOAELs for various molecular changes ranged between 1.7 and 11 mg/kg bw per day and for cellular changes between 4.25 and 6.6 mg/kg bw per day.

Strength (effect size, statistical significance, dose–response)

- The type of cellular molecular changes investigated varied widely across the studies examined. Although reliable, statistically significant and dose-dependent effects were often reported, the biological relevance of the magnitude of the observed effects is difficult to determine.

Conclusion

In spite of the diversity of molecular and cellular endpoints examined a clear integrated mode of action hypothesis could not be extracted. The biological relevance of molecular/cellular effects in DNT and NT studies reported at dose levels of approximately 2.0 mg/kg bw per day, respectively is difficult to determine.

$LOE6_{(D)NT:}$ Mechanistic in vitro studies for potential DNT

Very few mechanistic studies were identified. In an in vitro neuronal cell precursor proliferation assay, fluoride effects on neuronal proliferation were non-specific, i.e. reduced proliferation at a high concentration that also affected cell viability. Fluoride effects on Na^+/K^+ ATPase and adenylate cyclase inhibition in vitro have been reported in the mM range. Given the paucity of data, these studies were not considered further.

Conclusion

The paucity of mechanistic studies and the non-specific effects reported do not provide any reliable and relevant evidence to support effects of fluoride on the brain.

$LOE7_{(ADME)}$: Total dose, Kinetics, Target Tissue Concentrations, Other Considerations

Evidence on total exposure and target tissue exposure is needed to support their biological plausibility and context in which the reported DNT/NT effects in humans and animals are interpreted.

Relevance and Reliability

- In the human studies limited data were available for total intake (including drinking water, diet and dental care products). In these studies, fluoride concentration in drinking water was used as measure of exposure, often in combination with urinary fluoride concentration.
- Depending on the geographic origin and time period of the studies, exposure through dental care products can also be assumed; it is uncertain in studies from geographic areas where their use may not be standard practice.
- The relative contribution of these different sources to the total intake depends on the population under study and can vary considerably (diet, lifestyle and the time period when the study is conducted).
- Urine levels are relevant as biomarker of internal exposure, originating from all sources and they can be used to estimate total intake using reverse dosimetry.
- Reliability of animal studies is limited by the lack of well-characterised background intake in the control groups. Fluoride content in feed was not reported in most studies. A value of 16 mg/kg of commercial rodent chow was used as default when not reported, but this value is largely based on analytical detection limits rather than confirmed content. This leads to uncertainty of the NOAEL/LOAEL values.
- Evidence of brain exposure to fluoride is relevant to the interpretation of the study outcomes in the CNS and would increase their reliability.
- Brain concentrations of fluoride in animals were available in five studies, two of which also reported behavioural data, but were of low reliability.
- Fluoride crosses the placenta (fetal cord blood 60% of maternal blood). This is relevant to the interpretation of the effects during development and suggests that exposure to the developing brain is likely considering the blood–brain barrier during development is immature.

Consistency

- Fluoride content in feed was reported in only 3 of the 27 studies and ranged from 0.0003 to 20 mg/kg.
- Within the animal experiments reported, it is not possible to discriminate the effects of developmental exposure relative to postnatal exposure, as the experimental designs employed maintained exposure to the animal postnatally until the time of assessment.
- Brain estimates of fluoride studies varied by 2 orders of magnitude in 4 of the 5 animal studies with similar exposure regimes.
- Brain-to-plasma concentration ratios in non-perfused animals were reported in one study and ranged from 0.26 to 0.29 in the adult brain, indicating that brain concentrations may reach 20–30% of those attained in plasma (Whitford et al., 1990). These values are likely an overestimation of brain concentrations as no perfusion was performed (or reported).
- One study reported cerebrospinal fluid fluoride levels in humans that did not differ between fluorosis patients (0.20 ± 0.062 mg/L) and non-fluorosis individuals (0.17 ± 0.03 mg/L) (Hu & Wu, 1988). However, the reliability of the results reported in this study is questionable. No other relevant data are available.

Strength

- There is evidence that supports access of fluoride to the brain during development but evidence documenting quantitatively access of fluoride to the brain after development is limited. It can be expected that fluoride reaches the brain albeit reliable quantitative data are not available.

Conclusions

The total dose of fluoride in participants of human studies is uncertain and can be estimated using default factors and conservative assumptions. The total dose of animals is uncertain and may be overestimated using default feed concentrations. Fluoride accesses the brain during development and possibly in the adult, but the fraction of the dose reaching the brain is uncertain. Therefore, it is not possible to estimate the target tissue concentrations that are associated with the effects reported in vivo.

Integrate the evidence

Methods

Integration of lines of evidence was done based on expert judgement. Humans and animals/in vitro LOE were integrated independently before final overall integration of all (D)NT LOEs.

Results

Integration of $LOE1_{(D)NT}$ and $LOE2_{(D)NT}$:

- Based on the results from reliable prospective studies ($LOE1_{(D)NT}$), where two studies indicated an association between fluoride and lower IQ in children at drinking water fluoride concentrations from 0.7 mg/L to 1.4 mg/L and two other studies suggested no association, there is inconsistent evidence that fluoride may be adversely associated with neurodevelopmental outcomes in children at such low water concentrations. Such inconsistency in evidence is not unexpected in studies addressing low exposures in the general population where exposure range is narrow and changes in the outcome are modest.
- Although the observed effect size at low exposures (as observed in $LOE1_{(D)NT}$) is considered adverse, the modest effect size (loss of 1–4 IQ points) may result from residual confounding by other shared environmental factors. Factors such as parental education, which is a well-known determinant of IQ (Neiss et al., 2002) may co-vary across regions with low to moderate fluoride concentrations in drinking water causing bias in both directions. This was considered more relevant for $LOE1_{(D)NT}$ compared to $LOE2_{(D)NT,}$ as the latter had more studies to evaluate consistency and effect size was generally larger at higher exposure, which is less likely explained by residual confounding.
- The tier 1 cross-sectional studies ($LOE2_{(D)NT}$) provided additional evidence that fluoride is associated with lower IQ in children. Such associations were consistently observed at concentrations in drinking water above 1.5 mg/L.
- Based on the results from the cross-sectional studies (LOE2(D)NT), it is concluded that there is reliable and relevant evidence of an association between lower IQ scores in children born to women living in an area with high levels of fluoride in drinking water (> 1.5 mg/L), while findings were inconsistent and more uncertain at lower levels (< 1.5 mg/L).
- Although residual confounding may have been present in several of the studies in LOE2(D)NT, the effect size (≥ 4 points lower IQ) reported in these studies) indicates that there is an adverse association.
- No firm conclusions can be drawn from prospective or cross-sectional studies on neurobehavioural outcomes other than IQ (for LOE1(D)NT and LOE2(D)NT), due to heterogeneity of the outcomes and lack of replication in most instances.
- Overall, there is limited confidence in the evidence from LOE1(D)NT and LOE2(D)NT that exposures corresponding to drinking water concentrations below 1.5 mg/L are associated with impaired cognitive neurodevelopment in children and higher confidence in adverse effects associated with drinking water concentrations above 1.5 mg/L.

Integration of LOE3$_{(D)NT}$, LOE4$_{(D)NT}$, LOE5$_{(D)NT}$ and LOE6$_{(D)NT}$:

Considering the effects of fluoride reported in relation to neuronal cellular and molecular level, brain organ level and behavioural effects, within the available studies and the related information on relevance, reliability, consistency and strength as summarised above:

- Within the available rodent in vivo studies, the dose ranges indicative of possible onset of adverse effects in brain ranged within one order of magnitude (about 2.5–11.5 mg/kg bw per day). These values overlap among the developmental and adult animal studies.
- Available in vivo molecular and cellular level data appear primarily as mechanistically informative and several cellular/organ level data appear to be of low quantitative reliability (Xu et al., 2023; Yan et al., 2016; Agustina et al., 2019; Pulungan et al., 2018; Zhang et al., 2022; Niu et al., 2015; Zhang et al., 2023; Ran et al., 2023).
- Effects on absolute brain weight were reported in only two studies (one for cerebellum only) with significant difference from control observed in only one (Jiang et al., 2014), while brain weight was not affected in five other GLP rodent studies (Collins et al., 2001; NTP TR 393, 1990). As a result, the reported effects on brain weight are not considered reliable.
- Brain histology and morphometric data indicate organ level effects (Zhou et al., 2021, 2019; Zhao et al., 2022; Xu et al., 2023; Pulungan et al., 2018; Xiang et al., 2024; Bittencourt et al., 2023), but due to the complexity of the analysis they cannot be interpreted quantitatively for a RP derivation. However, behavioural LOAELs are identified at a similar dose of 3.5 mg/kg bw per day for developmental exposure (Jiang et al., 2014), and somewhat higher doses of 3.6 (Wang et al., 2023), 10.5 (Ran et al., 2021) and 11 mg/kg bw per day (Cao et al., 2019) for adult animal exposure.
- Available mechanistic in vitro data (LoE6) indicate effects at high concentrations only and are considered non-specific. Thus, LOE6 does not effectively contribute any weight to the overall WoE.
- Overall, adult and developmental neurotoxicity effects were reported between 2.5 and 11.5 mg/kg bw per day at cellular, organ and/or organism level (see Section 3.3.4.4). Notably, no behavioural effects were observed up to 2.1 mg/kg bw per day in a relatively well-conducted study (McPherson et al., 2018).
- Considering the uncertainties across different studies, methods and biological level of inquiry, a NOAEL or BMD from a single study may not represent an adequate RP. Rather this range of 2.5–11.5 mg/kg bw per day may be appreciated as the oral dose level where concern for DNT or NT begins to appear. The related uncertainty for a 'true' BMD could be represented by a log-normal distribution with a BMDL of 2.5 and BMDU of 11.5 mg/kg bw per day. This BMD including its uncertainty (BMDL/BMDU) may be used as a surrogate for a RP of potential effects on the central nervous system.
- In the case of fluoride, extrapolation from experimental neurodevelopmental outcomes in rodents to health-based guidance values protective for human specific cognitive functions is qualitatively and quantitatively uncertain.

Integration of LOE1$_{(D)NT}$, LOE2$_{(D)NT}$, LOE3$_{(D)NT}$, LOE4$_{(D)NT}$, LOE5$_{(D)NT}$, LOE6$_{(D)NT}$, LOE7$_{ADME}$

For neurodevelopment and neurotoxicity, there is reasonable confidence in the evidence from both human and animal studies suggesting that adverse effects on neurodevelopmental outcomes may occur at relatively high F exposures. Due to the uncertainty of the animal to human extrapolation the use of human evidence is considered more appropriate for establishing an RP. The Scientific Committee concluded that associations with adverse neurodevelopmental outcomes are uncertain at fluoride levels in drinking water below 1.5 mg/L, which are commonly found in Europe.

3.5.2 | Weight of evidence for lines of evidence (LOEs) on thyroid effects from human and animal studies

Thyroid hormones and function
Assemble the evidence
Select evidence
Systematic review of human, animal and in vitro *studies assessing the association between fluoride exposure effects on thyroid.*
Lines of evidence

$LOE1_{TH}$: Human studies

Results from 28 studies, of which 3 were prospective cohort studies while the others were all cross-sectional studies. Of these 28 studies, 8, 10 and 10 studies were assessed as Tier 1, 2 and 3, respectively.

$LOE2_{TH}$: Animal studies

6 studies were assessed for potential fluoride effects on thyroid histopathology or thyroid function. One was deemed irrelevant and of the remaining 5, only 1 study was considered with a low RoB.

$LOE3_{TH}$: In vitro studies

Changes in TSH-mediated activation of adenylate cyclase and cAMP by fluoride was examined in vitro in 9 studies. Membrane current generated by fluoride through the NIS was examined in 1 study.

Weigh the evidence

Methods

Reliability, relevance, internal validity (RoB), consistency across studies and the strength of the associations/effects were assessed for each study, as described in Section 3.5.1. Biological plausibility was evaluated across different lines of evidence for each health outcome category.

For the human studies, the mean TSH concentrations reported across studies were considered a comparable measure of fluoride impact on the thyroid. Correlations between mean TSH and mean thyroid hormones with mean fluoride exposure measures (in drinking water, urine or serum) reported in different studies were assessed. Individual measurements were not available.

Results

$LOE1_{TH}$: Human studies

- Changes in TSH were most frequently reported while fewer studies assessed the full thyroid hormone profile. Across these studies, fluoride in drinking water ranged from 0.15 to 6.2 mg/L. Most of the studies originated from China, Pakistan or India.
- The cross-sectional studies were assessed to be less prone to bias if 2 or more fluoride biomarkers were assessed and participants had been permanent residents of the recruitment area. More weight was given to these results.
- When comparing groups of children living in areas with low and high(er) water fluoride concentrations, higher TSH was reported in 11 out of 13 studies. Higher TSH were not accompanied by (statistically significant) lower mean FT4, where information on FT4 was available (8 studies).
- A similar trend of higher levels of TSH was observed for adults living in areas with higher fluoride in drinking water.
- In a prospective cohort study from Canada, pregnant women from fluoridated areas (water fluoride concentration 0.60 mg/L) had higher concentrations of fluoride in urine than women from non-fluoridated areas (water fluoride concentration 0.13 mg/L); a small but statistically significant association with lower serum FT4 was reported but no association with TSH. In the same study, women diagnosed with primary hypothyroidism had higher mean fluoride intakes than euthyroid women.
- A higher FT3/FT4, typically interpreted as a difference in thyroid hormone metabolism, was also suggested in a prospective study from Sweden.

Reliability & Relevance

Since measurable effects on thyroid function from chemical exposure are expected to occur within a few months and since participants in most studies were long-term residents in areas with low to high fluoride levels in drinking water, the cross-sectional nature of the available studies was not considered a major limitation. As such, most of the human studies were considered relevant to the assessment. The reliability of the studies was considered moderate mostly because reports including other hormone measures were rare and, when present, were in a direction inconsistent with reported TSH changes.

Consistency

Increasing fluoride exposures, measured either in urine or by consumption of drinking water, were consistently associated with higher circulating TSH in both children and adults. Associations with thyroid hormones were not as frequently reported and the results were less consistent (see Annex D.7, Figures C.7.1 and C.7.2).

Strength

The effect size corresponding to increase in mean TSH from ~2 mUI/L to ~3 mUI/L (see Figure 4) when comparing populations living in areas of varying fluoride levels in drinking water is considered biologically relevant as it reflects a substantial shift in mean TSH within a population. These associations were consistently observed at drinking water concentrations above 1.5 mg/L. If anything, these associations were observed at slightly higher concentrations compared to neurodevelopmental outcomes. In the absence of consistent associations with thyroid hormones, particularly FT4 (reported in fewer studies), the interpretation of the results observed for TSH in terms of adversity is subject to uncertainty.

Conclusion

Based on the reliability, relevance and strength of the evidence presented above, an association between fluoride concentrations in urine and in drinking water with higher TSH is observed in human cross-sectional studies. The significance of these associations is unclear.

LOE2 $_{TH}$: Animal studies

Only five studies were available for assessment of effects on the thyroid. Of these only one study was assessed as low risk of bias and four were of high risk of bias.

Reliability & Relevance

- The rat is considered an appropriate model relevant to humans for assessing thyroid effects.
- The only Tier 1 study available tested a low dose range of fluoride (up to 2.1 mg/kg bw per day) in rats and reported no effects on thyroid hormones.
- Evidence of effects of fluoride on thyroid hormones and histopathological changes in the thyroid gland were reported in the other studies at doses from 1.35 mg/kg bw per day or greater. However, the quality limitations (Tier 3) of these findings, introduce high uncertainty on the reliability of the findings.

Consistency

Effects reported in the four Tier 3 studies were consistent with each other but were not consistent with the absence of effect in the Tier 1 study.

Strength

The strength of the evidence is low for effects of fluoride on the thyroid but indicative of possible effects at high doses.

Conclusion

The results from few mostly Tier 3 animal studies provide insufficient evidence to support a thyroid effect.

LOE3 $_{TH}$: In vitro studies

- Fluoride activation of cAMP has been reported at high concentrations. This may be relevant to effects on thyroid cells (TSH also activates cAMP), but results are inconsistent across in vitro studies.
- Competition of fluoride with iodine for NIS transport, as a mode of action that may decrease thyroid hormone production, has been postulated but not demonstrated empirically. In vitro influx of fluoride through the NIS (assessed in oocytes expressing NIS cRNA) was minimal compared to iodine and other anions including bromide (Eskandari et al., 1997).
- Fluoride transport channels have been described in vitro but their presence/distribution/function in vivo remains to be seen.
- No in vitro assessment of fluoride in tests of the ToxCast battery has been conducted.

Reliability & Relevance

Studies designed to test the activity of fluoride on targets relevant to the thyroid are lacking.

Consistency

Number and relevance of studies are too limited for assessment.

Strength

The evidence is too limited to indicate effects on the thyroid.

Conclusion

Available in vitro evidence is insufficient to support a mode of action for fluoride effects on the thyroid.

Integrate the evidence

Methods

Integration of all lines of evidence (humans, animals and in vitro) was done based on expert judgement.

Results

The results from the human studies (LOE1$_{TH}$) suggest that living in areas with high fluoride exposure from drinking water (see Figure 4) is associated with elevated serum TSH concentrations but associations with thyroid hormones were inconsistent.

Animal studies (LOE2$_{TH}$) did not provide additional evidence to support adverse effects of fluoride on thyroid function, while the limited available in vitro data (LOE3$_{TH}$) indicate that fluoride is a poor competitor of iodine for uptake into the thyroid and does not support possible thyroid disrupting effects of fluoride through this mode of action.

In conclusion, the findings from human and the few animal and in vitro studies available on thyroid function are either too limited or inconsistent, which makes it difficult to draw clear conclusions. The human evidence suggests that higher fluoride exposure is associated with higher mean TSH levels in children and adults. These associations are observed at fluoride concentrations in drinking water similar or higher than those suggesting neurodevelopmental effects (> 1.5 mg/L) (see Figure 7).

3.5.3 | Weight of evidence for lines of evidence (LOEs) on bone effects from human and animal studies

Bone Cancer

Assemble the evidence

Select evidence

Systematic review of human studies assessing the association between fluoride exposure and bone cancer.

Lines of evidence

$LOE1_{BC}$: Human studies ($n = 9$)

Weigh the evidence

Methods

Reliability, relevance, internal validity (RoB), consistency across studies and the strength of the associations/effects were assessed for each study, as described in Section 3.5.1. Biological plausibility was evaluated across different lines of evidence for each health outcome category.

Results

$LOE1_{BC}$: A total of 6 out of 9 studies on osteosarcoma were of high risk of bias (Tier 3) and the remaining studies were of moderate risk of bias (Tier 2).
Out of these 9 studies, some association with increased odds of osteosarcoma with higher fluoride exposure was reported in two studies in adults from India with small sample sizes (< 30 cases and controls) (Kharb et al., 2012, Sandhu et al., 2011). Two larger studies (more than 100 cases and controls) in children and young adults did not find any association (Bassin et al., 2006; Archer et al., 2016). The remaining studies did not find any association with bone cancer either.

Reliability & Relevance

The available studies had high relevance but generally low reliability as majority of the studies (six out of nine) were high risk of bias.

Consistency

The studies consistently reported no association.

Strength

Overall, no association with cancer risk were reported.

Conclusion

The nine new studies identified since 2005 and the previous EFSA conclusion based on evidence published before 2005 (EFSA NDA Panel, 2005) do not support an association between fluoride exposure and bone cancer risk.

Bone health

Assemble the evidence

Select evidence

Systematic review of human studies assessing the association between fluoride exposure and bone health effects.

Lines of evidence

$LOE1_{BH}$: Fractures in human studies (five studies since 2003 and previous EFSA assessment)
$LOE2_{BH}$: Bone Mineral Density in human studies (11 studies since 2003 and previous EFSA assessment)
$LOE3_{BH}$: Other biomarkers of bone health in human studies
$LOE4_{BH}$: Animal studies organ level effects
$LOE5_{BH}$: Animal studies biochemistry

Weigh the evidence

Methods

Assessing consistency, strength (effect size, statistical precision and dose–response) and biological plausibility of findings within each line of evidence. This was partly done by taking internal validity (RoB) and other study characteristics into consideration. Reliability, relevance and consistency for animal studies are summarised in Table 25.

Results

$LOE1_{BH}$: Fractures in human studies

Therapeutic doses of fluoride (> 20 mg/day) have shown increased risk of fractures in postmenopausal women (EFSA NDA Panel, 2005). None of the new studies identified refuted those findings.

A total of five studies on fractures, published after 2005, were identified. Of these, four were observational and one was a small RCT ($n = 48$). One prospective cohort study (Helte et al., 2021) was assessed as of low risk of bias (Tier 1) while the remaining four studies were assessed as of high risk of bias (Tier 3).

- In the Tier 1 prospective study from Sweden (Helte et al., 2021) conducted in 4072 postmenopausal women, total fluoride intake from diet and drinking water was positively associated with incident of hip fractures (but not total fractures), with a hazard ratio of 1.6 (95% CI: 1.1, 2.3) when comparing the highest (median: 2.9 mg/day) to the lowest (1.4 mg/day) tertiles of exposure. Similar results were observed for urinary fluoride. Hazard ratios for major osteoporosis fractures were slightly elevated (likely due to the inclusion of hip fracture in this group), but the increase was not statistically significant.
- Of the remaining studies, all reported no clear association with fractures. For example, a large study (Nasman et al., 2013), also from Sweden, found no association between lifelong fluoride exposure through drinking water (< 0.3 mg/L to ≥ 1.5 mg/L) and hip fractures assessed in 2006 in women and men born between 1900 and 1919 ($n = 60{,}733$). Although residual confounding cannot be excluded, key risk factors (age and sex), county of residence and calendar year were adjusted for.
- Also, a prospective study in 1300 adults aged 20–90 years with relatively few cases of incident fractures (34 osteoporotic and 41 non-osteoporotic fractures) did not find an association but had low statistical power (Sowers et al., 2005). One study from the UK (Young et al., 2015), of ecological design and one case–control study (Kim et al., 2011) did not find an association with bone fractures.

Reliability & Relevance

All the studies on bone fractures were of high relevance but only one (Helte et al., 2021) had high reliability.

Consistency

Only one study from Sweden (Helte et al., 2021) of high quality (Tier 1) reported an association between low fluoride exposures (~ 3 mg/day) with increased risk of fractures, which was not supported by another large Swedish study. Other studies provided limited to no support.

Strength

The observed effect size in the study by Helte et al. (2021) was moderate (HR ~ 1.5 with wide confidence interval due to a limited number of hip fractures) but biologically relevant.

Conclusion

Although indications for increased risk of fractures were observed in one well-conducted study, the overall evidence is judged to be too limited to conclude on the presence of an association with fractures at low exposures (~ 3 mg/day) based on findings from a single study.

$LOE2_{BH}$: Bone Mineral density in human studies

As reviewed and concluded previously (EFSA NDA Panel, 2005) therapeutic doses of fluoride have been shown to increase bone mineral density. This effect has been observed in somewhat lower doses than those associated with fractures. The effect on bone mineral density coincides with increased propensity of fractures and suggests that the increase in bone mineral density associated with fluoride exposure results in decreased mechanical strength of the bone.

The 11 additional studies identified, published since 2003 and not included in the previous EFSA assessment (EFSA NDA Panel, 2005), all observational studies except one RCT, might suggest that changes in bone mineral density due to fluoride exposure may occur at lower concentrations than previously assessed.

- A study with small sample size ($n = 68$) showed a substantial improvement in T-score (−1.95 to −1.33) after 2 years of treatment with ~ 11 mg/day of fluoride in young adults with Crohn's disease (a risk factor for osteoporosis).
- In a study from Turkey recruiting 45 postmenopausal women with skeletal fluorosis who had been living in an endemic fluorosis area since birth and exposed to drinking water with ~ 2.6 mg fluoride/L, the mean bone mineral density of the spine and hip was 26% and 13%–16% higher, respectively, compared to 41 women from a control area exposed to fluoride in drinking water of ~ 0.5 mg/L (Yıldız et al., 2003). No significant differences in age, BMI and clinical markers of nutrition status were observed between the exposed and control women, but it is unclear how representative both the exposed women and the control women were.
- Furthermore, more modest significantly higher bone mineral density of ~ 1% and 2% in the lumbar spine and femoral neck, respectively, were observed in the prospective study of 4072 postmenopausal women from Sweden (Helte et al., 2021) exposed to fluoride around ~ 3 mg/day versus ~ 1 mg/day from diet and drinking water. However, for the femoral neck, women in the second and third tertile had similar bone mineral density.
- Significantly lower bone mineral density and bone strength of the femoral head were reported in subjects undergoing hip-replacement in Canada and exposed to low fluoridated water ($n = 53$, ~ 1 mg/L) compared to non-fluoridated water ($n = 39$). Fluoride content in the bone was ~60% higher in the exposed group (Chachra et al., 2010). However, this study is hampered by limited control for selection bias and confounding.
- Several publications from the Iowa Bone Developmental Study (US) did not report a clear correlation between fluoride intake from diet and drinking water and bone mineral density in children, some of which had been exposed to low fluoridated water (Levy et al., 2009; Levy et al., 2018; Lee, Hamilton, et al., 2024; Lee, Kim, et al., 2024; Saha et al., 2021).
- Several other studies from China, Turkey and Ethiopia using less precise measures of bone mineral density (i.e. speed of sound) reported some but divergent associations between fluoride exposure and bone mineral density (Gao et al., 2020; Godebo et al., 2020; Topuz et al., 2006).

Reliability & Relevance

The reviewed studies were considered reliable but of somewhat limited relevance for establishing a HBGV as the adversity of the observed bone mineral density increases associated with fluoride exposure (or treatment) are difficult to interpret. This is not to say that these changes may not be adverse but a threshold above which these changes increase propensity for fracture cannot be identified.

Consistency

The evidence consistently suggests that fluoride is associated with increased bone mineral density. This association appears present even at low (3 mg/day) exposures.

Strength

The observed effect size for bone mineral density ranged from small ~ 1% to relatively large 10% and was proportional to the exposure level.

Conclusion

The human evidence presented above suggests that changes in bone mineral density due to fluoride exposure may occur at lower concentrations than previously assessed. However, it is difficult to make judgement on the adversity of these findings as the threshold above which such changes may result in a decreased mechanical strength of the bone and corresponding fracture risk is not known.

$LOE3_{BH}$: Other biomarkers of bone health in human studies

- RCTs have shown that treatment with fluoride at around ~ 10 mg/day or above results in significant increases in serum osteocalcin concentrations and bone-ALP cross-linked type I collagen telopeptide (Reginster et al., 2003; Morabito et al., 2003; von Tirpitz et al., 2003). The limitation of these studies is that the comparison is based on the effect of fluoride given as co-treatment with other osteoporotic drugs, but these studies clearly showed that fluoride co-treatment leads to more elevated changes in these biomarkers than osteoporosis treatment alone.
- Similar changes in osteocalcin concentrations and bone-ALP have been reported in observational studies comparing subjects living in areas with elevated fluoride in drinking water (> 2.0 mg/L) with those living in areas with lower exposures (< 1.0 mg/L). Given the varying sample size and study designs the results from these studies have not been consistent.
- Some indications of fluoride being associated with higher PTH have also been reported but no strong conclusion can be made based on the few studies available.

Reliability & Relevance

The assessed studies were considered relevant, but their reliability was limited in terms of assessing adversity and due to the heterogeneity of the outcomes assessed.

Consistency

Due to the heterogeneity of the outcomes assessment of consistency is limited.

Strength

Varying effect sizes were reported some of which appeared biologically relevant.

Conclusion

Overall, the evidence presented above indicates that fluoride influences biomarkers of bone turnover reflecting bone health. However, the direction of the associations, reliability and consistency of some of these findings still requires further elucidation. In addition, interpreting differences in biomarkers of bone turnover becomes difficult when comparing small variations in fluoride exposure.

$LOE4_{BH}$: Bone strength in animal studies

- Two low risk of bias studies in rats showed decreased overall bone strength from 6 mg/kg bw per day after 30 days (Fina et al., 2018) and at 9.13 mg/kg bw per day after 180 days (Sharma et al., 2022)
- Increased fractures in trabecular bone starting from 6 mg/kg bw per day, and increased stiffness of cortical bone at 18 mg/kg bw per day within 30 days (Fina et al., 2018) with no effects on bone mineral density were reported
- Decreased bone volume (BV/TV) and Tb.Th were reported at 10 and 18 mg/kg bw per day, respectively without significant change in growth plate cartilage or cartilage histomorphometry (Lombarte et al., 2021)
- A study in BALB/c mice reported increased cancellous bone formation and Tb.Ar at the top dose (18.3 mg/kg bw per day: Chu et al., 2020).
- Decreased bone connectivity was observed in several studies.

Reliability & Relevance

Of 15 studies identified, 4 Tier 1 studies and 4 Tier 2 studies reported relevant effects on bone and dose-dependent increase of fluoride in bone as expected.

Consistency

The outcomes measured among studies were heterogeneous but together they indicate consistent changes in the direction of decreased bone strength and related markers from 6 mg/kg bw per day and above.

Strength

The reported changes were of sufficient effect size, in the same direction and statistically significant to be relevant.

Conclusion

There is relevant and reliable evidence for fluoride effects on bone strength in animals from about 6 mg/kg bw per day.

$LOE5_{BH}$: Biochemical changes included:

- Apoptotic nuclei in the subchondral trabecular bone at 18 mg/kg bw per day.
- Activation of Wnt/b-catenin signalling pathway in BALB/c mice shown as increased protein expression of markers (Wnt3a, phospho-GSK3b, b-catenin and Runx2) from 10.8 mg/kg bw per day.
- Increased autophagy in rat osteoblasts from 6 mg/kg bw per day and decreased p62 at 1.95 mg/kg bw per day. Autophagosomes and autolysosomes were observed by TEM at all doses.
- Changes in antioxidant enzyme activities (e.g. GSHPx, GSHRd, AChE, Cat, SOD) and/or other oxidative stress markers (lipid peroxidation), in one Tier 1 study from 4.67 mg/kg bw per day and one Tier 2 study at the top dose (21.5 mg/kg bw per day).
- Increased bone turnover as measured by increases in serum ALP and osteocalcin in BALB/c mice from 10.8 mg/kg bw per day.
- Limited but supporting evidence is reported in rabbits, as increased plasma ALP, ALT and AST from 50 mg fluoride/L in drinking water.

Reliability & Relevance

These data are of sufficient reliability. Although they provide evidence of changes in biomarkers of bone health, the interpretation of their relevance to humans is difficult due to the high dose levels at which they are observed.

Consistency

Considering the heterogeneity of the outcomes, consistency is difficult to evaluate.

Strength

Changes were observed at high doses and variable effect sizes.

Conclusion: Biochemical changes including apoptosis and autophagy, upregulated signalling pathways and oxidative stress occur at the highest dose levels tested.

Integrate the evidence

Methods

Integration of lines of evidence was done based on expert judgement. Humans and animal LOE were integrated independently before final overall integration of all lines of evidence on bone effects.

Results

Integration of $LOE1_{BH,}$ $LOE2_{BH}$, $LOE3_{BH}$

There is relatively strong and consistent evidence, from RCTs and observational studies, to suggest that fluoride exposure induces changes in bone mineral density and other biomarkers of bone health that may start at low exposures (~3 mg/day) (see Figure 7). Overall, the available evidence does not allow identification of a threshold where changes in bone mineral density or other biomarkers of bone health reflect adversity as increased risk of fractures.

One well-conducted study from Sweden suggested that low dietary intake (~3 mg/day) of fluoride might be associated with higher risk of hip fractures in postmenopausal women but, due to lack of support from other studies, no strong conclusions can be drawn on possible fracture risk at such low exposure. Of note however is the fact that the effect of fluoride on risk of fractures is likely to be causal, as suggested by results from RCTs (EFSA NDA Panel, 2005). Due to use of single high doses, establishing a HBGV from such trials requires several assumptions and is uncertain. Prospective cohorts conducted in populations at risk (such as postmenopausal women) are better suited to answer that question. The new evidence assessed here adds further support to that. However, findings from more than one study are needed for an assessment of consistency or concordance. The available evidence on fracture risk at low fluoride exposure is currently not sufficient to identify a quantitative threshold for establishing a HBGV.

Integration of $LOE4_{BH,}$ $LOE5_{BH}$

Based on the reliability, relevance and strength of the evidence presented above, fluoride exposure results in adverse effects on bone strength. This is particularly relevant to trabecular bone, such as loss of bone volume, loss of connectivity, decreased bone strength and increased bone fractures from approximately 6 and 10.8 mg/kg day in rats and mice, respectively.

Integration of $LOE1_{BH,}$ $LOE2_{BH}$, $LOE3_{BH,}$ $LOE4_{BH}$ $LOE5_{BH}$

Recent human data on the effects of fluoride exposure on bone health, particularly on bone mineral density, suggest that such effects may occur at fluoride exposures below the existing ULs. Evidence from animals is consistent with such effects on bone albeit changes are observed at higher doses compared to human studies. Even though one human study reported increased fracture risk at low exposures (upper quarter of fluoride intake, mean 3.2 mg/day), the overall evidence is judged to be too limited to conclude on the presence of an association with fractures at low exposures (see Figure 7).

3.5.4 | Weight of evidence for lines of evidence (LOEs) on dental fluorosis from human studies

Dental fluorosis

Assemble the evidence

Select evidence

Previous EFSA Opinion (2005) and assessments by national authorities (U.S. EPA, 2010; Health Canada 2024).

Lines of evidence

$LOE1_{DF}$: Dental fluorosis in children

Weigh the evidence

Methods

The relationship between fluoride and dental fluorosis is well established. Therefore, a systematic literature review was not conducted for this endpoint. Narrative assessment was conducted based on information provided previous assessments by other authorities and of the literature identified.

Results

- Dental fluorosis develops in children before the age of 8 years (and later for wisdom teeth).
- Demineralisation of teeth and mottling of enamel in a moderate or severe form is considered an adverse effect.
- The NDA Panel Opinion in 2005 based the UL for young children (1–8 years) on a prevalence of moderate to severe dental fluorosis of less than 5% in populations ingesting 0.08–0.12 mg fluoride/kg bw per day and described by Dean in 1942. The UL was set at 0.1 mg/kg bw per day (1–3 years: 1.5 mg/day and 4–8 years: 2.5 mg/day).
- The U.S. EPA (2010) established a reference dose (RfD) of 0.08 mg fluoride/kg bw per day based on the Dean index data, as '*the fluoride dose that will protect against severe dental fluorosis, clinical stage II skeletal fluorosis and skeletal fractures*'.
- As of 2024, the study by Dean (1942) is still considered to provide the best data to assess the risk of dental fluorosis (Health Canada, 2024). Health Canada estimated a BMCL of 1.56 mg/L fluoride in drinking water, based on 1% increase of moderate to severe dental fluorosis in the population, as a point of departure.
- Studies published after the EFSA NDA Panel 2005 Opinion, also showed a clear association between fluoride in urine and dental fluorosis and suggest it may occur at lower intake levels than those presented in the Dean study.

Conclusion

The Dean (1942) data on dental fluorosis are still the most robust data on dental fluorosis in children for quantitative modelling but evidence from later studies needs to be integrated for assessing risk for dental fluorosis in today's populations.

3.6 | Critical endpoints

Based on evaluation of relevant literature published since 2005 that met the inclusion criteria ($n = 445$ human studies and $n = 270$ animal studies), possible effects on the central nervous system (CNS), thyroid function and bone health were prioritised for systematic review ($n = 134$ human studies and $n = 59$ animal studies) as possible candidates for challenging the existing ULs for fluoride established in 2005 (EFSA NDA Panel, 2005).

New evidence on well-established adverse effects of fluoride, such as skeletal and dental fluorosis, were not subject to systematic literature review for several reasons. First, the direct link (or causality) between fluoride and these outcomes is well established. Second, in line with previous assessments (EFSA NDA Panel, 2005), it was judged that skeletal fluorosis likely occurs at higher exposures compared to exposures resulting in bone fractures for which the existing UL for adults is established. Third, the UL for children was based on dental fluorosis and screening of the new literature did not suggest that new evidence would substantially challenge previous studies published prior to 2003.

The applicability of the prioritised endpoints to different life stages is shown in Figure 1 (Section 2.3.3). Based on the outcome and conclusions of the weight of evidence assessment (see Section 3.5), dental fluorosis was selected as the critical endpoint for infants < 1 year, toddlers 1–3 years and children 4–8 years old. Effect on the CNS was also selected as a critical endpoint for which the most vulnerable life stages are pre- and post-development and the most relevant subpopulations for establishing a HBGV are pregnant women and infants, toddlers and children. The rationale for these selections is provided in the next two sub-sections.

3.6.1 | Dental fluorosis in infants, toddlers and children

Children are the most relevant population for dental fluorosis. It is well established that dental fluorosis occurs during a time window of permanent teeth development between birth and 3 years of age, and typically up to 8 years for development of first and second molars.[70] In this Opinion, dental fluorosis is considered relevant to infants (< 1 year), toddlers (1–3 years) and children (4–8 years). The existing UL for children 1–8 years is 0.1 mg/kg bw per day, based on the prevalence of moderate to severe dental fluorosis (EFSA NDA Panel, 2005). This value was based on estimated intake in children living in areas where fluoride in drinking water was up to ~2.0 mg/L and where prevalence of moderate to severe fluorosis was < 5%. A UL was not set for infants < 1 year of age.

In the previous EFSA assessment of fluoride when defining the existing UL for children (EFSA NDA Panel, 2005) and in more recent assessments by National Authorities (Health Canada, 2023; U.S. EPA, 2010) dental fluorosis has consistently been considered the most sensitive indicator for adverse effects associated with fluoride exposure. Furthermore, there are neither reliable nor sufficient data in the evidence assessed in this Opinion to indicate that potential effects on the CNS, thyroid or bone health are more sensitive than dental fluorosis for age groups of 1–3 and 4–8 years. As a result, the Scientific Committee considered dental fluorosis a relevant and reliable critical endpoint for all children up to 8 years of age (see also Figure 7).

3.6.2 | Central nervous system outcomes

Effects in the CNS were identified as a possible concern in the mandate for this assessment. They were prioritised as a critical endpoint to identify a RP for pregnant women due to concerns raised for the offspring from exposures during pregnancy, in both animal and human studies assessed in this Opinion.

Both the animal and human evidence suggests, despite uncertainties, that fluoride exposure through ingestion by pregnant females may adversely affect the developing brain of their offspring. Hence, for this critical endpoint the most relevant population is the fetus due to the vulnerability of the developing human brain. Pregnant women are identified as the most vulnerable adult population group. Since development continues postnatally, the possible adverse effects of fluoride on the CNS are also relevant for children of all age groups (see also Figure 7). However, there was insufficient evidence to directly assess this relationship.

Among all indicators of CNS effects reported, IQ in children was most consistently assessed in human studies. The overall evidence of neurodevelopmental effects in animal studies, including histopathological changes in brain, learning and memory tests, and organ and cellular level changes, provides additional support for changes in the CNS. However, the neurodevelopmental outcomes in animals were not considered reliable enough to be used independently from the human epidemiological data. This was due to uncertainties associated with study conduct, dose ranges tested and different nature of outcomes assessed in the animal studies. Due to these uncertainties the available human data were considered more suitable for establishing a HBGV.

Compared to the other endpoints prioritised for the human studies, effects in the CNS were considered more reliable and sensitive to be selected as critical endpoint compared to the new evidence reviewed for thyroid and the bone (increased risk of fractures). Neither of those two endpoints was considered more reliable for establishing a HBGV for children or adults for several reasons. For thyroid, changes in TSH observed in human studies were indicative of thyroid stress at

[70]The 3rd permanent molars (wisdom teeth) develop later.

intake levels above those where possible effects on the CNS appeared to occur. However, the associated changes in thyroid hormones were inconclusive and did not justify selection of thyroid as a critical endpoint. In addition, toxicological evidence was limited. For bone health, changes in bone mineral density that may affect bone strength and other biomarkers appeared to be continuous starting at low exposures. However, the evidence does not allow identification of a threshold reflecting adverse changes such as increased risk of fractures. New evidence indicating increased risk of fractures at low dietary fluoride intake was limited to one study which is insufficient to justify selection of fractures as a critical endpoint for adults. Supporting evidence from animal studies was also limited as those effects were observed at high doses, in addition to uncertainties associated with animal to human extrapolation.

Overall, adverse effects in the thyroid gland or bone are unlikely to occur in adults at exposures below those of pregnant women where effects on developing CNS of the offspring may start to occur (see also Figure 7).

3.7 | Derivation of a reference point

The approach for identification of a numerical RP based on adverse effects on dental fluorosis and neurodevelopment is presented. A schematic representation of the available evidence of adverse effects of fluoride relative to its concentration in drinking water and the identified reference points is presented in Figure 7.

3.7.1 | Dental fluorosis

Dental fluorosis was selected as the critical endpoint relevant for the identification of a RP for infants, toddlers and children. Unlike the evidence for neurodevelopment, existing data for dental fluorosis can be used for dose–response modelling. Such modelling is additional to the assessment previously performed when the existing UL for children was established (EFSA NDA Panel, 2005). For the existing UL, the RP was established based on expert judgement with reference to two studies in particular: a large study ($n = 5824$) examining dental fluorosis in children living in different areas in the US where fluoride concentrations in drinking water ranged from < 0.1 to 14.1 mg/L (Dean, 1942), and a dose–response assessment analysis performed on a separate set of studies conducted in US and Swedish children (Fejerskov et al., 1996).

Since 2005, when the UL for children was established (EFSA NDA Panel, 2005), the methodology for identifying a RP has evolved. Current EFSA guidance recommends dose–response modelling for establishing a RP when data allow (EFSA Scientific Committee et al., 2022). Dental fluorosis was re-assessed in the current assessment with Bayesian benchmark dose (BMD) modelling to refine the existing UL. Data of prevalence of dental fluorosis in children after oral exposure to fluoride obtained from the Dean study are eligible and were used for dose–response modelling (Dean, 1942).

3.7.1.1 | *Selection of a benchmark response for dental fluorosis*

The EFSA guidance on Bayesian BMD (2022) recommends that the BMR should reflect the onset of a human-relevant adverse effect, meaning that a response above the BMR is considered adverse. For fluoride, the endpoint of dental fluorosis is measured as incidence or prevalence of a defined severity grade (very mild, mild, moderate and severe). Therefore, two criteria should be considered for BMD modelling of dental fluorosis: the severity grade that is considered adverse and the level of its prevalence represented in the BMR.

Despite being described qualitatively in categories of severity grades, dental fluorosis occurs along a continuous scale of change in the tooth enamel in the individual and transitions from a non-adverse to adverse effect with increasing exposure. The grades of very mild and mild dental fluorosis indicate changes in the tooth enamel of minimal aesthetic impact, consisting of opaque white spots on the teeth and without an adverse health effect. However, moderate dental fluorosis reflects hypomineralisation of the tooth enamel, which is associated with increased brown staining and minor pitting of teeth, and subsequent increased risk of dental caries. This is generally accepted as an adverse effect on tooth integrity in addition to its considerable aesthetic impact.

Therefore, the Scientific Committee considered moderate and severe dental fluorosis as the relevant grades of onset of adversity and a BMR of 1% which indicates the prevalence of this adverse effect in the population studied. It is noted that milder forms of dental fluorosis would co-occur at a higher frequency in the population presenting 1% of moderate fluorosis. A more conservative modelling approach was also performed, based on a BMR of 5% for occurrence of the combined non-adverse grade of mild up to severe dental fluorosis, where the risk of developing moderate to severe dental fluorosis would be minimal (not expected to occur).

3.7.1.2 | *Results from the benchmark dose analyses*

Increase in moderate and severe dental fluorosis was modelled as function of drinking water concentrations, resulting in benchmark concentrations (BMC). The results from the Bayesian BMD analyses are summarised in Table 26.

Using a BMR of 1% for the prevalence of moderate and severe fluorosis, a $BMCL_{01}$ of 1.71 mg/L in drinking water was derived, with a $BMCU_{01}$ of 1.87 mg/L. When using a BMR of 5% for combined mild, moderate and severe dental fluorosis prevalence, a $BMCL_{05}$ of 1.41 mg/L in drinking water was derived, with a $BMCU_{05}$ of 1.54 mg/L. Both modelling approaches

have narrow credible intervals, with slightly narrower credible interval for the estimation of the BMC for mild and severe dental fluorosis (BMCU-BMCL = 0.13 mg/L) compared to moderate to severe dental fluorosis (0.16 mg/L). This is in part due to the broader range of the data for modelling an increase in mild to severe fluorosis compared to modelling an increase in moderate to severe fluorosis.

TABLE 26 Bayesian Benchmark dose modelling for dental fluorosis effects (all results calculated using Bridge sampling[a]).

Reference	Observed effect (BMR)	Dosing	BMCL	BMC	BMCU
Dean (1942)	Moderate and severe dental fluorosis (1%)	Drinking water fluoride levels 0.0 to 14.1 mg/L	$BMCL_{01}$ = 1.71 mg/L	1.79 mg/L	1.87 mg/L
Dean (1942)	Mild and severe dental fluorosis (5%)	Drinking water fluoride levels 0.0 to 14.1 mg/L	$BMCL_{05}$ = 1.41 mg/L	1.48 mg/L	1.54 mg/L

[a]Extended dose range assumption was not applied in order to avoid confidence intervals falling outside of the dose range tested.

Considering all the available evidence on dental fluorosis, the $BMCL_{01}$ of 1.71 mg/L of the scenario for a BMR of 1% for moderate and severe dental fluorosis is above the threshold for which moderate to severe fluorosis was reported in other similar studies (Driscoll et al., 1983, 1986; Galagan & Lamson Jr., 1953; Heifetz et al., 1988; Horowitz et al., 1984; Richards et al., 1967; Selwitz et al., 1995, 1998) conducted after the Dean study (U.S. EPA, 2010). Although the overall prevalence of severe fluorosis was low in these studies (≤ 3%), results suggest that such adverse events may occur at lower doses than 1.71 mg/L fluoride in drinking water and that a low BMR of 1% is still not sufficiently protective. This may be due to contribution of additional sources of fluoride exposure that became available after the introduction of water fluoridation, such that an equivalent *total* intake is reached at lower water fluoride concentrations. This suggests that while the data from the Dean (1942) are still robust, the total fluoride intake must be taken into account for accurate comparison. While the $BMCL_{05}$ of 1.41 mg/L from the second scenario (BMR 5% for mild and severe dental fluorosis) may be considered conservative, as it is not based on modelling of a strictly adverse event, it takes into consideration changes that occur in the pathway prior to the adverse event (tooth damage). As such, a BMR based on mild and severe fluorosis is more protective against adverse aesthetic effects (brown stains on teeth) in addition to adverse physiological effects. A more protective BMR scenario in terms of severity basis is also justified when the adverse effects are cumulative and non-reversible, as well as due to the uncertainty about the total fluoride intake in the available studies.

The Scientific Committee considered that the BMCLs of the two modelling scenarios are consistent with the previous assessments where BMRs of 0.5 to 5% have been modelled for moderate and severe fluorosis based on the Dean study (see Table 18, Section 3.2.6). The protective properties of fluoride against caries have been estimated to reach their maximum at water concentrations between 0.7 and 1.3 mg/L (Dean, 1942; U.S. EPA, 2010; EFSA NDA Panel, 2005), i.e. below the respective BMCLs of these scenarios. Therefore, either scenario proposed for the BMD modelling of dental fluorosis results in water fluoride concentrations that are not associated with dental fluorosis and leave a margin from levels established for the beneficial use of fluoride.

Based on the above, the Scientific Committee considers that the fluoride level of 1.4 mg/L in drinking water is an appropriate RP from which to establish a HBGV that is protective against dental fluorosis in children (Figure 7).

3.7.2 | Neurodevelopment

Characterising the relationship between fluoride exposure and neurodevelopmental outcomes is challenging for several reasons. Available data from animal studies were too uncertain for deriving a RP due to study design and conduct, outcome assessment and dose ranges tested. The set of available mechanistic in vitro studies was not comprehensive relative to current OECD guidance. Reliable data for kinetic in vitro to in vivo extrapolation modelling were also lacking. For the human studies, the main limitations were the small number of prospective studies conducted in pregnant women and children in areas with a sufficiently wide range of fluoride concentrations in drinking water and lack of studies with well-characterised (total) fluoride intake.

For the human studies, dose–response modelling was not considered informative due to the lack of a sufficient number of exposure categories for drinking water within the available studies. Access to data with individual participant urine measurements was also not possible. Despite this, it is possible to identify an exposure level above which neurodevelopmental adverse effects were consistent, based on the large evidence base and taking the totality of the evidence into consideration.

As summarised in the weight of evidence section, it was concluded that, at low fluoride exposure from drinking water (< 1.5 mg/L) evidence for an association of fluoride intake with adverse neurodevelopmental outcomes is inconsistent. The Scientific Committee concludes, based on a large number of studies in children, that exposure to fluoride concentrations in drinking water ≥ 1.5 mg/L is consistently associated with lower IQ scores in children. Therefore, 1.5 mg/L of fluoride in drinking water is identified as a RP, based on expert judgement after assessing all the available human evidence, as described in the weight of evidence section (Figure 7).

It is important to note that the RP of 1.5 mg/L in drinking water is subject to some uncertainty. First, possible adverse associations have been reported at concentrations around 1 mg/L in some prospective studies (see Section 3.2.2.1 and Table D.2 in Annex D). It was, however, concluded that at such low levels of exposure there is lower confidence in the evidence (see Weight of Evidence, Section 3.5.1). Secondly, this RP is not derived quantitatively. Despite these uncertainties, it is judged appropriate to use the value of 1.5 mg fluoride/L in drinking water as a RP, supported by the overall evidence that adverse effects are observed from exposures to fluoride in drinking water above this concentration. In addition, even in the absence of dose–response analyses from human data, this RP is considered more precise than what could be derived from animal data due to inter- and intra-species uncertainties.

Lastly, the available studies on CNS effects do not provide sufficient information for identification of a similar RP specifically for infants. The Scientific Committee considered that exposure of the infants and children in the human studies was subject to the same drinking water concentration as that of the mother, assuming continued residency in the same area. Therefore, the Scientific Committee considered that it was reasonable to use the same RP for infants, children and adults (including pregnant women).

In conclusion, based on expert judgement, a value of 1.5 mg/L fluoride in drinking water is considered to be supported by the currently available evidence to be used as a RP for infants, children and adults, including pregnant women (Figure 7).

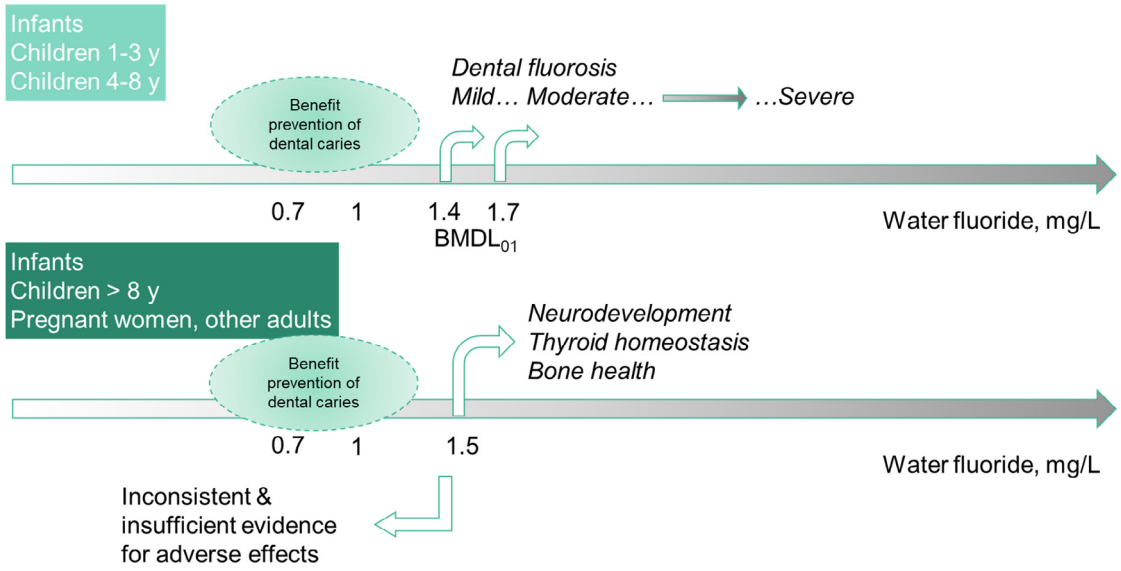

FIGURE 7 Overview of evidence on prioritised endpoints assessed in this opinion relative to evidence on benefit of fluoride assessed by the EFSA NDA Panel in 2013.

3.8 | Establishing health-based guidance values (HBGVs)[71] for children and adults

The Scientific Committee notes that the HBGVs for infants, children and adults are established based on evidence with different levels of confidence. The causality and dose–response relationship between fluoride and dental fluorosis is well established and the available data are sufficiently reliable for a quantitative characterisation of the RP. Therefore, for infants, toddlers and children (< 1, 1–3 and 4–8 years, respectively), a UL can be established.

There is confidence in the evidence from human studies that fluoride exposure during pregnancy corresponding to ≥ 1.5 mg/L fluoride in drinking water may adversely affect neurodevelopmental outcomes in the offspring. In addition, there is some evidence of adverse effects for the other prioritised endpoints above this concentration in adults. The heterogeneity of study designs and the lack of sufficient data to model the dose–response relationship for the neurodevelopmental outcomes (and the other prioritised endpoints) make quantitative characterisation of a RP difficult. Therefore, the available evidence is not sufficiently robust to identify a quantitatively supported threshold as a RP from which to derive a UL. The Scientific Committee considered that in the absence of a robust RP, a 'safe level of intake' can be established (EFSA NDA Panel, 2024). The safe level of intake is defined as '*the highest level of intake for which there is reasonable confidence for the absence of adverse effects*' and is used in the context of nutrients when data are not sufficiently robust to establish a UL. While the evidence below the safe level of intake does not allow conclusion on safety of fluoride, it is nonetheless considered inconsistent and insufficient to result in a lower HBGV and the terminology is adopted due to the absence of a quantitative RP. Despite some uncertainties around the RP of 1.5 mg/L fluoride in drinking water, there is reasonable confidence that this value is protective from adverse effects of fluoride. Notwithstanding the uncertainties in the evidence of

[71]The term HBGV is used in this opinion when referring to both safe level of intake and UL established for fluoride.

neurodevelopmental effects of fluoride, the overall evidence is sufficiently strong to warrant a lower HBGV for adults than the previously established UL of 7 mg/day (EFSA NDA Panel, 2005).

The RPs derived for neurodevelopmental outcomes and dental fluorosis of 1.5 and 1.4 mg fluoride/L in drinking water, respectively, are essentially the same and could result in HBGVs according to standard dose extrapolation from drinking water concentrations. However, as noted previously, the effects reported in the literature are not only the result of fluoride present in drinking water, but of the total fluoride body burden of the participants in the human studies and of experimental animals. Attributing the effects observed in human studies exclusively to fluoride in drinking water would not be correct (it would be an overestimation of fluoride toxicity). Therefore, the HBGVs must represent the total intake of fluoride; water is one of the contributing sources, in addition to fluoride from foods and dental care products (see Tables 34 and 35).

Translating the RP for fluoride in drinking water to total intake of the population under study from all sources (water, foods and dental care products) rests mostly on assumptions for total liquid intake. This is because in cases where water fluoride concentrations are elevated, drinking water is by far the main contributor to total exposure, exceeding intake from food and dental care products (see Section 3.9.3). Several factors influence intake of water including temperature and physical activity. The Scientific Committee considered that adequate intakes provided in the Scientific Opinion on Dietary Reference Values for water (EFSA NDA Panel, 2010) can be used as estimates of total liquid intake to derive a safe level of intake. This opinion derived Adequate Intake of liquids[72] for different age groups. The Adequate Intake for water takes into account *total water* intake from drinking water, beverages and food moisture, assuming moderate environmental temperature and physical activity levels (physical activity level of 1.6). It is understood in this context that food moisture refers to the water content of food as consumed, naturally occurring or changed during food preparation. The NDA Panel also estimated that food contributes to around 20%–30% of total fluid intake while drinking water and beverages around 70%–80%.

Lastly, the RPs used to establish a UL for children and safe level of intake for adults are obtained from human studies; hence no interspecies extrapolation factor is applicable. The Scientific Committee concluded that a UF to account for interindividual variability is not needed because the evidence is obtained from diverse, relevant and sensitive human populations.

3.8.1 | Tolerable upper intake level for infants, toddlers and children ≤ 8 years

For dental fluorosis in children, the relevant age group is between birth to 8 years old. For this age group the Adequate Intake for total water from all sources for boys and girls is 800–1000 mL/day for infants 6–12 months, 1300 mL/day for ages 1–3 years and 1600 mL/day for ages 4–8 years (EFSA NDA Panel, 2010).

Assuming that all foods and beverages contain water with fluoride concentrations equal to the RP of 1.4 mg/L derived for the critical endpoint of dental fluorosis, the total water contribution to fluoride intake would be up to 1.4 mg/day for infants 6–12 months, 1.82 mg/day for toddlers 1-3 years and 2.24 mg/day for children 4–8 years. These estimates do not include the contribution of fluoride that was naturally occurring in food consumed without addition from fluoridated water by the children of the Dean study.

It is important to note that assuming that all liquid intake would derive from water with a fluoride concentration of 1.4 mg/L is most likely leading to an overestimation of the exposure. The reason being that even during the time of the Dean's study the children exposed to drinking water with varying fluoride levels would have consumed foods and beverages (including dairy) with low or no fluoride content or that would have come from outside their living area. This is a source of uncertainty. A reasonable assumption can be made that 20% of the liquid intake would be obtained from beverages with negligible/no fluoride, or that originated from outside the area of residence. This results in an estimated intake from water of 1.12 mg/day for infants 6–12 months, 1.46 mg/day for toddlers 1–3 years and 1.79 mg/day for children 4–8 years. This approach (20% of the liquids consumed does not contain fluoride) is supported by reported actual drinking water consumption data from several countries in the EU, which is e.g. 1 L/day instead of the default value for all liquids of 1.6 L/day for children 4–8 years (EFSA Comprehensive Consumption database).

The contribution of fluoride from foods to the total intake of fluoride of children in the Dean study is uncertain. The range of food-derived fluoride intake estimated in the present exposure assessment may be used as a surrogate, as it may both underestimate and overestimate the food-derived fluoride intake in children in the 1940's (different food commodities; absence of supplementation; higher range of possible background contamination as indicated by the broader drinking water fluoride range that reached 14.1 mg/L). Alternatively, the estimated total intake by the children of the Dean study as presented by U.S. EPA (2010) may be adopted, where food at the time of the Dean study was estimated to contribute 0.01 mg/kg bw per day of fluoride, based on a mean fluoride content of 0.5 mg/kg in solid foods of representative diets, caloric intakes and body weights. This fluoride intake from food corresponds to around 0.09 mg/day for infants of 6–12 months, 0.12 mg/day for 1–3 years and 0.20 mg/day for 4–8 years. This increases the total fluoride intake from food and water to

[72]'Water requirement varies between individuals and according to environmental conditions. Therefore, only adequate intakes have been defined for specific age groups from a combination of observed intakes in population groups with desirable osmolarity values of urine and desirable water volumes per energy unit consumed' (EFSA NDA Panel, 2010).

1.2 mg/day, 1.6 mg/day and 2.0 mg/day, for age groups 6–12 months, 1–3 years and 4–8 years, respectively. These intakes correspond to 0.14, 0.13 and 0.10 mg/kg bw per day, respectively[73] (Table 27).

For infants < 6 months, the total liquid intake is estimated to be 100–190 mL/kg bw per day (EFSA NDA Panel, 2010). For the purpose of these calculations, the liquid intake is assumed to be 0.7 L/day for < 6 months, based on the average value of the total liquid intake range (150 mL/kg bw per day) and on the body weight of infants < 3 months.[74] Food intake was not applied as it is represented by milk intake which is covered by the total daily liquid intake. Due to limited liquid sources for infants < 6 months and uncertainties regarding the choice of breast milk or infant formula, the 20% reduction is not applied to the total intake estimation in this age group. Hence, the total intake for age groups < 6 months is estimated to be 1.0 mg/day corresponding to 0.2 mg/kg bw per day, based on the body weight of younger infants (< 3 months) and applied to infants < 6 months (Table 27).

In the estimations of total intake of fluoride associated with dental fluorosis in children of the Dean study, addition of fluoride from dental care products would not be appropriate as the Dean study from which the RP was derived (Dean, 1942) was conducted in a time period predating the commercialisation of fluoridated dental care products. Therefore, the contribution of dental care products is not included in the calculations for the total intake in infants and children to establish a UL based on dental fluorosis.

Based on the above, the Scientific Committee established ULs of 1.0 mg/day for all infants (< 1 year) based on the total intake of infants < 6 months, 1.6 mg/day for toddlers 1–3 years and 2.0 mg/day for children 4–8 years (Table 29).

3.8.2 | Safe level of intake for adults and children > 8 years

The Scientific Committee recognised that the existing UL of 7 mg/day for adults is not sufficiently protective as it was based on a significantly increased risk of non-vertebral fractures observed in randomised clinical trials of pharmacological interventions, where fluoride tablets were administered at a relatively high dose (34 mg/day) for 4 years and were also associated with side effects. New evidence indicates that adverse effects in bone as well as the thyroid and the CNS are observed at intakes below the existing UL (see Sections 3.5.1–3.5.3).

In this opinion, an RP of 1.5 mg/L fluoride in drinking water has been identified as the concentration above which there is reasonable confidence for adverse effects in the CNS of the fetus born to exposed pregnant women, based on the critical endpoint of neurodevelopment of the offspring. Therefore, this RP is the basis of the HBGV for adults and age groups > 8 years. For adults, the adequate intake of total liquid (drinking water, beverages and food moisture) has been estimated at 2.0 L/day and 2.5 L/day for females and males, respectively (EFSA NDA Panel, 2010). For this assessment, 2.0 L/day for females will be used as approximation of total liquid intake. Using the RP of 1.5 mg/L for neurodevelopment, the corresponding intake for adults from drinking water would be 3.0 mg/day assuming all liquid intake (drinking water, beverages, food moisture) would come from water with that concentration.

As described above for children, assuming that all liquid intake would derive from water with fluoride of 1.5 mg/L is most likely leading to overestimation of the total intake in the study participants. A reasonable assumption can be made that intake of fluoride from water was < 100%; if only 80% of fluid intake came from water with fluoride concentration of 1.5 mg/L and the remaining 20% from beverages with negligible fluoride content, the corresponding intake is reduced to 2.4 mg/day. The contribution from food alone (excluding beverages) under the non-fluoridated water scenario is estimated to be around 0.64 mg/day on average (range 0.43–1.0 mg/day) (see Appendix F.1.). Contribution of fluoride from dental care products may also be reasonably assumed in the populations of the studies, contributing on average 0.3 mg/day (Table 35). Fluoridated salt may not have been a common source of fluoride for the populations studied, thus it cannot be assumed that it appreciably contributed to the total dose in the participants of these studies. Differences in dietary patterns between the EU consumption data and the countries where the studies were conducted introduce uncertainty about the contribution of food to the total intake of fluoride; this was taken into consideration in the uncertainty assessment of the safe level of intake. Therefore, the estimated total fluoride intake in the study populations adds up to 3.3 mg/day[75] (Table 28).

In the NDA Scientific opinion on DRVs for water (NDA, 2010) it is stipulated that 'adolescents of 14 years and older are considered as adults with respect to adequate water intake.' In addition, the adequate water intake for children 9–13 years of age is estimated to be 2100 mL/day for boys and 1900 mL/day for girls. As this is in the same range as the water intake of adults used in calculating total intake, the total intake of 3.3 mg/day is applicable to age groups > 8 years of age.

An alternative approach for estimating total intake of fluoride in study participants consuming water with a fluoride concentration of 1.5 mg/L can be based on the reported urine fluoride concentrations in these studies and mass balance calculation (see Section 3.9.3.3 for details). The total fluoride intake estimated using urine concentrations of fluoride corresponding to 1.5 mg/L fluoride in water (see Section 3.2.1) resulted in a range of 1.6–5.6 mg/day. The estimated total fluoride

[73]Default body weights of 8.8 kg, 12 kg and 20 kg for age groups 6–12 months, 1–3 years and 4–8 years, respectively (EFSA Scientific Committee, 2012).

[74]Default body weights of 4.8 kg and 6.7 kg for age groups < 3 months and 3–6 months, respectively. The calculations are based on the body weight of the younger infants (EFSA Scientific Committee, 2012).

[75]((1.5 mg/L × 2 L/day) × 0.80) + 0.6 mg/day + 0.3 mg/day.

intake derived from default factor calculations and addition of mean food and dental care products (3.3 mg/day) is within this range.

For infants, the RP for CNS effects can be extrapolated to the infant total intake using inputs of default liquid intake and food intake, as presented above (Section 3.8.1). The total fluoride intake of infants exposed to drinking water with a fluoride concentration of 1.5 mg/L may be calculated using a default liquid consumption of 0.7 L/day and 1 L/day (100–190 mL/kg bw) for < 6 months and 6–12 months, respectively, and corresponds to 1.05 and 1.5 mg/day, respectively. As for the other age groups, it can be reasonably assumed also for infants 6–12 months that only 80% of fluid intake came from water with a fluoride concentration of 1.5 mg/L and the remaining 20% from beverages with negligible fluoride content, so that the corresponding fluoride intake is reduced to 1.2 mg/day. This assumption was not applied to infants < 6 months (see also Section 3.8.1). The contribution from food alone for infants 6–12 months under the non-fluoridated water scenario is estimated to be around 0.023 mg/kg bw per day, corresponding to 0.20 mg/day on average. It is noted that the food intake was not applied to children younger than 6 months as it is represented by milk intake which is covered by the daily liquid intake. No contribution from fluoridated dental care products was added to the total intake of infants in the studies on DNT because the use of these products in infants is uncertain and may have increased the resulting HBGV unnecessarily (less conservative). In addition, as noted in the exposure section, the use of fluoridated discretionary salt was not reported for infants. The resulting total intakes for infant age groups < 6 months and 6–12 months are 1.1 and 1.4 mg/day, respectively, corresponding to 0.22 and 0.16 mg/kg bw per day, respectively (Table 25).

The Scientific Committee concluded that, for CNS effects, the estimated total intake in the study populations of 3.3 mg/day can be used as a safe level of intake for pregnant women to limit fetal exposure to fluoride from maternal intake and resulting body burden. Based on the weight of evidence (Section 3.5) and as noted in Section 3.6.2, adverse effects in the thyroid gland or bone are unlikely to occur in adults at exposures below the exposures in pregnant women where effects on developing CNS may start to occur. The safe level of intake is therefore considered protective against other possible adverse outcomes and hence applies to all adults. In the absence of specific data for other age groups, the safe level of intake also applies to all age groups older than 8 years and is protective against possible adverse effects in the CNS, thyroid or bone (Table 29).

The Scientific Committee concluded that, for CNS effects, the estimated total intake of 1.1 and 1.4 mg/day in the study populations could also be used as safe levels of intake for infant age groups < 6 months and 6–12 months, respectively. However, the Scientific Committee recognised that the total intakes calculated for infants based on dental fluorosis and for potential CNS effects, respectively are based on similar RP values. The intake estimates differ in consideration of fluoride contributed from food in studies on CNS effects and the older study on dental fluorosis. Since a UL is a more robust HBGV, the UL of 1.0 mg/day based on dental fluorosis is selected over the safe level of intake based on potential CNS effects in infants (Table 29). In addition, the UL based on dental fluorosis is lower (more protective) than the safe level of intake.

TABLE 27 Total intake for infants and children from relevant lines of evidence.

Weight of evidence				Intake in mg/day						Intake in mg/kg bw per day					
Line of evidence	No concern/ evidence of effects	Evidence of adverse effect	Relevant population	Equivalent human dose (RefPoint)	20% discounting for non-F-DW	Human dose from food	Human dose from dental products	Human dose from discr.F-salt	Reference value (mg/day)	Equivalent human dose	20% discounting for non-F-DW	Human dose from food	Human dose from dental products	Human dose from discr.F-salt	Reference value (mg/kg bw per day)
Human DNT	Insufficient evidence for an association between exposure to Fin pregnancy and lower IQ in areas with no or low drinking water fluoridation, i.e. < 1.5 mg/L	Consistent findings from cross-sectional studies for an association with lower IQ in areas with elevated drinking water levels > 1.5 mg/L	Infants 0–6 months	1.05	NA	NA	NA	NA	1.05	0.22	NA	NA	NA	NA	0.22
			Infants 6-12 months	1.5	1.20	0.20	NA	NA	1.40	0.17	0.14	0.023	NA	NA	0.16
Dental fluorosis	Dean study: fluoride protective under 1.5 mg/L	Dean study for moderate to severe fluorosis with BMR of 5%, BMDL of 1.4 mg/L; BMD of 1.48; BMDU of 1.54 mg/L	Infants 0-6 months	1.0	NA	NA	NA	NA	1.0	0.20	NA	NA	NA	NA	**0.20**
			Infants 6-12 months	1.4	1.12	0.09	NA	NA	1.2	0.16	0.13	0.01	NA	NA	0.14
			Children, 1–3 years	1.82	1.46	0.12	NA	NA	1.6	0.15	0.12	0.01	NA	NA	**0.13**
			Children, 4–8 years	2.24	1.8	0.2	NA	NA	2.0	0.11	0.09	0.01	NA	NA	**0.10**

Notes: Default body weights of 8.8 kg, 12 kg and 20 kg for age groups 6–12 months, 1–3 years and 4–8 years, respectively. The calculations for infants < 6 months are based on the body weight of 4.8 kg of the younger infants < 3 months (EFSA Scientific Committee, 2012). See text for further details.

TABLE 28 Total intake for adults from relevant lines of evidence.

Weight of evidence				Intake in mg/day						Intake in mg/kgbw per day					
Line of evidence	No concern/ evidence of effects	Evidence of adverse effect	Relevant population	Equivalent human dose (RefPoint)	20% discounting for non-F-DW	Human dose from food	Human dose from dental products	Human dose from discr.F-salt	Reference value	Equivalent human dose	20% discounting for non-F-DW	Human dose from food	Human dose from dental products	Human dose from discr.F-salt	Reference value adult (70 kg)
Human DNT	Insufficient evidence for an association between exposure to F in pregnancy and lower IQ in areas with no or low drinking water fluoridation, i.e. < 1.5 mg/L	Consistent findings from cross-sectional studies for an association with lower IQ in areas with elevated drinking water levels > 1.5 mg/L	Pregnant women and infants	3	2.40	0.60	0.3	0	3.30	0.04	0.03	0.01	0.005	0	**0.047**
Human Thyroid	No reliable evidence for changes in thyroid hormones or TSH at levels of fluoride < 1.5 mg/L water	uF 1.5 mg/L urine; at wF > 2 mg/L TSH is consistently increased by 30%or more in several studies	Children and adults	3	2.40	0.60	0.3	0	3.30	0.04	0.03	0.01	0.005	0	0.047
Human Bone Mineral Density	Modest changes start around 3 mg/day (diet and water) (1-2% increase), but these may not be	Substantial increase at 2.7 mg/L drinking water (IC) (14-20%) supported by findings in	Adults, postmenopausal women	3	NA	NA	0.3	0	3.30	0.04	NA	NA	0.005	0	0.047
Human Bone Fractures	Reference group of 1.4 mg/day (diet and water)	Modest effects at 3 mg/day (one observational study)	Adults, postmenopausal women	3	NA	NA	0.3	0	3.30	0.04	NA	NA	0.005	0	0.047
Animals (D)NT Behaviour	No evidence of adverse effects up to 2 mg/kg bw per day (incl. feed)	2.5 mg/kg bw per day (MWM); M/F; in standard diet (incl. 1.5 mg/kg bw per day from feed)	Pregnant women, children and infants	1.75	NA	NA	NA	NA	1.75	0.025	NA	NA	NA	NA	0.025
Animals (D)NT Organ/ Brain	NOAEL 2.5 mg/ kg bw	Organ level effects (decreased brain weight by 5%) not reliable	Pregnant women, children and infants	2.8	NA	NA	NA	NA	2.8	0.04	NA	NA	NA	NA	0.040
Animals (D)NT Molecular/ Cellular	Generally, no effects < 2 mg/kg bw per day	Molecular and cellular changes reported at > 2 mg/kg bw per day	Pregnant women, children and infants	1.4	NA	NA	NA	NA	1.4	0.02	NA	NA	NA	NA	0.020
Animals Thyroid	1.8 mg/kg bw per day	NA (only one reliable study reporting no effects up to 1.8 mg/kg bw).	Children and adults	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Animals Bone	Molecular changes at 2 or 4.7 mg/ kg bw per day not considered adverse	Effects start at 6 mg/kg bw per day for decreased bone strength	Adults, postmenopausal women	2.8	NA	NA	NA	NA	2.8	0.04	NA	NA	NA	NA	0.040

Notes: Default body weight for adults of 70 kg. Extrapolation factor of 100 applied from animal dose to human equivalent. See text for further details.

TABLE 29 Tolerable upper intake levels (UL) and safe levels of intake for all age groups.

Age group	Tolerable upper intake level (UL) (mg/day)	Safe level of intake (mg/day)
Infants (< 1 year)	1.0	
Toddlers (1–3 years)	1.6	
Children (4–8 years)	2.0	
Age groups > 8 years		3.3
Adults		3.3

3.9 | Exposure assessment

3.9.1 | Exposure assessment to fluoride from dietary sources

Tables 30 and 31 show the summary statistics of the estimated chronic dietary exposure to fluoride (in units of mg/kg bw per day and mg/day, respectively) for each age group for the basic scenario based on LB/MB/UB mean water fluoride concentrations as calculated from the available concentration data in food and drinking water (see Section 2.2.2.4).

TABLE 30 Mean and P95 (LB/MB/UB) chronic dietary exposure to fluoride for the basic scenario (mg/kg bw per day).

Range of mean dietary exposure (mg/kg bw per day)							
		LB		MB		UB	
Age group	N surveys	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Infants	11	0.01	0.04	0.01	0.04	0.01	0.04
Toddlers	15	0.02	0.03	0.02	0.03	0.02	0.03
Other children	19	0.02	0.03	0.02	0.03	0.02	0.03
Adolescents	21	0.01	0.02	0.01	0.02	0.01	0.02
Adults	22	0.01	0.02	0.01	0.02	0.01	0.02
Elderly	19	0.01	0.02	0.01	0.02	0.01	0.02
Very elderly	14	0.01	0.02	0.01	0.02	0.01	0.02
Range of P95 dietary exposure (mg/kg bw per day)							
		LB		MB		UB	
Age group	N surveys	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Infants	9	0.03	0.08	0.03	0.08	0.04	0.08
Toddlers	13	0.03	0.05	0.03	0.06	0.03	0.06
Other children	19	0.02	0.05	0.02	0.05	0.02	0.05
Adolescents	20	0.01	0.03	0.01	0.03	0.01	0.03
Adults	22	0.01	0.03	0.01	0.03	0.01	0.03
Elderly	19	0.01	0.03	0.01	0.03	0.01	0.04
Very elderly	10	0.01	0.03	0.01	0.03	0.01	0.03

TABLE 31 Mean and P95 (LB/MB/UB) chronic dietary exposure to fluoride for the basic scenario (mg per day).

Range of mean dietary exposure (mg/day)							
		LB		MB		UB	
Age group	N surveys	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Infants	11	0.09	0.28	0.10	0.30	0.10	0.31
Toddlers	15	0.25	0.37	0.26	0.39	0.28	0.41
Other children	19	0.31	0.57	0.32	0.60	0.33	0.63
Adolescents	21	0.44	0.78	0.44	0.82	0.45	0.85
Adults	22	0.47	1.14	0.48	1.17	0.50	1.19
Elderly	19	0.44	1.30	0.46	1.32	0.47	1.35
Very elderly	14	0.57	1.22	0.58	1.24	0.59	1.27

TABLE 31 (Continued)

Range of P95 dietary exposure (mg/day)							
		LB		MB		UB	
Age group	N surveys	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Infants	9	0.26	0.44	0.28	0.47	0.30	0.48
Toddlers	13	0.36	0.62	0.39	0.65	0.42	0.68
Other children	19	0.45	1.07	0.46	1.09	0.47	1.11
Adolescents	20	0.68	1.48	0.70	1.49	0.71	1.52
Adults	22	0.73	2.22	0.74	2.26	0.75	2.31
Elderly	19	0.71	2.40	0.71	2.45	0.72	2.50
Very elderly	10	0.95	2.08	0.97	2.11	0.99	2.13

Mean dietary exposure to fluoride in the basic scenario ranged from 0.01 mg/kg bw per day in infants, adolescents, adults, elderly and very elderly per day to 0.04 mg/kg bw per day in infants. The 95th percentile of dietary exposure ranged from 0.01 mg/kg bw per day in adolescents, adults, elderly and very elderly to 0.08 mg/kg bw per day in infants (Table 30).

Mean dietary exposure to fluoride in the basic scenario ranged from 0.09 mg per day in infants to 1.35 mg per day in the elderly. The 95th percentile of dietary exposure ranged from 0.26 mg per day in infants to 2.50 mg per day in the elderly (Table 31).

TABLE 32 Mean and P95 chronic dietary exposure to fluoride for the three water fluoridation scenarios (mg/kg bw per day).

Range of mean dietary exposure (mg/kg bw per day)							
		P95 scenario		LEGAL limit 1 scenario		LEGAL limit 2 scenario	
Age group	N surveys	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Infants	11	0.02	0.07	0.03	0.13	*	*
Toddlers	15	0.03	0.06	0.04	0.12	*	*
Other children	19	0.02	0.04	0.02	0.09	*	*
Adolescents	21	0.01	0.02	0.01	0.06	0.01	0.08
Adults	22	0.01	0.02	0.02	0.05	0.02	0.07
Elderly	19	0.01	0.03	0.01	0.04	0.01	0.06
Very elderly	14	0.01	0.03	0.02	0.04	0.02	0.06
Range of P95 dietary exposure (mg/kg bw per day)							
		P95 scenario		LEGAL limit 1 scenario		LEGAL limit 2 scenario	
Age group	N surveys	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Infants	9	0.07	0.11	0.09	0.23		
Toddlers	13	0.04	0.11	0.10	0.21		
Other children	19	0.03	0.07	0.03	0.16		
Adolescents	20	0.02	0.04	0.03	0.09	0.03	0.19
Adults	22	0.01	0.04	0.03	0.09	0.03	0.17
Elderly	19	0.01	0.05	0.03	0.07	0.03	0.15
Very elderly	10	0.02	0.05	0.03	0.07	0.04	0.12

*As according to Directive 2003/40/EC, 'water containing more than 1.5 mg fluoride/L is not suitable for regular consumption by infants and children under seven years of age', the 'Water legal limit 2' scenario was assessed only for older age groups.

Mean dietary exposure to fluoride in the water fluoridation scenario using the P95 fluoride concentration in water as derived from EFSA occurrence database (700 microgram/L) ranged from 0.01 mg/kg bw in adolescents, adults, elderly and very elderly to 0.07 mg per day in infants. The 95th percentile of dietary exposure ranged from 0.01 mg/kg bw in adults and elderly to 0.11 mg/kg bw in infants and toddlers (Table 32).

Mean dietary exposure to fluoride in the water fluoridation legal limit 1 scenario using maximum fluoride concentration in tap water set by legislation (1500 microgram/L) ranged from 0.01 mg/kg bw per day in adolescents and the elderly to 0.13 mg/kg bw per day in infants. The 95th percentile of dietary exposure ranged from 0.03 mg/kg bw per day in other children, adolescents, adults, elderly and very elderly to 0.23 mg/kg bw per day in infants (Table 32).

Mean dietary exposure to fluoride in the water fluoridation legal limit 2 scenario using maximum fluoride concentration in drinking water set by legislation (1500 microgram/L) and for bottled water (5000 microgram/L) ranged from 0.01 mg/kg

bw per day in adolescents and the elderly to 0.08 mg/kg bw per day in adolescents. The 95th percentile of dietary exposure ranged from 0.03 mg/kg bw per day in adolescents, adults and the elderly to 0.19 mg/kg bw per day in adolescents (Table 32).

TABLE 33 Mean and P95 chronic dietary exposure to fluoride for the three water fluoridation scenarios (mg per day).

Range of mean dietary exposure (mg/day)							
		P95 scenario		LEGAL limit 1 scenario		LEGAL limit 2 scenario	
Age group	N surveys	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Infants	11	0.18	0.55	0.26	0.84		
Toddlers	15	0.35	0.71	0.61	1.45		
Other children	19	0.37	0.83	0.42	2.09		
Adolescents	21	0.49	1.23	0.80	2.79	0.81	4.32
Adults	22	0.56	1.75	1.30	3.50	1.32	5.47
Elderly	19	0.53	1.89	1.05	3.20	1.08	4.72
Very elderly	14	0.73	1.76	1.07	2.88	1.32	4.19
Range of P95 dietary exposure (mg/day)							
		P95 scenario		LEGAL limit 1 scenario		LEGAL limit 2 scenario	
Age group	N surveys	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Infants	9	0.59	0.78	0.81	1.52		
Toddlers	13	0.50	1.31	1.28	2.38		
Other children	19	0.52	1.55	0.63	3.51		
Adolescents	20	0.83	2.40	1.75	4.92	1.80	10.92
Adults	22	0.84	3.15	2.44	5.95	2.46	12.38
Elderly	19	0.80	3.26	2.20	5.25	2.20	10.85
Very elderly	10	1.36	2.88	1.86	4.43	2.73	9.56

Mean dietary exposure to fluoride in the water fluoridation scenario using the P95 fluoride concentration in water as derived from EFSA occurrence database (700 microgram/L) ranged from 0.18 mg per day in infants to 1.89 mg per day in the elderly. The 95th percentile of dietary exposure ranged from 0.50 mg per day in toddlers to 3.26 mg per day in the elderly (Table 33).

Mean dietary exposure to fluoride in the water fluoridation legal limit 1 scenario using maximum fluoride concentration in water set by legislation (1500 microgram/L) ranged from 0.26 mg per day in infants to 3.5 mg per day in adults. The 95th percentile of dietary exposure ranged from 0.63 mg per day in other children to 5.95 mg per day in adults (Table 33).

Mean dietary exposure to fluoride in the water fluoridation legal limit 2 scenario using maximum fluoride concentration in water set by legislation (1500 microgram/L) and for bottled water (5000 microgram/L) ranged from 0.81 mg per day in the adolescents to 5.47 mg per day in adults. The 95th percentile of dietary exposure ranged from 1.80 mg per day in the adolescents to 12.38 mg per day in adults (Table 33).

Detailed mean and P95 dietary exposure estimates for all age groups and dietary surveys in each of the four scenarios are presented in Tables C.5 (basic scenario) and C.6 (water fluoridation scenarios) in Annex C.

Fluoride intake through food and water estimated in this opinion are in the same range as the intake documented by the EFSA NDA Panel (2013) although at the time of the NDA Opinion dietary data at the individual level for the EU were not available and intakes were extracted from EU and non-EU (USA) literature referring to specific countries. Some assumptions on consumption of food and water and of fluoride concentration, in particular fluoride concentration in water used to assess the dietary exposure, can impact considerably the assessment and thus making a generic comparison difficult.

3.9.1.1 | *Main contributors*

The main contributing food categories to the dietary exposure to fluoride across the different age groups in the basic scenario were assessed among food categories at level 1 of the foodex2 classification for most of the food categories with the exception of 'Water and water-based beverages' (level 2 of the foodex2 classification) and 'Coffee, cocoa, tea and infusions' (level 3 of the foodex2 classification).

Main contributing food categories were considered those that had the highest number of surveys in which they contributed more than 10% to the total exposure in the basic scenario. They included 'Grains and grain-based products' ($n = 113$), 'Milk and dairy products' ($n = 71$), 'Tea beverages' ($n = 66$, contribution up to 63%) and 'Drinking water' ($n = 56$). 'Food products for young population' was also a main contributor for infants, contributing between 19% and 61% to the exposure across the 11 infant surveys. Table 34 shows the number of surveys in which each food category contributed more than 10% to the total exposure in the basic scenario.

TABLE 34 Number of surveys per age group in which the identified food category contributed more than 10% to the total exposure to fluoride and in brackets the percentage (%) contribution range across surveys. In brackets by each age group header, the total number of surveys for that age group.

Food	Infants (11)	Toddlers (15)	Other children (19)	Adolescents (21)	Adults (22)	Elderly (19)	Very elderly (14)	N survey > 10%
Grains and grain-based products	5 (1.9–16)	15 (13.6–29.5)	19 (16.2–31)	21 (15–33.8)	22 (11.7–27.8)	18 (9.9–28)	13 (9.6–29.5)	113
Milk and dairy products	10 (9.3–38.7)	15 (11.8–29.1)	19 (10.4–35.6)	13 (7.3–27.8)	6 (5.6–17.1)	5 (4.7–15.7)	3 (4.5–11.1)	71
Tea beverages	1 (0–19.9)	4 (0–15.7)	8 (0–29.7)	13 (0.3–34.1)	16 (2.3–47.9)	13 (5.8–58.7)	11 (1–63.2)	66
Drinking water	3 (2.9–37.8)	4 (5.2–21.3)	8 (0–21.7)	11 (3.5–22.5)	14 (4.9–24.7)	9 (3.6–24.8)	7 (2.3–15.7)	56
Food products for young population	11 (18.7–61)	5 (1.6–26.2)	(0–0)	(0–0)	(0–0)	(0–0)	(0–0)	16
Fish, seafood, amphibians, reptiles and invertebrate	(0–0)	2 (0.8–12.6)	2 (0.6–13.2)	1 (0.9–11.9)	2 (1.2–13.8)	3 (1.8–15.8)	1 (0.5–15.5)	11
Meat and meat products	(0–0)	(0–0)	1 (4.8–10)	2 (4.7–12.9)	4 (4.4–14)	1 (4.2–11.9)	3 (3.9–11.6)	11
Vegetables and vegetable products	2 (1.3–11.9)	1 (3.1–11.1)	(0–0)	(0–0)	2 (2–10.6)	2 (2.2–12.3)	2 (1.8–16.7)	9
Composite dishes	(0–0)	(0–0)	2 (0.1–29.9)	(0–0)	1 (0–12.2)	1 (0–10.8)	1 (0–11.1)	5
Coffee beverages	(0–0)	(0–0)	(0–0)	(0–0)	(0–0)	1 (0.5–11.3)	1 (0.4–11.8)	2
Water based beverages	(0–0)	(0–0)	(0–0)	1 (0.8–11)	(0–0)	(0–0)	(0–0)	1

'Grains and grain-based products', 'Milk and dairy products', 'Tea beverages' and 'Drinking water' are the food categories with the highest number of surveys that contributed more than 10% to the dietary exposure to fluoride. 'Food products for young population' (Infant and follow-on formulas) and 'Milk and dairy products' are the main contributors for infants. 'Milk and dairy products', 'Grains and grain-based products' are the main contributors for toddlers and other children. 'Grains and grain-based products', 'Milk and dairy products' and 'Tea beverages' are the main contributors for adolescents. 'Grains and grain-based products', 'Tea beverages' and 'Drinking water' are the main contributors for adults, elderly and the very elderly.

For adults, elderly and very elderly 'Tea beverages' contributed in some surveys more than 45% to the overall exposure (up to 63%). 'Food products for young population' contributed up to 61% to the exposure across the 11 surveys on infants. 'Drinking water' contributed up to 38%, 66%, 79% and 92% in the basic, water P95, legal limit 1 and legal limit 2 scenarios respectively (Annex C). Additional water contribution from cooking pasta and rice, and from food to be reconstituted and beverages that were reported in the ready-to-eat and ready-to-drink form, would increase the highest water contribution across surveys and scenarios with 1%–2%.

Looking at concentration of fluoride in tea ingredients (95,000 microgram/kg) and in water (LB = 185 microgram/L), and considering a dilution factor of 75, it can be estimated that about 15% of the exposure coming from tea beverages could be attributed to water in the basic scenario at the LB concentration of fluoride in water.

Similarly, looking at concentration of fluoride in pasta and similars (945 microgram/kg) and rice (628 microgram/kg) and water (LB = 185 microgram/L), about 20% to 29% of the exposure coming from pasta and rice could be attributed to water in the basic scenario at the LB concentration of water. Figure 8 shows the percentage contribution of the main food categories to the total exposure of fluoride for each survey for adults, toddlers and infants in the basic and the water P95 scenarios. Figure 9 provides a comparison of the contribution of the main food categories to the total exposure to fluoride, in mg/kg bw per day, for each survey for adults, toddlers and infants in the basic and the water P95 scenarios.

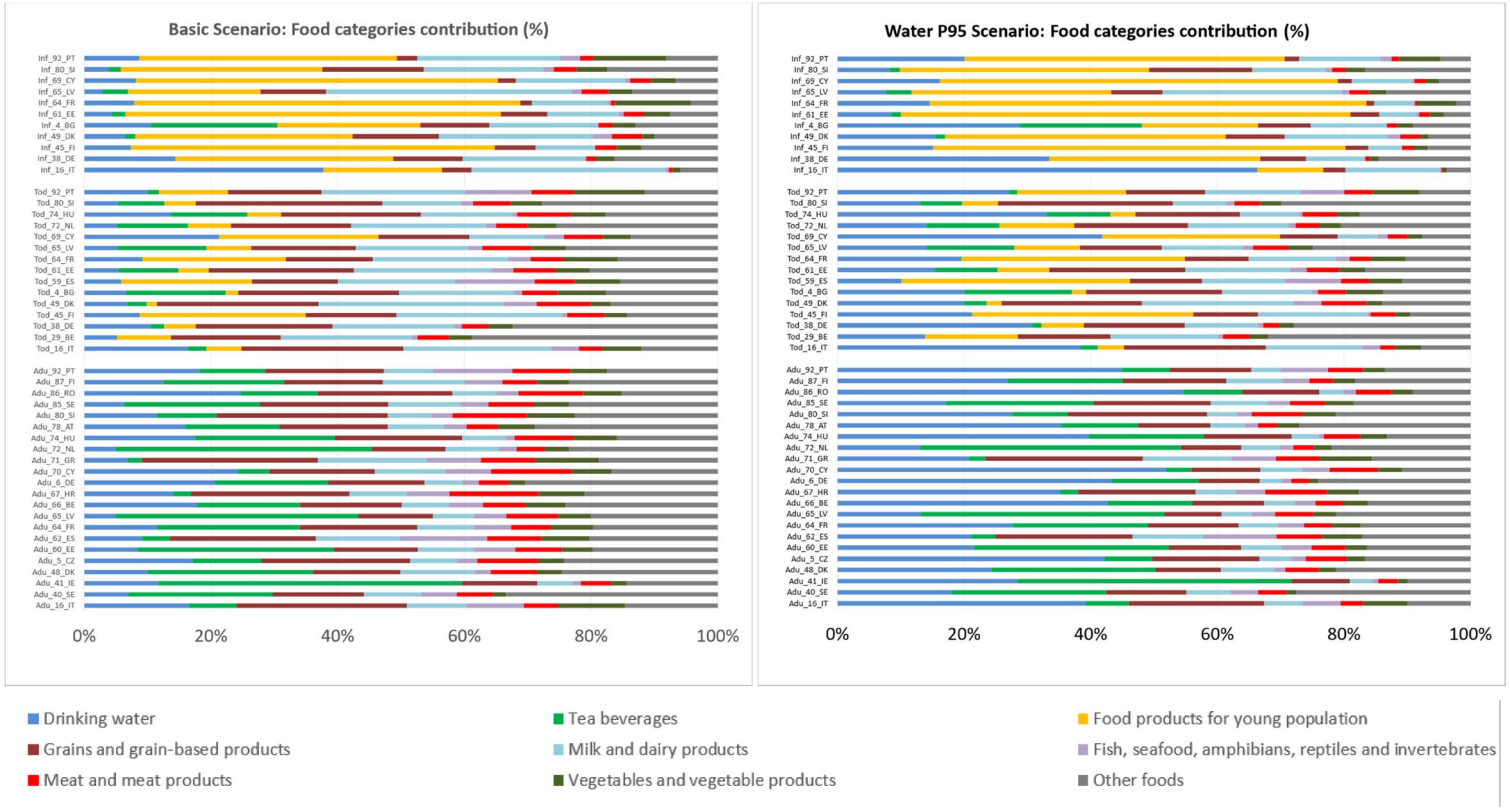

FIGURE 8 Basic scenario (left) and Water P95 scenario (right): Percentage contribution of food categories to the total exposure to fluoride for each survey for adults, toddlers and infants.

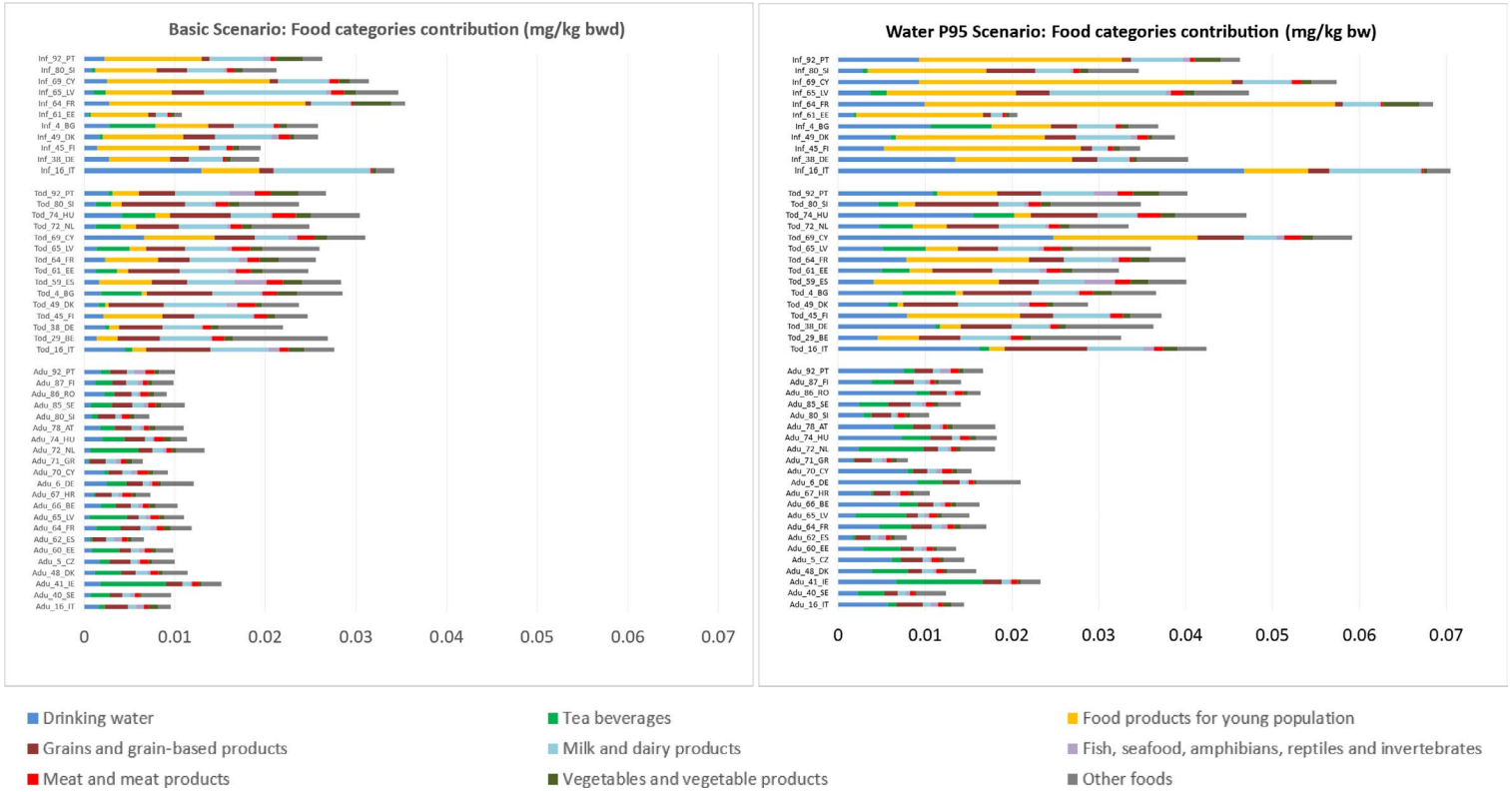

FIGURE 9 Basic scenario (left) and Water P95 scenario (right): Contribution of food categories to the total exposure to fluoride for each survey for adults, toddlers and infants (mg/kg bw per day).

Details on the contribution of these categories across age groups and surveys for the basic scenario are given in Table C.7 in Annex C.

3.9.1.2 | *Dietary exposure to fluoride through discretionary salt*

According to the NDA Panel, the amount of fluoride ingested per person per day from the use of fluoridated salt is estimated to be 0.50–0.75 mg in Germany and 1 mg in France (EFSA NDA Panel, 2013).

Using the identified concentration level of fluoride in fluoridated salt extracted from the literature (i.e. 250 mg/kg), the dietary exposure to fluoride through discretionary salt consumption added at home from average to P75 would range from 0.30 to 0.38 mg F/day in children and from 0.38 to 0.56 mg F/day in adults.

3.9.1.3 | *Dietary exposure assessment of exclusively breast-fed infants*

For the fluoride exposure assessment of infants below 6 months of age exclusively fed via breast milk, an age of 3 months was selected, assuming an average body weight of 4.8 kg, with an estimated milk average daily consumption of 800 mL per day and a high consumption of 1200 mL per day of breast milk (EFSA Scientific Committee, 2012). This was combined with the mean occurrence levels of fluoride retrieved from literature.

Concentrations of fluoride in breast milk were obtained from literature, and specifically from studies in which there is no indication that the subjects lived in high fluoride areas (less than 1 mg/L in the drinking water). The breast milk fluoride concentrations reported by Sener et al. (2007) from Turkey ranged from 3 to 11 μg/L with a mean of 6 μg/L. From another Turkish breast milk survey, Koparal et al. (2000) have reported a slightly higher fluoride concentration with a mean of 19 μg/L. Similar levels of fluoride concentrations of breast milk and colostrum with 8 μg/L have been reported by Spak et al. (1983), Faraji et al. (2014) and H. Poureslami et al. (2016) have reported from Iran a mean breast milk fluoride concentration of 2.2 μg/L and of 6 μg/L respectively. Chuckpaiwong et al. (2000) from Thailand reported a mean breast milk fluoride concentration of 17 μg/L. Dabeka et al. (1986) from Canada showed that the concentration of fluoride in breast milk is related to the fluoride content of the drinking water consumed by the mothers. For communities with 1 mg/L fluoride in the drinking water ('fluoridated communities') and with less than 0.2 mg/L fluoride in the water ('non-fluoridated communities'), mean fluoride levels in the breast milk were, respectively, 9.8 μg/L and 4.4 μg/L. Esala et al. (1982) have reported breast milk fluoride levels in Sweden of 7 μg/L and 5 μg/L for Finland and EFSA (EFSA NDA Panel, 2013) reported from a German publication a mean concentration of 3–4 μg/L from areas with low fluoride in the drinking water (< 0.2 mg/L, Bergmann et al., 1994). These EU fluoride concentrations in breast milk are consistent with the SCHER reported concentrations of fluoride in breast milk that ranged from 3.8 to 7.6 μg/L (SCHER, 2011).

Overall, it is noted that breast milk fluoride concentrations reported in literature are in line with the WHO reported levels of fluoride in breast milk that range from < 2 to 100 μg/L with most mean values from European countries being below 10 μg/L (Ekstrand et al., 1984; Esala et al., 1982; Memba et al., 2021; Poureslami et al., 2016; Spak et al., 1983; WHO/IPCS, 2002). Some EU papers were found to report considerably higher concentrations of fluoride in breast milk (around 500 μg/L in Campus et al. (2014); Pasternak et al. (1998)). The differences regarding the subjects who participated in these studies and differences in the fractions of breast milk that were analysed did not allow comparison of the results from these studies and to give a comprehensive justification of the high concentrations measured. The above inconsistencies combined with evidence suggest that there is minor transfer of fluoride from plasma to breast milk (Ekstrand et al., 1981).

Based on a reported mean concentration of fluoride in breast milk as previously described for European countries up to 9.8 μg/L rounded at 10 μg/L, the dietary exposure to fluoride ranged from 0.002 mg/kg bw per day for infants with an average milk consumption to 0.0025 mg/kg bw per day for infants with a high milk consumption.

3.9.2 | Non-dietary sources of exposure

3.9.2.1 | *Exposure assessment to fluoride from the use of toothpaste*

It is estimated that in adults less than 10% of the toothpaste is ingested whereas the estimated intake by children below 6 years old may be 40%–100% depending on the study and age group. Ellwood and Cury (2009) reported average ingestion in children of 48% in 2–3 years old, 42% in 4 years old, 34% in 5 years old and 25% in 6 years old. Cochran, Ketley, Duckworth, van Loveren, et al. (2004) reported in two studies from seven European countries that children between 1.5 and 2.5 years old ingested an estimated average of 64%–84% of the toothpaste dispensed. For the children between the ages of 2.5–3.5 years, the percentage was between 53% and 82%. For the children over 3.5 years, the percentage was between 39% and 67%. The authors noted that the percentage fluoride ingested was significantly related to age group and country and that a high percentage of children in all age groups appeared to ingest between 80% and 100% of the toothpaste dispensed (up to 70% for children age of 1.5–2.5 years and up to 40% for children age of in 2–5–> 3.5 years). For children over 6 years old, it could be reasonably assumed that the spitting response is well developed with an estimation of the toothpaste ingested at the same percentage of 10% as reported for adults (SCHER, 2011).

The mean fluoride ingested during toothbrushing, according to a mean concentration for regular toothpaste ranging from 1221 to 1399 mg F/kg, a twice daily brushing and the percentage fluoride ingested from the average amount dispensed of 0.4 g (1.5–2.5 years), 0.53 g (2.5–3.5 years) and 0.6 g (> 3.5 years), was from 0.01 to 0.03 mg F/kg bw/day in the 1.5–2.5 years, 0.01–0.04 mg F/kg bw/day in the 2.5–3.5 years and 0.01–0.03 mg F/kg bw per day in the > 3.5 years Cochran et al. (2004a). Another publication from Zohoori et al. (2012) reported similar results where the fluoride ingested by England

children group of 4–6 years was from 0.02 to 0.03 mg F/kg bw per day for an average daily amount of toothpaste dispensed of 0.7 g. Djukic-Cosic et al. (2019) also reported among 3 years old children in Belgrade, daily exposure to fluoride from the use of toothpaste ranging from 0.09 up to 0.63 mg F/day. Overall, it can be concluded that the resulting dietary average exposure to fluoride from the use of toothpaste in children would be up to 0.36 mg F/day for a 11.9 kg default body weight (EFSA Scientific Committee, 2012) for children below 3 years old and up to 0.69 mg F/day for a 23.1 kg default body weight (EFSA Scientific Committee, 2012) for children between 3 and 10 years old.

For adults, the daily exposure levels have been estimated by the Scientific Committee using the daily amounts of toothpaste applied on average from measured values published by (Hall et al., 2007) of 2.1 g per day corresponding to 30 mg/kg bw per day (average body weight of 67 kg) with a standard concentration level of 1500 mg F/kg and a factor of 10% ingestion of toothpaste after brushing and rinsing the teeth. The resulting daily average exposure to fluoride from the use of toothpaste would be 0.3 mg F/day corresponding to 0.005 mg F/kg bw per day for a 67 kg adult person. The daily amounts of toothpaste applied at the P95 daily from measured values published by Hall et al. (2007) of 3 g per day corresponding to 49 mg/kg bw per day (average body weight of 67 kg) with the standard concentration level of 1500 mg F/kg and the factor of 10% ingestion of toothpaste after brushing and rinsing the teeth. The resulting daily P95 exposure to fluoride from the use of toothpaste would be 0.45 mg F/day corresponding to 0.007 mg F/kg bw per day.

For other population groups (children < 6 years old, and children ≥ 6 years old and < 15 years old) and in absence of published data related to the P95 amount of toothpaste applied when brushing tooth, the Scientific Committee agreed to apply consistently through all population groups the ratio of 1.6 observed from B. Hall et al. (2007) for adults between the P95 and the average amount of toothpaste applied when brushing teeth during a day in order to provide reliable estimates of the P95 exposure for all other population groups based on their reported daily average amount of toothpaste applied. This ratio is consistent with the ratio of 2 generally applied by the approach taken by JECFA in absence of available reliable P95 exposure data (IPCS/WHO, 2019).

For children ≥ 6 years old and < 15 years old, in absence of available data related to the amount of toothpaste applied when brushing teeth, it is assumed by the Scientific Committee that the daily amount applied is similar to the average amount applied from measured values published by B. Hall et al. (2007) for adults of 2.1 g per day with a standard concentration of 1500 mg F/kg and a factor of 10% ingestion of toothpaste after brushing and rinsing the teeth. The resulting daily exposure to fluoride from the use of toothpaste would be on average 0.3 mg F/day corresponding to 0.007 mg F/kg bw per day for a 43.4 kg average default body weight (EFSA Scientific Committee, 2012). For the P95, the resulting daily exposure would be up to 0.48 mg F/day (average of 0.3 mg F/day multiplied by 1.6) corresponding to 0.01 mg F/kg bw per day.

For children between 1.5 and 6 years, the daily exposure levels have also been estimated by the Scientific Committee using the daily amounts of toothpaste applied on average twice daily from measured values published by Cochran et al. (2004b) and Fatemeh V. Zohoori et al. (2012) of 0.8 g per day (1.5–3 years) and 1.2 g per day (3–6 years) with a standard concentration level of 1500 mg F/kg and a conservative factor of 100% ingestion of toothpaste after brushing the teeth. The resulting daily average exposure to fluoride from the use of toothpaste is up to 0.8 mg F/day corresponding to 0.07 mg F/kg bw per day for a 11.9 kg default body weight (EFSA Scientific Committee, 2012) for children < 3 years old and up to 1.1 mg F/day corresponding to 0.05 mg F/kg bw per day for a 23.1 kg average default body weight (EFSA Scientific Committee, 2012) for children between 3 and 10 years old. For the P95, the resulting daily exposure is up to 1.3 mg F/day (average of 0.8 mg F/day multiplied by 1.6) corresponding to 0.11 mg F/kg bw per day for children below 3 years old and up to 1.8 mg F/day (average of 1.1 mg F/day multiplied by 1.6) corresponding to 0.08 mg F/kg bw per day for children between 3 and 10 years old.

For children between 6 months (first tooth) and < 1.5 years and in absence of available data for this age group, the daily exposure levels have been estimated by the Scientific Committee using the recommendations from EAPD (Toumba et al., 2019) that report twice daily brushing with an amount of toothpaste applied of 0.125 g/brushing and a standard concentration level of 1000 mg F/kg with the conservative factor of 100% ingestion of toothpaste after brushing and rinsing the teeth. The resulting daily average exposure to fluoride from the use of toothpaste is then up to 0.3 mg F/day corresponding to 0.03 mg F/kg bw per day for a 8.8 kg default body weight for this age group (EFSA Scientific Committee, 2012). For the P95, the resulting daily exposure is up to 0.48 mg F/day (average of 0.3 mg F/day multiplied by 1.6) corresponding to 0.05 mg F/kg bw per day.

Overall, when considering all the available data and the most conservative exposure approach, the Scientific Committee concluded that the daily mean exposure to fluoride from the use of toothpaste will be up to 0.3 mg F/day for children between 6 months (first tooth) and < 1.5 years, up to 0.8 mg F/day for children between 1.5 and 3 years old, up to 1.1 mg F/day for children between 3 and < 6 years old, up to 0.3 mg F/day for children between 6 and 15 years old and up to 0.3 mg F/day for adults over 15 years. At the P95, the daily exposure will be 0.5 mg F/day for children between 6 months (first tooth) and < 1.5 years, up to 1.3 mg F/day for children between 1.5 and 3 years old, up to 1.8 mg F/day for children between 3 and 6 years old, up to 0.5 mg F/day for children between 6 and 15 years old and up to 0.5 mg F/day for adults over 15 years.

Table 35 summarises the mean and the P95 daily exposure to fluoride from the use of toothpaste expressed in mg F/day and in mg F/kg bw per day across all population groups.

TABLE 35 Mean and P95 daily oral exposure to fluoride ion from the use of toothpaste across age groups. Estimates are rounded to one decimal.

mg F/day					
	6 months to < 1.5 years[a]	1.5 to < 3 years[b]	3 to < 6 years[c]	6 to < 15 years[d]	> 15 years[e]
Mean	0.3	0.8	1.1	0.3	0.3
P95[f]	0.5	1.3	1.8	0.5	0.5
mg F/kg bw per day					
	6 months to < 1.5 years[a]	1.5 to < 3 years[b]	3 to < 6 years[c]	6 to < 15 years[d]	> 15 years[e]
Mean	0.03	0.07	0.05	0.007	0.005
P95[f]	0.05	0.11	0.08	0.01	0.007

[a]Fluoride concentration of 1000 mg/kg in toothpaste, 0.125 g of toothpaste per brushing, twice daily brushing and 100% ingestion of toothpaste ingested after brushing and rinsing the teeth, default body weight of 8.8 kg (EFSA Scientific Committee, 2012).

[b]Fluoride concentration of 1500 mg/kg in toothpaste, 0.40 g of toothpaste per brushing, twice daily brushing and 100% ingestion of toothpaste ingested after brushing and rinsing the teeth, default body weight of 11.9 kg (EFSA Scientific Committee, 2012).

[c]Fluoride concentration of 1500 mg/kg in toothpaste, 0.6 g of toothpaste per brushing, twice daily brushing and 100% ingestion of toothpaste ingested after brushing and rinsing the teeth, default body weight of 23.1 kg (EFSA Scientific Committee, 2012).

[d]Fluoride concentration of 1500 mg/kg in toothpaste, 1.05 g of toothpaste per brushing, twice daily brushing and 10% ingestion of toothpaste ingested after brushing and rinsing the teeth, default body weight of 43.4 kg (EFSA Scientific Committee, 2012).

[e]Fluoride concentration of 1500 mg/kg in toothpaste, 1.05 g of toothpaste per brushing, twice daily brushing and 10% ingestion of toothpaste ingested after brushing and rinsing the teeth, body weight of 67 kg (Hall et al., 2007)

[f]A multiplicative factor of 1.6 was applied consistently to the average daily exposure through all age groups to estimate the P95 based on the ratio observed between the P95 and the average amount of toothpaste applied for adults (Hall et al., 2007).

3.9.2.2 | *Exposure assessment to fluoride from the use of other dental/oral care products*

From the reported data, it is noted that use of F-containing oral tablets of 0.25 mg per day are commonly recommended for 3–6 year olds and 0.5 mg per day for 6–16 year olds. Intakes up to 1.5 mg per day have been reported in adults and children > 12 years assessed in clinical setting to be at higher risk of caries.

Based on the WHO recommendation of use of fluoride varnish of two to four times per year and a fluoride concentration of 22,500 mg F/kg, the additional exposure coming from such application for young children (6 months–6 years olds) can be estimated to be up to 0.06 mg F/day and for adults up to 0.25 mg F/day.

3.9.3 | Total aggregated oral exposure to fluoride ion from all major sources (food and non-food sources)

Water, food, discretionary fluoridated salt and oral hygiene products (mainly toothpaste) were considered in the assessment of the total aggregated oral exposure for fluoride ion from all major sources.

Table 36 summarises the total aggregated oral exposure to fluoride ion from all major sources (water, food including discretionary fluoridated salt and dental care products) with the mean contribution ranges of major sources to total aggregated oral exposure for different exposure scenarios (basic and P95, legal limit 1, legal limit 2 water fluoridation) across age groups (min-max estimates expressed in mg F/day and/or in mg F/kg bw per day).

3.9.3.1 | *Basic scenario*

Table 36 shows that the total aggregated oral mean exposure for fluoride ion from all major sources ranged from 0.02 mg F/kg bw per day in adolescents and adults up to 0.10 mg F/kg bw per day in toddlers. At the P95, total aggregated oral exposure ranged from 0.02 mg/kg bw per day in adults up to 0.14 mg F/kg bw per day in toddlers.

Mean discretionary salt intake contributed to the total aggregated oral exposure to fluoride ions from all sources from 15% in other children to 33% in adults.

The mean contribution of dental care products to the total aggregated oral exposure to fluoride ions from all sources ranged from 15% in adults to 75% in infants and toddlers.

The contribution of dietary sources (food and drinking water) to the total aggregated oral exposure to fluoride ranged from 19% in children to 66% in adults.

3.9.3.2 | *Water fluoridation scenarios (P95, Legal limit 1 and 2)*

Table 36 shows that the total aggregated oral exposure for fluoride ion from all major sources in the three water fluoridation scenarios, ranged on average from 0.02 mg F/kg bw per day (P95 water scenario) in adults to 0.19 mg F/kg bw per day (legal limit 1 water scenario) in toddlers. At the P95, total aggregated oral exposure ranged from 0.02 (P95 water scenario mg F/kg bw per day) in adults to 0.27 mg F/kg bw per day (legal limit 2 water scenario) in adolescents.

In comparison to the basic scenario, the contribution from discretionary salt and dental care products to the total aggregated oral exposure to fluoride ions from all sources decreased following the increase of the water contribution in the three water fluoridation scenarios in all population groups. Dental care products contributed up to 70%, 60% and 21% in the P95, legal limit 1 and legal limit 2 scenarios respectively. Discretionary salt contributed up to 32%, 22% and 22% in the P95, legal limit 1 and legal limit 2 scenarios respectively. Dietary sources (food and drinking water) contributed up to 74%, 84% and 89% in the P95, legal limit 1 and legal limit 2 scenarios respectively.

TABLE 36 Total aggregated oral exposure for fluoride ion from all major identified sources (water, food including discretionary salt and dental care products) for different exposure scenarios (basic and P95, legal limit 1, legal limit 2 water fluoridation) across age groups (min–max estimates).

mg F/day										
	Infants		**Toddlers**		**Other children**		**Adolescents**		**Adults**	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
Basic scenario (a)	0.10–0.30	0.28–0.47	0.26–0.39	0.39–0.65	0.32–0.60	0.46–1.09	0.44–0.82	0.7–1.49	0.46–1.32	0.71–2.45
P95 water fluoridation scenario	0.18–0.55	0.59–0.78	0.35–0.71	0.50–1.31	0.37–0.83	0.52–1.55	0.49–1.23	0.83–2.40	0.53–1.89	0.80–3.26
Legal limit 1 water fluoridation scenario	0.26–0.84	0.81–1.52	0.61–1.45	1.28–2.38	0.42–2.09	0.63–3.51	0.8–2.79	1.75–4.92	1.05–3.50	1.86–5.95
Legal limit 2 water fluoridation scenario (b)	NC	NC	NC	NC	NC	NC	0.81–4.32	1.80–10.92	1.08–5.47	2.20–12.38
Discretionary fluoridated salt (c)	NA	NA	NA	NA	0.30	0.38	0.30	0.38	0.38	0.56
Dental care products (d)	0.3	0.5	0.8	1.3	1.1	1.8	0.3	0.5	0.3	0.5
Total aggregated oral exposure ranges for fluoride ion from all sources with basic scenario (e)	**0.40–0.60**	**0.60–0.80**	**1.06–1.19**	**1.56–1.69**	**1.72–2.00**	**2.42–2.70**	**1.04–1.42**	**1.30–2.09**	**1.14–2.00**	**1.39–3.13**
Total aggregated oral exposure ranges for fluoride ion from all sources with P95 water fluoridation scenario (e)	**0.48–0.85**	**0.89–1.08**	**1.15–1.51**	**1.65–2.11**	**1.77–2.23**	**2.47–2.93**	**1.09–1.83**	**1.43–3.00**	**1.21–2.57**	**1.48–3.94**
Total aggregated oral exposure ranges for fluoride ion from all sources with legal limit 1 water fluoridation scenario (e)	**0.56–1.14**	**1.11–1.82**	**1.41–2.25**	**1.91–3.18**	**1.82–3.49**	**2.52–4.91**	**1.40–3.39**	**2.23–5.52**	**1.73–4.18**	**2.03–6.63**
Total aggregated oral exposure ranges for fluoride ion from all sources with legal limit 2 water fluoridation scenario (e)	**NC**	**NC**	**NC**	**NC**	**NC**	**NC**	**1.41–4.92**	**2.36–11.5**	**1.76–6.15**	**2.25–13.1**

TABLE 36 (Continued)

mg F/day	Infants	Toddlers	Other children	Adolescents	Adults
Mean contribution ranges of **discretionary salt** to total aggregated oral exposure from all sources with **basic scenario** (%)	NA	NA	17.43–15.01	28.74–21.20	33.46–18.96
Mean contribution ranges of **dental care products** to total aggregated oral exposure from all sources with **basic scenario** (%)	75.31–50.18	75.35–67.37	63.91–55.05	28.74–21.20	26.42–14.97
Mean contribution ranges of **discretionary salt** to total aggregated oral exposure from all sources with **P95 water fluoridation scenario** (%)	NA	NA	16.98–13.43	27.42–16.36	31.52–14.80
Mean contribution ranges of **dental care products** to total aggregated oral exposure from all sources with **P95 water fluoridation scenario** (%)	62.95–35.20	69.36–53.15	62.27–49.23	27.42–16.36	24.89–11.69
Mean contribution ranges of **discretionary salt** to total aggregated oral exposure from all sources with **legal limit 1 water fluoridation scenario** (%)	NA	NA	16.50–8.59	21.5–8.85	22.01–9.09
Mean contribution ranges of **dental care products** to total aggregated oral exposure from all sources with **legal limit 1 water fluoridation scenario** (%)	53.25–26.39	56.71–35.58	60.49–31.48	21.5–8.85	17.38–7.18

TABLE 36 (Continued)

mg F/day	Infants		Toddlers		Other children		Adolescents		Adults	
Mean contribution ranges of **discretionary salt** to total aggregated oral exposure from all sources with **legal limit 2 water fluoridation scenario** (%)	NC		NC		NC		21.32–6.09		21.56–6.18	
Mean contribution ranges of **dental care products** to total aggregated oral exposure from all sources with **legal limit 2 water fluoridation scenario** (%)	NC		NC		NC		21.32–6.09		17.02–4.88	
mg F/kg bw per day										
	Infants		**Toddlers**		**Other children**		**Adolescents**		**Adults**	
	Mean	**P95**	**Mean**	**P95**	**Mean**	**P95**	**Mean**	**P95**	**Mean**	**P95**
Basic scenario (a)	0.01–0.04	0.03–0.08	0.02–0.03	0.03–0.06	0.02–0.03	0.02–0.05	0.01–0.02	0.01–0.03	0.01–0.02	0.01–0.03
P95 water fluoridation scenario	0.02–0.07	0.07–0.11	0.03–0.06	0.04–0.11	0.02–0.04	0.03–0.07	0.01–0.02	0.02–0.04	0.01–0.03	0.01–0.05
Legal limit 1 water fluoridation scenario	0.03–0.13	0.09–0.23	0.04–0.12	0.1–0.21	0.02–0.09	0.03–0.16	0.01–0.06	0.03–0.09	0.01–0.05	0.03–0.09
Legal limit 2 water fluoridation scenario (b)	NC	NC	NC	NC	NC	NC	0.01–0.08	0.03–0.19	0.01–0.07	0.03–0.17
Discretionary fluoridated salt (c)	NA	NA	NA	NA	0.013	0.016	0.006	0.008	0.005	0.008
Dental care products (d)	0.03	0.05	0.07	0.11	0.05	0.08	0.007	0.01	0.005	0.007
Total aggregated oral exposure ranges for fluoride ion from all sources with basic scenario (e)	0.04–0.07	0.06–0.11	0.09–0.1	0.13–0.14	0.08–0.09	0.11–0.12	0.08–0.09	0.03–0.11	0.02–0.03	0.02–0.04
Total aggregated oral exposure ranges for fluoride ion from all sources with P95 water fluoridation scenario (e)	0.05–0.1	0.1–0.14	0.1–0.13	0.14–0.17	0.08–0.1	0.11–0.13	0.08–0.1	0.04–0.12	0.02–0.04	0.02–0.06

TABLE 36 (Continued)

mg F/kg bw per day	Infants		Toddlers		Other children		Adolescents		Adults	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
Total aggregated oral exposure ranges for fluoride ion from all sources with legal limit 1 water fluoridation scenario (e)	0.06–0.16	0.12–0.26	0.11–0.19	0.15–0.28	0.08–0.16	0.11–0.23	0.09–0.13	0.05–0.17	0.02–0.06	0.03–0.1
Total aggregated oral exposure ranges for fluoride ion from all sources with legal limit 2 water fluoridation scenario (e)	NC	NC	NC	NC	NC	NC	0.09–0.16	0.06–0.27	0.02–0.08	0.03–0.18

Abbreviations: NA, not available; NC, not considered.

[a]The basic scenario does not include fluoridated bottled and tap water. Ranges refer to MB exposure estimates (Tables 16 and 17). For infants, children aged > 12 months and for adults (including elderly and very elderly age groups).

[b]According to Directive 2003/40/EC, 'water containing more than 1.5 mg fluoride/L is not suitable for regular consumption by infants and children under seven years of age', the Water legal limit 2 scenario was assessed only for older age groups, and infants, toddlers and other children were not considered in the Legal limit 2 water fluoridation scenario.

[c]High level estimates for exposure linked to discretionary salt consumption are based on P75 instead of P95.

[d]Mean and P95 daily oral exposure from the use of toothpaste reported from Table 33.

[e]The approach used for estimating high percentiles of aggregated exposure from all sources adds at country and survey level the highest high level of exposure from one source to the mean exposure values for the other oral exposure sources (EFSA, 2011; EFSA Scientific Committee, 2014).

3.9.3.3 | *Alternative approach to calculate total aggregated intake of fluoride from all sources based on study data*

It is possible to estimate total fluoride intake from urinary fluoride (uF) concentration data using either a physiologically based kinetic (PBK) model (reverse dosimetry) or a mass balance approach, as urinary fluoride concentration reflects fluoride intake from all sources. Given the limitations of existing PBK models (see Section 3.1.4), the mass balance approach was selected to estimate total fluoride intake based on uF concentrations in children and adolescents from epidemiological studies conducted in areas with drinking water fluoride (wF) concentrations of approximately 1.5 mg/L (range: 1.4–1.7 mg/L). Reported mean uF values in these studies ranged from 0.67 to 2.4 mg/L.

Total fluoride intake was estimated using the biomonitoring equivalent equation (Hays et al., 2018):

$$\text{total}F = \frac{uF \times V24}{\text{fue}}$$

Where totalF = total fluoride intake (mg/kg bw per day), uF = urinary fluoride concentration (mg/L), V24 = daily urinary flow (0.02 L/kg bw per day) and fue = urinary excretion fraction of fluoride (60%).

Using the observed range of uF concentrations, this approach estimated total fluoride intake to range from 0.022 to 0.08 mg/kg bw/day, corresponding to 1.6 to 5.6 mg/day for a 70 kg adult. This range aligns with estimated total aggregated oral exposure range for fluoride from all sources under the legal limit 1 water fluoridation scenario (1.5 mg/L) in adults, which is 1.7 to 4.2 mg/day (min-max range of mean aggregate exposure) (Table 36).

It is worth noting that this approach does not account for fluoride mobilised from bone into plasma and subsequently excreted in urine. Consequently, the estimated total intake may slightly overestimate the actual intake due to fluoride released from bone, which could lead to a underestimated risk assessment.

4 | RISK CHARACTERISATION

The ULs based on dental fluorosis of 1.0 mg/day for infants < 1 year, 1.6 mg/day for toddlers 1–3 years and 2.0 mg/day for 4–8-year old children, and the safe level of intake based on CNS of 3.3 mg/day for adults and all age groups > 8 years were compared to the estimated aggregated exposures in these populations.

The aggregated mean exposure range in infants (< 1 year) under the basic water fluoride scenario (0.4–0.6 mg/day), as well as the mean exposure range (0.48–0.85 mg/day) of the P95 water fluoride scenario are below the UL for infants. The P95 range of exposure under the basic water scenario (0.6–0.8 mg/day) is below the UL for infants < 1 year, while the top

of the P95 range of exposure under the P95 water fluoride scenario (0.89–1.08 mg/day) slightly exceeds the UL for infants < 1 year. The main contributing source to these exposures is dental care products (35 to 75% on average and up to 83% at the P95), assuming 100% ingestion.

The aggregated mean exposure range in toddlers (1–3 years) under the basic water fluoride scenario (1.06–1.19 mg/day), as well as the mean exposure range (1.15–1.51 mg/day) of the P95 water fluoride scenario are below the UL for toddlers. The P95 range of exposure under the basic water scenario (1.56–1.69 mg/day) reaches and slightly exceeds the UL and the P95 range of exposure under the P95 water fluoride scenario (1.65–2.11 mg/day) results in 28.5% likely exceedance of the UL. The main contributing source to these exposures is dental care products (53 to 69% on average and up to 80% at the P95), assuming 100% ingestion.

The aggregated mean exposure range in children (4–8 years) under the basic water fluoride scenario (1.72–2.00 mg/day), as well as the mean exposure range of the P95 water fluoride scenario (1.77–2.23 mg/day) reaches and slightly exceeds, respectively, the UL for children 4–8 years. The P95 range of exposure under the basic water scenario (2.42–2.7 mg/day) and the P95 range of exposure under the P95 water fluoride scenario (2.47–2.93 mg/day) result in 18% likely and 69% likely exceedance of the UL for this age group, respectively. The main contributing source to these exposures is dental care products (49 to 62% on average and up to 75% at the P95), assuming 100% ingestion (< 6 years).

The aggregated mean (1.04–2.00 mg/day) and P95 (1.30–3.13 mg/day) exposure ranges in adults and children > 8 years under the basic water fluoride scenario, as well as the mean exposure range (1.09–2.57 mg/day) of the P95 water fluoride scenario are below the safe level of intake for adults and groups > 8 years. The P95 range of exposure (1.43–3.94 mg/day) of the P95 water fluoride scenario is below the safe level of intake for adults and children > 8 years, although the upper end of this range results in exceedance of the safe level of intake in adults. The main contributing sources to these exposures are diet (food plus water fluoridation from 44% to 74% in average and up to 84% at the P95), discretionary fluoridated salt (15%–31% in average) and dental care products (12%–25% in average), assuming 10% ingestion.

The Scientific Committee concluded that mild dental fluorosis may occur in infants exposed to fluoride at the P95 range of the P95 water scenario and in toddlers exposed to fluoride at the P95 range of the basic and the P95 water scenario, including a conservative assumption of 100% ingestion of dental care product.

For children 4–8 years, the exposure exceedances may result in mild dental fluorosis in the molar teeth which develop during this period, including a conservative assumption of 100% ingestion of dental care product by children < 6 years.

For adults the safe level of intake is exceeded at high levels of exposure associated with high contributions from all the following sources combined in descending order: drinking water, diet, fluoridated discretionary salt and ingested dental care products.

The mean and P95 exposure estimated on the basis of the legal limit 1 for water fluoridation exceeds the UL established for infants, toddlers and children 4–8 years and the safe intake level for adults and age groups > 8 years. Such exceedance occurs because the RPs (1.4 or 1.5 mg/L, respectively) derived for estimating these HBGVs were based on fluoride concentrations in drinking water which correspond to the current legal limit for drinking water.

The Scientific Committee concluded that the risk for adverse effects in the CNS from fluoride exposure is related to ingested fluoride from any source. Risk from exceedance of the safe level of intake does not apply to use of fluoridated dental care products if not ingested. This assessment includes a conservative assumption of 100% ingestion of dental care product by children < 6 years.

Although the beneficial effects of fluoride are out of scope of this mandate, the Scientific Committee noted that the most effective protection against dental caries is obtained from topical applications of fluoride (see Section 3.2.6).

5 | UNCERTAINTY ANALYSIS

Uncertainty analysis for this opinion was conducted according to the EFSA Guidance (EFSA Scientific Committee et al., 2018a, 2018b) in order to identify and assess the overall impact of all identified uncertainties on the main conclusions of the assessment.

In a first step, expert judgements were used to assess the uncertainty of the hazard assessment and the exposure assessment for fluoride separately. A semi-formal expert knowledge elicitation (EKE) protocol was applied to discuss and assess the sources and impact of major uncertainties, as well as to quantify the overall uncertainties of the exposure and hazard estimates. In a second step, the uncertainties resulting from hazard and exposure assessments were combined to assess the uncertainties in the risk characterisation and the final conclusions. The detailed EKE results on the uncertainty of the risk assessment of fluoride are provided in Annex F.

5.1 | Uncertainty of Hazard assessment

The purpose of the analysis was to assess the overall uncertainty of the HBGVs established in this opinion, such as ULs of 1.0 mg/day for infants < 1 year, 1.6 mg/day for toddlers 1–3 years and 2 mg/day for children 4–8 years and the safe level of intake of 3.3 mg/day for adults and children > 8 years.

The overall uncertainty was assessed based on uncertainties identified for each line of evidence within the weight of evidence approach (Sections 3.5.1–3.5.4). The total intake associated with the reported potential effects for each line of

evidence was considered a 'potential HBGV' that would have resulted from each prioritised endpoint if selected as critical endpoint. 'Potential HBGVs' were calculated for relevant lines of evidence for each age group based on the endpoints considered relevant, including neurodevelopment, thyroid function and bone health for adults and for children and additionally dental fluorosis for children (not relevant for adults). Bone cancer was not included as no association with fluoride emerged in the weight of evidence assessment (see Sections 3.5.1–3.5.4). The calculations for adults are shown in Table 26 (Section 3.8). For children, the calculations of total intake are shown separately for children 1–3 years and 4–8 years in Table 25 (Section 3.8) for dental fluorosis and F.1.2 - F.1.3 (Annex F.1) for all other lines of evidence. For infants, calculations of total intake are presented in Table 25 (Section 3.8) for the lines of evidence of neurodevelopment and dental fluorosis. It was noted that, while 'potential HBGVs' were calculated from all lines of evidence for children for the purpose of the uncertainty analysis, the endpoint of dental fluorosis received the highest weight toward establishing UL for children (see Section 3.8 and Tables F.1.2 and F.1.3 in Annex F.1). The experts considered that the uncertainties in the evidence for children were also applicable to infants and consideration of all other lines of evidence for infants was superfluous. The total intakes in units of mg/kg bw per day were used in the uncertainty analysis for comparability between human and animal sources. The human equivalent dose from animal studies was estimated using the deterministic default regulatory assessment factor of 100.

The relative contribution of each line of evidence to the uncertainty around the established HBGVs for children and adults was weighed based on expert judgement of the panel of experts in the EKE session for uncertainty of hazard assessment. The uncertainty assessment of the HBGVs was conducted using the Roulette Method and individual weights for the lines of evidence as described below. The final output was obtained by fitting a distribution to the composite of the judgements of the experts.

In a first stage, the experts were presented with the 'potential HBGVs' (total intakes) from each line of evidence and were asked to weigh these for establishing HBGVs for children and adults, taking into account the identification of the most sensitive endpoint, the limitations of the study type and the quality of the full body of evidence used. The weights were to represent the expert's judgement of the confidence in, priority and relative contribution of a line of evidence for establishing a HBGV. Capitalising on the reasoning developed in the Weight of Evidence sections (3.5.1–3.5.2) for the identified strengths and limitations of the evidence the following question was posed to the experts:

> '*Based on the overall strength, limitations, reliability, consistency and relevance of each line of evidence assign to each a weight as a basis for establishing a HBGV*'.

The lower the attributed weight, the lower the confidence an expert had for using the respective line of evidence as a basis for a HBGV and the lower its impact on the risk assessment. The median weights and minimum–maximum range assigned to each line of evidence by the experts who participated in the EKE session are shown in Tables F.1.1–F.1.4 in Annex F.1. A visual representation of the range of the weights provided by the individual experts was developed to illustrate the prioritisation for the evidence available on hazard for adults and children as shown in Annex F, Figures F.1 and F.2.

In a second stage, the experts were asked to describe the uncertainty within each line of evidence for each age group, in the form of a range around the respective 'potential HBGV' using the Roulette method. The uncertainty of human dose estimates derived from animal data included the standard default uncertainty factors. This exercise was performed separately for evidence for adults, children 1–3 years and children 4–8 years. The experts were asked the following question:

> '*What is the uncertainty distribution of the "potential HBGV" derived from each line of evidence?*'

The higher the uncertainty in the line of evidence the wider the range of 'potential HBGVs' on either or both directions of the calculated value presented.

Finally, the individual weights and uncertainty distributions per line of evidence assigned by each expert were used to assess the uncertainty from all lines of evidence by weighted superposition. The experts were asked to review the outcome of the calculation to express their overall uncertainty:

> *'Considering the weight given to each line of evidence, what is the overall uncertainty of the HBGV for adults/children derived from all relevant lines of evidence?'*

A distribution of likely HBGVs (UL or safe level of intake) was obtained from each expert for each age group. The individually weighted total uncertainties of the UL and safe level of intake were reviewed and discussed. It was concluded that differences between the experts were minor, and no revision of answers was needed. The final distribution for the respective HBGV for each age group (UL or safe level of intake) was obtained by fitting a parametric distribution to the average of the individual answers, as shown in Annex F (Figures F.1.3–F.1.5).

It was estimated that the median safe level of intake for adults is 3.6 mg/day (0.051 mg/kg bw per day), with a 90% certainty interval of 2.5 to 5.1 mg/day (0.036–0.072 mg/kg bw per day) (Table 37 and Figure F.1.3).

It was estimated that the median UL for toddlers of 1–3 years is 1.5 mg/day (0.124 mg/kg bw per day), with a 90% certainty interval of 0.9 to 2.4 mg/day (0.077–0.199 mg/kg bw per day) (Table 37 and Figure F.1.4).

It was estimated that the median UL for children 4–8 years is 2.1 mg/day (0.107 mg/kg bw per day), with a 90% certainty interval between 1.4 and 3.2 mg/day (0.072–0.159 mg/kg bw per day) (Table 37 and Figure F.1.5).

The different UL values result from calculations of total intake (Table 27 and Tables F.1.2 and F.1.3). Hence, the 90% ranges of certainty around the ULs are similar for children 1–3 years and for 4–8 years. Rather than repeating the Roulette method exercise for infants, the experts were asked whether the range of 90% certainty around the UL for infants is likely to be substantially different (wider or narrower) than that of children. The experts concluded that the 90% certainty range that resulted from expert judgement around the ULs for children 1–3 years was considered applicable to the UL for infants (see Table 25 in Section 3.8 and Table F.1.4 in Annex F.1).

It was estimated that the median UL for infants < 1 year is 0.9 mg/day (0.124 mg/kg bw per day), based on the younger age group of < 6 months, with a 90% certainty interval between 0.6 and 1.5 mg/day (0.077–0.199 mg/kg bw per day) (Table 37).

The uncertainty of human dose estimates derived from animal data was not considered within this EKE. Instead, a standard extrapolation factor was used. A separate characterisation using a refined, data-based probabilistic approach is described in Appendix D.3. It informs on relative contributions of uncertainty from BMD modelling, from animal to human data extrapolation and from human variability modelling, indicating that uncertainty from human variability appears to be the main source. Moreover, the uncertainties from the use of animal data versus human data are quantitatively contextualised and visualised.

TABLE 37 Uncertainty ranges of UL for infants and children and safe level of intake for adults and children >8 years.

		Uncertainty range (mg/day)		
	HBGV (mg/day)	5%	Median (50%)	95%
Infants (< 1 year)	1	0.6	0.9	1.5
Toddlers (1–3 years)	1.6	0.9	1.5	2.4
Other Children (4–8 years)	2	1.4	2.1	3.2
Adults (> 18 years); > 8 years	3.3	2.5	3.6	5.1

5.2 | Uncertainty of exposure assessment

The purpose of the analysis was to assess the uncertainty of aggregated exposure to fluoride in the EU population by age group based on the 'basic' water scenario (see Table 34 in Section 3.9.3.1). This scenario is representative of exposure of the average person in the EU population (including a fraction of individuals living in fluoridated water areas). Exposure estimates based on the three fluoridation scenarios (P95, legal limit 1 and legal limit 2) represent EU sub-populations living in areas where water is fluoridated and are not representative of the general EU population, therefore not included in the uncertainty analysis. The uncertainty of exposure assessment was evaluated for each major exposure source separately, such as drinking water, food (without water), discretionary fluoridated salt and fluoridated dental care products (see Section 3.9.3) and for the age group where it was considered relevant.

Toddlers was the age group with the highest estimated mean aggregated exposure (toddlers, 12–36 months: 0.09–0.1 mg F/kg bw per day)[76] and was assessed for all sources, while uncertainty factors were extrapolated from one age group to another where considered applicable (see Annex F.2).

Separate assessments were done for the different exposure pathways using the Quartile Method. The total intake was finally calculated as sum of the pathways via Monte-Carlo-Simulation assuming no correlation.

The EKE session was focused on assessing potential uncertainties of **the mean** exposure estimate in each of these sources of exposure (using the 'median survey' point estimates). For this purpose, the exposure contribution of 'Diet' (food + drinking water) was separated into food and drinking water as shown in Table 38 for toddlers (together with discretionary salt and dental care products). See also Appendix F for the respective data on food and drinking water for adults, toddlers 1–3 years, children 4–8 years and infants (Tables F.1–F.4).

TABLE 38 Fluoride intake in toddlers (mg/kg bw per day).

	Mean			P95		
Source	Min survey	Median survey	Max survey	Min survey	Median survey	Max survey
Diet (including drinking water of Basic scenario and food)	0.0220	0.0260	0.0310			
Drinking water (Basic scenario)	0.00126	**0.00187**	0.00659	0.00305	0.00409	0.01690
Food (excluding drinking water)	0.0197	**0.0236**	0.0267			
Discretionary salt		**NA**			NA	
Dental care products		**0.07**			0.11	

[76]The assessment of toddlers relies on consumption surveys of 15 MS: Belgium, Bulgaria, Cyprus, Denmark, Estonia, France, Finland, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia and Spain.

The uncertainty evaluation for exposure through food considered that the data obtained from the EFSA food composition database represent free fluoride concentrations and not total fluoride, based on a comparison of the concentrations in the main food contributors with reported values in the scientific literature obtained with appropriate analytical methodology (measuring free fluoride). The composition data are not left-censored. The main uncertainties were related to analytical limitations in fluoride quantification in some matrices where fluoride is adsorbed or aggregated on proteins, such as milk and dairy products, leading to possible underestimation (factor reported as 1 to 1.5 in Spano et al. (2023)), or in matrices where the presence of other factors lead to possible overestimation of fluoride, such as interference with IC measurements in tea (factor reported as 0.1 to 1 in Janiszewska and Balcerzak (2013); Zhou et al. (2018)) (see Annex F.2.1).

The WG estimated that the plausible range of the multiplicative factor of the mean toddler intake from food was 0.87–1.19 with 90% certainty, while the median estimate of the true intake from food remained nearly unchanged (factor of 1.04). The same multiplicative factor was considered applicable to 'Other children' (4–8 years) and adult groups based on the assumption of similar food composition. The uncertainty of exposure from food was assessed separately for infants based on the contribution of milk as the predominant or exclusive food source. The range of the multiplicative factor of the mean infant intake from food was 0.92–1.38 with 90% certainty, while the median estimate of the true intake from food indicated slight underestimation (factor of 1.15).

The uncertainty evaluation for exposure through drinking water considered that the analytical measurement of fluoride in drinking water is performed according to the regulatory standards. Concentration data of fluoride ions in drinking water were available from 8 MS.[77] Exposure may be underestimated if fluoride water levels are higher in MS who did not submit data or MS with water fluoridation programmes are under-represented. Information on water fluoridation in EU MS indicated that it is implemented in Ireland (population coverage 74%), Spain (3%) and Portugal (1%). Similarly, exposure through drinking water may be overestimated if fluoride water levels are lower in MS who did not submit data (see Annex F.2.2).

The WG estimated that the plausible range of the multiplicative factor of the mean intake of toddlers from drinking water was 0.83–1.38 with 90% certainty, with a median factor of 1.1. The same multiplicative factor was considered applicable to all other age groups based on the absence of differences in uncertainties for drinking water as a source of exposure.

The uncertainty evaluation for exposure through fluoridated discretionary salt considered that the diet of toddlers may include foods containing fluoridated discretionary salt similar to the food of 'Other children' or lower. The dietary average to P75 exposures to fluoride in children and adults through discretionary salt consumption added at home range between 0.30 to 0.38 mg/day and 0.38 to 0.56 mg/day, respectively. The calculation of the multiplicative factor was not possible due to the missing intake from this source for toddlers in the current exposure assessment (Table 35). The WG considered that the EU average consumption of discretionary salt is higher in some MS and lower in other, that the market share of fluoridated salt may be increased or decreased since the time of available consumption data (2006) and that there may be a tendency for avoidance of discretionary salt, particularly fluoridated, in toddler diets in the EU (see Annex F.2.3).

Therefore, the WG estimated that the plausible range of the mean fluoride intake in toddlers[78] from fluoridated discretionary salt is between 0.10 and 0.28 mg/day with 90% certainty, with a median estimate of the true intake from discretionary salt of 0.16 mg/day.

Fluoride intake from discretionary salt was not considered relevant to infants based on the assumption that infants don't consume discretionary salt. For adults, the plausible range of the mean fluoride intake from fluoridated discretionary salt was assessed with a multiplicative factor range of 0.34–1.90 with 90% certainty, and a median estimate of 1.03. The uncertainty factor derived for adults was also considered applicable to 'Other children' (4–8 years old).

The uncertainty evaluation for exposure through toothpaste considered that the average amount of toothpaste per application is either higher or lower, usage of fluoridated toothpaste by toddlers may be higher or lower than recommended and that ingestion may be lower than 100% which is assumed in the exposure assessment (see Annex F.2.4). The WG estimated that the plausible range of the multiplicative factor of the mean intake from toothpaste was 0.32–0.96 with 90% certainty, with a median factor of 0.61.

The uncertainty factor derived for toddlers was also considered applicable to infants. For adults, the plausible range of the multiplicative factor of the mean intake from toothpaste was 0.52–1.44 with 90% certainty, with a median factor of 0.92. The uncertainty factor derived for adults was also considered applicable to 'Other children' (4–8 years).

Taken together the plausible range of the overall uncertainty multiplicative factor for aggregated exposure of toddlers through four sources is 0.88 with a 90% range between 0.65 and 1.15 (Table 39 and Annex F.2.5). The overall ranges of intake from each source for toddlers are shown in Annex Figure F.2.5.1 and their relative contribution in Figure F.2.5.2.

[77] Belgium, Germany, Malta, Italy, Spain, Hungary, Ireland, Slovakia.

[78] Based on 12 kg average body weight for toddlers.

TABLE 39 Summary outcome of EKE for uncertainty of exposure assessment.

	Current assessment estimated intake (mg/day)		Elicited range of uncertainty assessment for the mean consumer (mg/day)	
	Mean consumer[a]	P95 consumer	Median	90% Certainty range
Food without water				
Infants (< 1 year)	0.17	[b]	0.22	0.18–0.27
Toddlers (1–3 years)	0.28		0.30	0.25–0.35
Other children (4–8 years)	0.35		0.37	0.31–0.43
Adults (> 18 years); > 8 years	0.61		0.67	0.55–0.76
Drinking water				
Infants (0–12 months)	0.02	0.07	0.02	0.015–0.025
Toddlers (1–3 years)	0.02	0.05	0.027	0.020–0.034
Other children (4–8 years)	0.03	0.09	0.04	0.03–0.05
Adults (> 18 years); > 8 years	0.09	0.25	0.11	0.08–0.14
Discretionary salt				
Infants (< 1 year)	0	0	0	0
Toddlers (1–3 years)	0	0	0.16	0.10–0.28
Other children (4–8 years)	0.30	0.38	0.31	0.10–0.57
Adults (> 18 years); > 8 years	0.38	0.56	0.39	0.13–0.72
Dental care products				
Infants (< 1 year)	0.22	0.37	0.08	0.04–0.13
Toddlers (1–3 years)	0.83	1.31	0.32	0.17–0.50
Other children (4–8 years)	1.00	1.60	0.87	0.50–1.36
Adults (> 18 years); > 8 years	0.35	0.49	0.31	0.17–0.48

[a]Median value of the available food consumption surveys.

[b]Mean intake via 'food without water' is calculated by intake via food minus intake via water. (Not valid for the calculation of the P95 intake).

5.3 | Uncertainty of risk assessment

The overall uncertainty in the assessment of risk from fluoride intake in each age group was based on the combined uncertainties related to total oral intake (exposure uncertainty distribution) and the respective HBGVs for each age group (UL or safe level of intake) (Annex F.3). The uncertainty ranges of the HBGVs for each age group are presented in Table 37. The uncertainty distributions of total intakes for each age group are presented in Table 40 (the sum of all oral sources shown in Table 39).

The uncertainty around the level of risk was defined as the 'likelihood of concern' and was calculated as a probability that the range of mean total intake (representing the average consumer) exceeds the respective HBGV as established in Section 3.8 (Table 40 and Annex Table F.3.3). Likelihood of concern was also estimated based on exceedance of the median HBGV resulting from the uncertainty of the hazard assessment in Section 5.1.

It was estimated that there is a 18% likelihood of exceedance of the UL for toddlers by the mean total intake of other children (4–8 years) (or 82% likely not exceeded). There is no concern of exceedances of the HBGVs for the other age groups by the mean total intake ranges (100% likely not exceeded). When comparing the mean total intake with the median HBGV for each age group, the likelihood of no concern was 99.7%, 98%, 80.8% and 99.9%, for infants, toddlers, other children and adults, respectively (Table 40 and Annex Table F.3.3).

Likelihood of concern was also estimated as the probability of the 95% total intake exceedance of the range of HBGVs (see Annex Table F.3.4). It was estimated that the established ULs for infants (< 1 years), toddlers (1–3 years), other children (4–8 years) and safe level of intake for adults (and children > 8 years) were 100%, 71.5%, 30.7% and 100% likely not exceeded. There is 28.5% and 69.3% likelihood that the UL for toddlers (1–3 years) and other children (4–8 years) is exceeded by the 95% of total intake. It was estimated that the median HBGVs for infants, toddlers, other children and adults were 95.1%, 56.5%, 45% and 97.7% likely not exceeded. There is 43.5% and 55% likelihood, respectively, that the median ULs for toddlers (1–3 years) and other children (4–8 years) are exceeded by the 95% of total intake (Annex Table F.3.4).

TABLE 40 Uncertainty of risk from fluoride intake based on the mean of the basic drinking water scenario.

		Uncertainty range of mean total intake (mg/day)[b]				Exceedance of the established HBGV			Exceedance of the median HBGV	
	Estimated mean total intake (mg/day)[a]	5%	Median (50%)	95%	Established HBGV (mg/day)[c]	Likelihood of concern (%)	Likelihood of no concern (%)	Median HBGV (mg/day)[d]	Likelihood of concern (%)	Likelihood of no concern (%)
Infants (< 1 year)	0.40	0.27	0.35	0.44	1.0	0.0%	100.0%	0.9	0.3%	99.7%
Toddlers (1–3 years)	1.14	0.73	1.00	1.31	1.6	0.0%	100.0%	1.5	11.0%	89.0%
Other children (4–8 years)	1.68	1.14	1.65	2.22	2.0	18.1%	82.0%	2.1	19.2%	80.8%
Adults (> 18 years) and children > 8 years	1.43	1.12	1.47	1.85	3.3	0.0%	100.0%	3.6	0.1%	99.9%

[a]Sum of median survey of mean basic drinking water scenario and mean of other oral sources, as described in Section 3.9.3.

[b]As estimated in Section 5.2.

[c]As established in Section 3.8.

[d]As estimated in Section 5.1.

6 | CONCLUSIONS

The Scientific Committee concluded the following:

Chemistry and analytical methods

- In this opinion, the term 'fluoride' refers to non-organic (i.e. non-covalently bound fluorine.
- A variety of analytical methods have been used to detect and quantify fluoride, most commonly ISE and IC. Sample preparation and measurement interpretation are critical to ensure accurate fluoride quantitation.

Fluoride kinetics

- Soluble fluoride salts (e.g. sodium fluoride) are rapidly and almost fully absorbed (up to 90% in rats). Calcium, aluminium and magnesium in foods can reduce fluoride absorption due to complex formation.
- Approximately 99% of the body's fluoride is stored in bone, where it can be slowly released during bone growth, resorption or remodelling.
- The low brain-to-plasma ratio and minimal fluoride presence in the brain indicate a relatively impermeable blood–brain barrier.
- In pregnant women, fluoride crosses the placenta and reaches the fetus, with fetal cord blood levels at ~60% of maternal blood concentrations. In breast milk it reaches typically 30%–40% of maternal blood concentrations.
- Fluoride is eliminated from blood via bone uptake and renal excretion in a biphasic manner, with 36% and 55% of absorbed fluoride deposited in calcified tissues in adults and children, respectively.
- Renal clearance varies by age with about 60% of absorbed fluoride excreted in the urine in adults and 45% in children.

Hazard assessment

- The effects of fluoride on dental fluorosis and skeletal fluorosis are well established and did not require further systematic literature review.
- Instead, new and emerging evidence on effects on the CNS, thyroid function and bone were prioritised for assessment.
- Based on all the available evidence, dental fluorosis remains the most sensitive adverse health effect associated with fluoride exposure in children < 8 years.
- For the CNS, evidence from human and animal studies indicates that there are adverse effects associated with exposures to fluoride in utero and/or early life, making pregnant women and infants the most vulnerable populations for exposure.
- There is evidence that adverse effects in the CNS may occur at fluoride concentrations in drinking water above 1.5 mg/L.
- The evidence of adverse effects in the CNS at fluoride concentrations in drinking water below 1.5 mg/L is inconclusive.
- A similar conclusion was reached for effects on thyroid function; however, the evidence of effects on the thyroid was less robust.
- The data on bone health assessed in this opinion (including fractures and bone mineral density) suggest that effects on bone may occur at higher fluoride exposures than those reported for CNS effects.

Hazard characterisation

- Based on benchmark dose modelling of dental fluorosis data, a RP of 1.4 mg/L was derived.
- A tolerable upper intake level for infants (< 1 year), toddlers (1–3 years) and children (4–8 years) was established of 1.0 mg/day, 1.6 mg/day and 2.0 mg/day, respectively, based on dental fluorosis.
- The current evidence does not allow derivation of a quantitative RP for effects on the CNS. However, based on expert judgement, a RP of 1.5 mg/L was derived based on the weight of evidence for effects on CNS. Despite some uncertainties around this value, there is reasonable confidence that it is protective for adverse effects of fluoride.
- Despite lack of quantitative RP for CNS effects, a safe level of maternal intake could be determined, based on the overall weight of evidence and expert judgement, to limit exposure of the fetus via the placenta and infants via breastfeeding.
- A safe level of intake of 3.3 mg/day was established, based on the RP of 1.5 mg/L, applicable to all adults and children ≥8 years.

Exposure assessment

- Aggregated oral intake of fluoride by age group is based on fluoride from major sources, including food, drinking water, fluoridated discretionary salt and ingested dental care products (100% ingestion by children < 6 years).
- Food products for young population contributed between 19 and 61% of the total intake for infants above 6 months. For infants below 6 months, intake was based on exclusively breast-fed infants.
- Intake from drinking water is based on data submitted to EFSA of current drinking water fluoride concentrations in European countries representing naturally occurring fluoride in drinking water or water fluoridation. Based on data submitted to EFSA, > 86% of samples contain < 0.3 mg/L fluoride and > 97% contain < 0.7 mg/L fluoride.

- Intake from drinking water was also assessed according to scenarios assuming water fluoridation up to the legal limits of 1.5 mg/L (tap or bottled) or 5 mg/L (bottled). These scenarios indicate the highest potential intakes.
- Contribution to the total aggregated oral exposure to fluoride from dietary sources (food and drinking water) ranged from 19% in children to 66% in adults, discretionary salt from 15% in other children to 33% in adults, and ingested dental care products from 15% in adults to 75% in infants and toddlers.
- In the water fluoridation scenarios, the higher contribution of water results in higher contribution of dietary sources overall by up to 74%–89% and lower contribution of the other sources.

Uncertainty analysis

- A source of uncertainty in the established ULs and safe level of intake is the calculation of total intake from the water concentration that impact the estimation of the safe level. Based on expert knowledge elicitation:
- The UL for infants < 1 year of 1.0 mg/day has a 90% certainty interval of 0.6 to 1.5 mg/day.
- The UL for toddlers 1–3 years of 1.6 mg/day has a 90% certainty interval of 0.9 to 2.4 mg/day.
- The UL for children 4–8 years is 2.0 mg/day has a 90% certainty interval of 1.4 to 3.2 mg/day.
- The safe level of intake for adults and children > 8 years of 3.3 mg/day has a 90% certainty interval of 2.5 to 5.1 mg/day.
- Uncertainties in the exposure assessment were related to analytical methods for food composition data, amount of fluoridated salt consumed, amount of toothpaste used and ingestion of product.
- The 90% certainty range for the aggregated exposure for infants through drinking water, food (excluding drinking water), fluoridated salt and dental care products can be 34% below to 8% above the estimated value, with a tendency of overestimating the exposure by 14% (median).
- The 90% certainty range for the aggregated exposure for toddlers through drinking water, food (excluding drinking water), fluoridated salt and dental care products can be 36% below to 15% above the estimated value, with a tendency of overestimating the exposure by 12% (median).
- The 90% certainty range for the aggregated exposure for children 4–8 years through drinking water, food (excluding drinking water), fluoridated salt and dental care products can be 32% below to 32% above the estimated value, with a tendency of overestimating the exposure by 2% (median).
- The 90% certainty range for the aggregated exposure for adults (and children > 8 years) through drinking water, food (excluding drinking water), fluoridated salt and dental care products can be 22% below to 29% above the estimated value, with a tendency of underestimating the exposure by 2% (median).

Risk characterisation

- The estimated mean and P95 intakes of fluoride based on typical drinking water concentrations in Europe (basic water scenario) do not exceed the established HBGVs, except for the top of the P95 range that exceeds the UL for toddlers (slightly) and children 4–8 years.
- Mild dental fluorosis may occur in toddlers and children 4-8 years (molar teeth) exposed to fluoride at the top of the P95 range of the basic water scenario and in infants, toddlers and children 4-8 years (molar teeth) at the P95 range of the P95 water scenario, assuming 100% ingestion of fluoridated dental care products.
- For adults, the safe level of intake may be exceeded at high levels of exposure associated with high contributions from all the following sources combined in descending order: drinking water, food, fluoridated discretionary salt and dental care products.
- The mean and P95 exposure estimated on the basis of the legal limit for water fluoridation exceeds the UL established for infants, toddlers and children 4–8 years and the safe intake level for adults and age groups > 8 years. Such exceedance occurs because the RPs (1.4 or 1.5 mg/L, respectively) derived for estimating these HBGVs were based on fluoride concentrations in drinking water which correspond to the current legal limit for drinking water. As such the current legal limit for fluoride in drinking water is not sufficiently protective.

7 | RECOMMENDATIONS

The Scientific Committee identified the following recommendations.

- Additional evidence to be generated to determine the biological activity of fluoride on molecular and cellular targets in order to support mode(s) of action of fluoride relevant to the endpoints assessed and assist in the interpretation of the biological relevance of the effects reported in human and animal studies.
- Additional well-conducted prospective studies at exposures lower than those associated with a drinking water concentration of 1.5 mg/L to strengthen the current evidence of adverse effects in the CNS during pre- and postnatal development.
- Studies designed to evaluate the kinetics of fluoride in different age groups, including disposition to and from the bone and distribution to the brain under chronic exposure conditions, particularly to the fetus during pregnancy and infants during lactation.

– Based on the outcome of this assessment, a re-evaluation of the current legal limit of fluoride in drinking water seems warranted.

ABBREVIATIONS

AAS	atomic absorption spectroscopy
AI	adequate intake
ADHD	attention-deficit/hyperactivity disorder
ADI	acceptable daily intake
AOP	adverse outcome pathways
AROI	acceptable range of oral intake
CNS	central nervous system
DAPs	differentially abundant proteins
DEGs	differentially expressed genes
DNT	developmental neurotoxicity
DRV	dietary reference value
HBGV	health-based guidance value
HPT	hypothalamus–pituitary–thyroid
HRV	heart rate variability
IC	ion chromatography
ICP-MS	inductively coupled plasma mass spectrometry
IPCS	International Programme on Chemical Safety (World Health Organization)
ISE	ion selective electrode
IVB	in vitro battery
LB	lower bound
LOAEL	lowest observed adverse effect levels
LOD	limit of detection
LOE	line of evidence
LOQ	limit of quantification
MB	middle bound
MIP-OES	microwave-induced plasma optical emission spectroscopy
MRLs	maximum residue levels
NTP	National Toxicology Program
NOAEL	no observed adverse effect level
OCCO	dense child cohort
OPMCSA	Office of Prime Minister's Chief Science Advisor
PARCI–MS	plasma assisted reaction chemical ionisation mass spectrometry
PBK	physiologically based kinetic
POD	point of departure
PTH	parathyroid hormone
RP	Reference Point
RoB	risk of bias
ROCF	Rey–Osterrieth complex figure
SCF	Scientific Committee on Food
sF	serum fluoride
SCHER	Scientific Committee on Health and Environmental Risks
T3	triiodothyronine
T4	thyroxine
TBG	thyroxin-binding globulin
TBPA	thyroxine-binding pre-albumin
TPO	thyroid peroxidase
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
UB	upper bound
UF	uncertainty factor
uF	urinary fluoride
UL	tolerable upper intake level
wF	water fluoride
WHO	World Health Organization

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SUPPORTING INFORMATION

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APPENDIX A

Overview of methods of analysis for fluoride

TABLE A.1 Fluoride methods of analysis.

Method	Pre-treatment	Sensitivity (mg/kg or mg/L)	Applicable matrix	Comment
ISE	Total ionic strength adjustment buffer	0.001–0.1	Water, water-based beverages, urine, high-water matrices (EURL-SRM, 2023; Helte et al., 2021; Kassahun & Chandravanshi, 2019; Belete et al., 2017; Goschorska et al., 2016; Abuhaloob et al., 2015; Fojo et al., 2013)	Method used by EU Reference Laboratory on Residues of Pesticides (high-water matrices) (EURL-SRM, 2023)
	Total ionic strength adjustment buffer following silicon-facilitated diffusion		All matrices (EURL-SRM, 2023; Martínez-Mier et al., 2011, 2017; Zohoori & Maguire, 2016; Abuhaloob et al., 2015)	Method used by EU Reference Laboratory on Residues of Pesticides (dry matrices) (EURL-SRM, 2023)
	Total ionic strength adjustment buffer following alkaline fusion up to 525°C		All matrices (Dagnaw et al., 2017; Dessalegne & Zewge, 2013)	There is no evidence that C-F bond breaks at this temperature. Evaporation of fluoride may result in lower recoveries.
Ion Chromatography (IC)	Direct measurement	0.0004–0.6	Water (Dovidauskas et al., 2020; Murray et al., 2018; Szmagara & Krzyszczak, 2019; Turhan et al., 2019)	Method used by U.S. EPA for water analysis (U.S. EPA, 2007)
	Dilution in water	0.5	Wine (Alkaya et al., 2019)	In wine samples sensitivity is based on the lowest point in the calibration curve
	2g of tea in 100 of boiling water	0.1–1.0 1.0	Tea infusions (Janiszewska & Balcerzak, 2013; Milovanovic et al., 2018; Minca et al., 2013)	In tea infusions IC appeared to overestimate the occurrence of fluoride because of interference from unknown species
	Dilution (media not specified)	1.0	Salt (Ning & Zhang, 2014)	
	Alkaline fusion at 550°C, water extraction, sulfuric acid distillation, hydrochloric acid extraction and adjustment of pH	0.2	Antarctic krill (Zhao et al., 2015)	Extracting ash with water only results in 3 × lower recoveries
Colorimetry: SPADNS zirconium alizarin red S	Direct measurement Sample fusion with sodium carbonate or digestion with perchloric acid	0.1 Not available	Water (Faraji et al., 2014) Tea infusions (Essebbahi et al., 2020) Algae, seaweed (El-Said & El-Sikaily, 2013; El Zokm et al., 2021)	SPADNS Method used by U.S. EPA for water analysis (U.S. EPA, 2017) Evaporation of fluoride may result in lower recoveries.
Particle induced gamma-ray emission	Samples dried at 60°C, powdered and pressed with cellulose and lithium carbonate to form pellets	9	Rice (Dhorge et al., 2017)	Not sensitive enough

TABLE A.1 (Continued)

Method	Pre-treatment	Sensitivity (mg/kg or mg/L)	Applicable matrix	Comment
Total fluorine methodologies HR-CS GFAAS ICP-QMS MIP-OES	Apply heating from 700°C to 8000°C	0.018 (LOD) 0.032 (LOD) 1.1 (LOD)	Wine (Ozbek & Akman, 2015) Tea infusions (Guo et al., 2020) Tea infusions (Akhdar et al. 2021)	Appropriate as proxy for fluoride content in water samples and other beverages such as tea infusions for which contribution of covalently bonded fluorine should be low (see Section 1.4.4). Not appropriate as proxy for fluoride content in cereals and vegetables, in which considerable contribution from fluorine-containing pesticides is not negligible.

[a]TISAB not added/not reported to be added.
[b]Analysis of fluoride in different milk fractions is reported but protocol is not described in the paper.
[c]Inductively coupled plasma-mass spectrometry.
[d]Microwave-induced plasma optical emission spectrometry.
[e]High resolution continuum source atomic absorption spectrometry.
[f]High resolution continuum source graphite furnace atomic absorption spectrometer.
[g]Atomic absorption spectrometry.

APPENDIX B

Thyroid homeostasis, state of knowledge

Thyroid hormone synthesis is regulated by the hypothalamus through the release of the TRH, which stimulates the pituitary gland to release TSH. This, in turn, stimulates the synthesis and release of T4 and, to a lesser extent, T3, through iodination of thyroglobulin tyrosine residues catalysed by thyroid peroxidase (TPO). Iodine uptake in the thyroid is mediated by the sodium-iodide symporter (NIS). When T4, T3 and iodide concentrations are high, the hypothalamus reduces TRH production, followed by reduced TSH and T4 and T3 production and release.

Outside the thyroid, part of T4 is de-iodinated to the more active T3 and the inactive or reverse T3 by three tissue-specific deiodinase isoforms expressed in liver, kidneys, muscles, thyroid, pituitary gland, brown adipose tissue and central nervous system. More than 99% of T4 circulates in blood reversibly bound to transport proteins [thyroxin-binding globulin (TBG), thyroxine-binding pre-albumin (TBPA) and albumin], while the small fraction that is unbound or free T4 (FT4), is metabolically active and available to tissues. Peripheral (extrathyroidal) T3 has a lower binding affinity for plasma proteins and therefore a more rapid turnover and a shorter half-life than T4 (1 day compared to 7 days), resulting in lower circulating total concentrations of T3 compared to total T4.

B.1 | Thyroid disease and iodine deficiency

Thyroid diseases are among the most common endocrine disorders worldwide. Females are about five to eight times more likely to have a thyroid condition than males (Garmendia Madariaga et al., 2014). Hypothyroidism is the most common thyroid disease, particularly in areas of low iodine intake. The prevalence of hypothyroidism in Europe was estimated to be 3.05% and of hyperthyroidism 0.75%, based on studies published between 1975 and 2012 (Garmendia Madariaga et al., 2014). Overt or primary hypothyroidism is defined as TSH concentrations above the reference range[79] and FT4 levels below the reference range. Hyperthyroidism is defined as low TSH and high FT4 (Taylor et al., 2018). Subclinical hypo- and subclinical hyperthyroidism are defined by high and low TSH, respectively, compared to the reference range, but with levels of FT4 within the reference range.

Iodine deficiency and auto-immune disease (Hashimoto's disease) account for the vast majority of cases of primary hypothyroidism (Taylor et al., 2018). On a population basis, iodine deficiency is defined by the WHO as median urinary iodine concentration (ul) below 100 μg/L. A urinary concentration between 100 and 200 μg/L represents adequate iodine nutrition, whereas concentrations ≥ 300 μg/L present a risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease) (WHO, 2013). In pregnancy, the recommended threshold for ul is 150 μg/L (WHO, 2013).

B.2 | Thyroid hormones in pregnancy

Thyroid function is critical during pregnancy as thyroid hormones regulate development and particularly brain development. Sub-optimal maternal thyroid function is therefore associated with poor growth and neurodevelopment of the fetus, in addition to adverse reproductive effects such as infertility and miscarriage (Griebel-Thompson et al., 2023). Insufficiency of thyroid hormones during pregnancy can produce adverse health effects in the offspring, including specific neuropsychological functions, depending on the developmental window during which the deficiency occurs and the brain region most sensitive during that window. Even modest degrees of thyroid hormones disruption experienced in utero can result in neuropsychological deficits in children despite normal thyroid status at birth (Haddow et al., 1999). When thyroid hormone insufficiency occurs after birth, language and memory skills are most predominantly affected (Gilbert et al., 2012; Zoeller & Rovet, 2004). These actions of thyroid hormones are mediated by a combination of thyroid hormone receptor (TR) isoforms that exhibit specific temporal and spatial patterns of expression during animal and human brain development (Zoeller, 2007). Evidence supporting a critical role of thyroid function during pregnancy in offspring neurodevelopment is also obtained from animal models of chemically induced hypothyroidism, such as by exposure to thyroid peroxidase inhibitor propylthiouracil (PTU) (Goodman & Gilbert, 2007). A dose-dependent bilateral cellular malformation, also known as heterotopia, was seen in neurons developed between GD17-19 within the white matter of the corpus callosum in rats. The malformation was observed at modest maternal thyroid insufficiency (around 45% reduction in T4 and no change in T3) (Goodman & Gilbert, 2007). Thyroid hormone insufficiencies present during the perinatal period (GD19-PN2) are sufficient to induce a heterotopia in the PTU rodent model (O'Shaughnessy et al., 2019). Heterotopia represent errors in neuronal migration and are present in many animal models of neurodevelopmental disorders and in humans with genetic mutation (Carabalona et al., 2012; Rosen et al., 2013) and associated with neurological impairments, intellectual disability and seizure disorders (Watrin et al., 2015).

The fetal thyroid is not fully functional until mid-pregnancy (18–20 weeks) and the fetus depends entirely on the maternal thyroid hormones during the first trimester of pregnancy. Thus, placental transfer of maternal thyroid hormones during early pregnancy (first trimester) is crucial, as is the maternal iodine status (Hall et al., 2023; Korevaar et al., 2016). Adequate

[79]Generally accepted range for individual blood TSH concentrations: 0.4–4.0 mUI/L; for FT4: 9.0–24.0 pmol/L (0.6992–1.8645 ng/dL); for TT4: 64–154 nmol/L (4.9720–11.964 μg/dL).

supplies of thyroid hormones are necessary throughout pregnancy with higher brain function. A relationship between FT4 and child IQ and grey matter volume indicated an optimal FT4 and TSH concentration during pregnancy of 15.5 pmol/L and 1.7 mIU/L respectively (Jansen et al., 2019; Korevaar et al., 2016).

B.3 | Evidence on the role of thyroid in development in human studies

Epidemiological evidence provides further support for the importance of maintaining normal thyroid function during pregnancy for optimal neurodevelopment and report an association of lower cognitive measures in offspring with low maternal FT4, but not with TSH. A meta-analysis of data from 9036 mother–child pairs from three prospective birth cohorts (INMA, Spain; Generation R, Netherlands; and ALSPAC, UK) reported that maternal hypothyroxinemia (i.e. normal TSH and low FT4 (< 2.5th percentile)) was associated with a 3.9 point lower non-verbal IQ and 2.1 point lower verbal IQ in offspring (Levie et al., 2018).

In a Dutch study, women with gestational FT4 concentrations above P95 (95th percentile of the distribution; median 22.5 pmol/L) had lower median TSH concentrations (0.60 mIU/L) compared to women with median FT4 concentrations between P5 and P95 (FT4 of 14.9 pmol/L and TSH of 1.39 mIU/L). Child IQ and mean total grey matter volume were highest at a maternal FT4 concentration of around 15.5 pmol/L (read from the graph). Sensitivity analyses showed cut-off values for low and high FT4 concentrations and decreased offspring IQ at P11 and P88. In the paper, concentrations FT4 for P10 and P90 are given as multiple of means of respectively 0.80 and 1.28 (resulting in 11.9 and 19.1 pmol/L or 0.92 and 1.48 ng/dL) (Korevaar et al., 2016). The associations for grey matter volume were most evident at 8 weeks gestation and were no longer seen after about 14 weeks of gestation (Jansen et al., 2019). The optimal maternal TSH concentration was 1.7 mIU/L (read from graph) for child mean total grey matter volume (cm^3) and mean cortex volume. A linear relationship was found between mean total grey matter volume at age 10 years and child IQ measured at age 6 years (after adjusting for confounders) (Jansen et al., 2019).

In a subset ($n = 3839$) of pregnant women from the prospective Generation R Study in the Netherlands, the relationship between maternal thyroid function early in pregnancy (< 18 weeks) and child IQ and brain morphology was assessed at age 6–8 years. Maternal FT4 (but not TSH) concentrations showed inverted U-shaped associations between maternal FT4 and children's IQ (reduction by 1.4–3.8 points; $p = 0.004$), grey matter volume ($p = 0.006$) and cortex volume ($p = 0.001$) (Korevaar et al., 2016). In another subset of the Generation R Study ($n = 5100$ women), women with severe hypothyroxinemia ($n = 136$; FT4 < 5th percentile with normal TSH) during gestational weeks 6–18 had almost four-fold increased odds of having an autistic child at the age of 6 years (parent scoring) (Román et al., 2013). In this study, women with maternal mild hypothroxinemia ($n = 127$) in early pregnancy had children with 7% higher scores for attention-deficit/hyperactivity disorder (ADHD) symptoms (thus, more ADHD symptoms) in 8-year old children, independent of possible confounders (Modesto et al., 2015).

Pop et al. (2003, 1999) compared 1196 children from Dutch Caucasian women with hypothyroxinaemia (FT4 below the lowest P10 and TSH within the reference range of 0.15–2.0 mIU/L) at 12 weeks of pregnancy, with children from women with FT4 between P50-P90 at 12 weeks of gestation (Amsterdam Born Children and Their development study). Child mental development and motor function (using the Bayley Scales of Infant Development) was delayed compared to controls at the age of 1 year (10 and 8 points on the mental and motor scale, respectively) and at the age of 2 years (8 and 10 points, respectively). This study underlined the importance and continuation of FT4 during early pregnancy because normal FT4 concentrations later in pregnancy did not lead to differences compared to controls.

Vrijkotte et al. (2017) concluded from TSH and FT4 concentrations obtained from pregnant Dutch women at 13 weeks of pregnancy ($n = 3988$) participating in the Amsterdam Born Children and Their Development study, that a 1 pmol/L increase in maternal FT4 resulted in a reduced birthweight of 33.7 and 16.1 g for males and females, respectively. Noten et al. (2015) assessed, using data from the same study, school performance at age 5 years old. Maternal hypothyroxinaemia (i.e. a maternal FT4 at < P10 of distribution) was associated with a 1.61 (95% CI: 1.05–2.47)-fold increased odds of (adjusted) subnormal arithmetic performance. There was no association found with TSH.

In a subsample of the Danish National Birth Cohort ($n = 7624$), 2276 women had a child diagnosed with seizures, specific developmental disorder (SDD), autism spectrum disorder (ASD) and/or ADHD (Andersen & Olsen, 2017). Blood samples taken at (median) week 9 of gestation were analysed for FT4 and TSH. In 12.5% of women abnormal thyroid function was found; this was higher for cases of ASD but not for other neurodevelopmental disorders. Maternal hypothyroidism (TSH > 10 mIU/L) was a risk factor for epilepsy in the child and both maternal hypothyroidism and hyperthyroidism were risk factors for ASD in the child. A low concentration of FT4 was related with ASD and ADHD in girls but not in boys (Andersen & Olsen, 2017).

Korevaar et al. (2016) studied T4 as a potential marker for maternal thyroid function during pregnancy because it is considered to be more stable than FT4 from the second trimester of pregnancy onwards and better reflects changes in the hypothalamic–pituitary-thyroid axis. A subset of 5647 mother–child pairs from the Generation R study were selected for whom data on maternal TSH, FT4 and T4, sampled at 13.2 weeks (median) of pregnancy were available. They found A log-linear association of TT4 and FT4 with TSH. T4 concentrations were highly variable in the first half of pregnancy and weakly related to maternal TSH. There was no association of maternal T4 or no better association than with FT4, with pre-eclampsia, premature delivery, birthweight or offspring IQ. The authors concluded that maternal FT4 concentrations is preferred over TT4 in the assessment of maternal thyroid function during the first half of pregnancy (Korevaar et al., 2016).

U.S. EPA used a biologically based dose–response model (BBDR), which describes the relationship between decreased thyroid hormone in early pregnancy and later neurodevelopmental effects (e.g. IQ decrease) in the offspring for the risk assessment and derivation of a maximum contaminant level goal (MCLG) of perchlorate in drinking water (Clewellet al., 2019; U.S. EPA, 2019). Based on the data of Korevaar et al. (2016), US EPA's model predicted that a decrease in FT4 concentration of 0.25 pmol/L would result in a 2% decrease in IQ points. The model was developed to protect the fetus in the first trimester of pregnancy of mothers with low iodine intake (i.e. 75 μg/kg bw per day) and low FT4 concentrations (i.e. at P10 of a distribution for individuals with an iodine intake of 75 μg/kg bw per day) and weak TSH feedback strength (i.e. reduced to be approximately 60% less effective than for the median individual).

B.4 | Guidance for the identification of endocrine disruptors

In 2018, European Chemicals Agency (ECHA) and European Food Safety Authority (EFSA) with support from the Joint Research Centre (JRC) published the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (ECHA/EFSA, 2018). There are quantitative species-specific differences in the systemic regulation of thyroid hormones levels between experimental animals and humans. In an appendix, guidance is provided on how to address specific thyroid related DNT concerns, i.e. (1) substances inducing histopathological changes in the thyroid, with or without changes in the circulating levels of thyroid hormones, would pose a hazard for human thyroid hormone insufficiency in adults as well as pre- and postnatal neurological development of offspring, (2) substances that alter the circulating levels of T3 and/or T4 without histopathological findings would still present a potential concern for neurodevelopment and (3) in the absence of substance-specific data which provide proof of the contrary, humans and rodents are considered to be equally sensitive to thyroid-disruption. In Summer 2018, ECETOC convened a 'Special T4 Task Force' to evaluate the scientific uncertainty with regard to the assessment of thyroid disruption. The Task Force concluded that serum TSH and fT4 concentrations depend on population, assay and laboratory; generally accepted ranges for normal values do not exist (Sauer et al., 2020). However, the human data confirmed an association between altered maternal serum FT4 and/or TSH and increased risk for child neurodevelopmental impairment. Whether altered maternal serum levels of total T4, free or total triiodothyronine are 'adverse' or indicative of transient physiological adaptations of the thyroid hormone system, or parameters unrelated to serum thyroid hormones are more relevant indicators of such effects was still to be answered (Sauer et al., 2020). Also, if the neurodevelopmental findings observed in human studies are caused by the same MoA is still not clear (Marty et al., 2021). The evidence was considered insufficient to establish quantitative boundaries of thyroid hormone concentrations in blood indicative of increased risk for neurodevelopmental impairment of children. The thyroid hormone system might have increased sensitivity during pregnancy (Sauer et al., 2020).

In a second review, the ECETOX Task Force (Marty et al., 2021) reviewed thyroid-related adverse outcome pathways (AOPs) with adverse neurodevelopmental outcomes in mammals. Also this review did not identify a single physiologically relevant normal range for FT4 (or TSH), or quantitative boundaries for maternal FT4 reduction (and/or TSH increase) indicating an increased risk for neurodevelopmental impairment in the children. It was considered whether a window of susceptibility could be identified in the first trimester of pregnancy, but this could not be confirmed (Marty et al., 2021). In the five linear thyroid-related AOPs, reduced serum T4 is a key event and also a mandatory parameter in five OECD TGs (OECD TGs 408, 414, 421, 422 and 423). It is unclear if TT4 (the standard parameter in rodent studies), FT4 (the standard parameter in human studies), TT3 or FT3 is the most sensitive or reliable parameter for thyroid hormone balance resulting in adverse neurodevelopmental effects (Marty et al., 2021; Sauer et al., 2020).

As described in further detail by Li et al. (2019), assuming two groups with 10 animals (rodents) each (a common group size in regulatory toxicity studies) and the maximum allowed intra-assay coefficient of variation (e.g. 25% for T3/T4 and 35% for TSH as per OECD TG 407) in one group and about half of this coefficient of variation in the other group (to reflect biological variability), T3/T4 decreases of approx. 25% and a TSH increase of approx. 40% as compared to the concurrent controls can be detected as statistically significant, and can therefore be considered 'reasonably detectable' in a common regulatory setting. Changes in concentrations TSH and T4 in rats and humans that exceed normal ranges, can be detected with roughly the same sensitivity (Marty et al., 2021).

In a third review (Marty et al., 2022) patterns of thyroid- and brain-related effects were examined in rats upon gestational/lactational exposure to 14 substances causing thyroid hormone imbalance by four different modes-of-action (inhibition of thyroid peroxidase, sodium-iodide symporter and deiodinase activities, enhancement of thyroid hormone clearance) or to dietary iodine deficiency. The authors concluded that for rats, thresholds of ≥ 60%/≥ 50% offspring serum thyroxine reduction and ≥ 20% and statistically significant offspring serum triiodothyronine reduction indicated an increased likelihood for statistically significant neurodevelopmental effects; accuracies: 83% and 67% when excluding electro-physiology (and gene expression) (Marty et al., 2022). In the last paper (Melching-Kollmuss et al., 2023) describe a proposal for a Thyroid Function-Related Neurodevelopmental Toxicity Testing and Assessment Scheme (Thyroid-NDT-TAS) that meets the European Commission Endocrine Disruptor Criteria.

B.5 | Species differences

The young adult rat is considered a sensitive species, and several substances disrupt the HPT axis in this species. This is mainly due to the faster decline of the hormone reservoir in rats, which occurs within days compared to the more stable reservoir in humans. The half-lives of T3 and T4 in rats are 6 h and 12–24 h, respectively, compared to half-lives of 1 and

7 days, respectively, in humans. As a result, the homeostatic upregulation of TSH and related changes are more immediate in the rat and serve as sensitive indicators of a disturbance. In addition, there are species differences in thyroid hormone binding proteins, where thyroid binding globulin (TBG) is the main transporter in humans whereas transthyretin (TTR; a.k.a. prealbumin) is the predominant protein in rats (and a secondary binding and transport protein in humans). TBG acts also as a reservoir for thyroid hormone, due to its high affinity for the thyroid hormone. Only the free hormones (about 0.3% or even less for T3) are biologically active at the tissue and cellular level (Bartsch et al., 2018; Fosteret al., 2021). In humans, about 75% of T4 is bound to TBG, 20% to transthyretin (TTR) and 5% to albumin while in the young adult rat, TTR is the predominant serum-binding protein where 75% of the T4 is bound to TTR and 25% to albumin. However, in the neonatal rat, T4 is primarily bound to TBG as in humans and thus although this species-difference is often highlighted, it is based only on data from adult animals.

In young adult rats, a 10-fold higher tT4 supplementation (20 mg/kg bw) is needed to maintain health in the absence of a functional thyroid compared to humans (2.2 mg/kg bw) (Capen, 1997). The tT4 half-life is significantly shorter in young adult rodents (12–24 h in rat, 12–18 h in mouse) compared to humans (5–9 days) due to higher reserves of thyroid hormone in humans in the form of TBG which is essentially absent in the young adult rodents. This, however, is not the case in the developing rodent where TBG also functions as a transport protein (Döhleret al., 1979). In contrast, TBG in humans, monkeys and dogs, has a 1000-fold higher affinity for T4 compared to TTR. These differences result in thyroid hormone kinetic differences, higher turnover, higher clearance rates with higher rate of thyroid hormone production, smaller reserve capacity of thyroid hormones in adult rodents compared to humans and by extension higher basal TSH level, a more active thyroid gland and consequently higher sensitivity of rodents to thyroid hormone inhibition compared to humans (Bartsch et al., 2018; Lewandowski et al., 2004; McClain, 1989). Distinct histological differences of the thyroid gland are observed between rats and other species, including humans and non-human primates (McClain, 1995). In rodents, unlike humans, the follicles have a tall cuboidal epithelium lining, contain smaller amounts of thyroglobulin colloid and have higher hormone synthesis and secretion activity (Suttie, 2017). The quantitative morphological and physiological differences observed in rat support a faster rate of thyroid hormone synthesis and turnover in response to perturbations compared to humans (U.S. EPA, 1998).

Nevertheless, despite the low level of TBG, mice appear less sensitive than rats (Kato et al., 2003) to the impact of T3 glucuronidation on decreased serum T4, increased serum TSH and proliferation of follicular cells (Hoodet al., 2003; Klaassen & Hood, 2001). Furthermore, rats and mice showed a different pattern of T4 and T3 binding proteins in blood with a greater binding of thyroid hormone to prealbumin in rats (transthyretin) and to postalbumin in mice (Capen, 1997, 1999).

Species differences have been demonstrated in vitro for NIS activity, with rat NIS being more efficient/effective in I-uptake than human NIS (Dayem et al., 2008; Heltemes et al., 2003) In addition, species differences have been observed in TPO activity, with non-human primates, humans, guinea pigs and chicken being more resistant to inhibition than rats (Capen, 1999).

Substantial species differences have also been reported with respect to thyroid hormone availability to the brain, as a result of differences in cell type and expression of different thyroid hormone transmembrane transporters, including their regulation, especially during development but also in adult brain (Wirth et al., 2014). The implications of these differences have not been fully assessed.

TSH serum concentrations differ between males and females in the rat and mouse, with 3-fold higher TSH concentrations in male compared to female rats (Foster et al., 2021; Kieffer et al., 1976). The histology of the female rat thyroid gland displays a greater number of large colloid-filled follicles and a flattened epithelial lining and is more like non-human-primates (Foster et al., 2021). In addition, the binding capacity of TTR for thyroid hormones in female rats is lower than in male rats due to oestrogen binding (Emerson et al., 1990).

APPENDIX C

In vitro evidence of fluoride effects

The *OECD Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity* In-Vitro *Testing Battery* (DNT IVB, OECD Series of Testing and Assessment No. 377) provide the scientific and practical framework for the interpretation of these in vitro DNT data. The DNT IVB covers neurodevelopmental processes, which have been agreed within a series of scientific workshops as being key for a healthy brain development: neuronal (stem or progenitor) cell proliferation, differentiation, migration, myelination, synaptogenesis, network function and apoptosis. A disturbance thereof represents a cellular toxicity that compromises an organism's capacity to compensate for additional stressors.

Case studies using the DNT IVB data have been developed at OECD level (they are annexed to the *OECD Initial Recommendations* document) and are further in progress for various regulatory problem formulations. These case studies may be used as a series of authoritative, case specific references towards the standardised use of the DNT IVB.

Within the Annex of this OECD WNT approved document, the detailed characterisations of the in vitro assays are provided. These assays represent the currently best state of the art for in vitro DNT methods.

The results of the DNT IVB assay of the US EPA CompTox Database presenting one 'positive hit', in a neural progenitor cell proliferation assay, i.e. NPC1a (neural progenitor cells) are shown in Table C.1 and Figure C.1.

TABLE C.1 In vitro assays reporting a positive hit for developmental neurotoxicity markers.

Reference	Intended target	Cell type	Assay-name	Hit	BMC 30 [μM]	BMCL-BMCU [μM]
Masjosthusmann et al. (2020); data recalculated according to Keßel et al. (2023)	NPC proliferation	Primary human feotal neural progenitor cell	IUF_NPC1a_ proliferation_ BrdU_72hr_dn	Yes, unspecific	1023	820–1848
	NPC viability		BMC30 viability (NPC1)	Yes	1589	983–2276

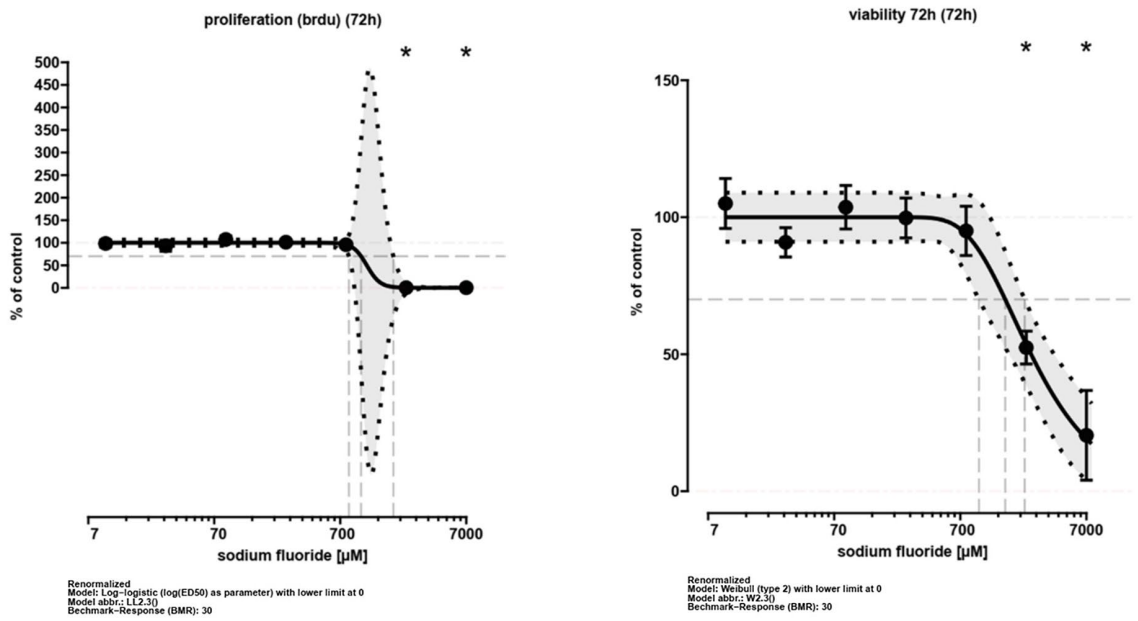


FIGURE C.1 Dose–Response Relationships for the NPC1a proliferation assay (left panel) and for the corresponding NPC1a viability assay (right panel).

Results of other DNT IVB assays of the US EPA CompTox Database with fluoride are presented in Table C.2.

TABLE C.2 *In vitro* assays negative for developmental neurotoxicity markers.

Reference	Intended target	Cell type	Assay-name	Hit	BMC [uM]	BMCL-BMCU [uM]
Masjosthusmann et al. (2020) with data-assessment pipeline according to Keßel et al. (2023)	NPC migration	Primary human neural progenitor cell	**BMC10 migration distance radial glia (NPC2a; 72 h)**	**Yes**	**13.5**	**2.4 - NA**
			BMC10 migration distance radial glia (NPC2a; 120 h)	No	n.a.	n.a.
			BMC30 migration distance neurons (NPC2b)	No	n.a.	n.a.
			BMC30 migration distance neurons (NPC2b, 120 h)	No	n.a.	n.a.
			BMC30 migration distance oligodendrocytes (NPC2c, 120 h)	No	n.a.	n.a.
	NPC differentiation		**BMC30 neuronal differentiation (NPC3, 120 h)**	**Yes**	**12.8**	**4.1 - NA**
			BMC30 neurite length (NPC4, 120 h)	No	n.a.	n.a.
			BMC30 neurite area (NPC4, 120 h)	No	n.a.	n.a.
			BMC30 oligodendrocyte differentiation (NPC5, 120 h)	No	n.a.	n.a.
	NPC2-5 cytotox & viability control		BMC30 cell number (120 h, NPC2-5)	No	n.a.	n.a.
			BMC10 cytotoxicity (72 NPC2-5)	No	n.a.	n.a.
			BMC10 cytotoxicity (120 h; NPC2-5)	No	n.a.	n.a.
			BMC30 viability (120 h; NPC2-5)	No	n.a.	n.a.
US-EPA Comptox	Proliferation	Human embryonic stem cell line	CCTE_Mundy_HCI_hNP1_Pro	No	n.a.	n.a.
	Apoptosis		CCTE_Mundy_HCI_hNP1_Casp3_7	No	n.a.	n.a.
	hNP1 viability control: cell count		CCTE_Mundy_HCI_hNP1_CellTiter	No	n.a.	n.a.
	Neurite outgrowth: initiation*	Human embryonic stem cell line derived	CCTE_Mundy_HCI_hN2_NOG (	No	n.a.	n.a.
	Synaptogenesis and neurite maturation#	rat primary cortical cell	CCTE_Mundy_HCI_Cortical_Synap&Neur)	No	n.a.	n.a.
	Neuronal network function: connectivity & bursting		CCTE_Shafer_MEA	No	n.a.	n.a.

*Cell count, neurite length, branching points.

#Mature synapse count, mature neuron count; neurite associated synapses/neuron count; neurite associated synapses/unit length of neuron; neurite length.

APPENDIX D

Data modelling

D.1 | Bayesian benchmark dose (BMD) modelling for dental fluorosis in children

Selection of the BMR

The EFSA Guidance on BMD (2022) recommends that the BMR should reflect the onset of a human-relevant adverse effect, meaning that a response above the BMR is considered adverse.

The Scientific Committee selected a BMR of 5% for occurrence of mild to severe dental fluorosis in children and a BMR of 1% for moderate to severe dental fluorosis (see Section 3.7.1).

Specification of deviations from default assumptions

All analyses using a covariate were performed by Laplace approximation.

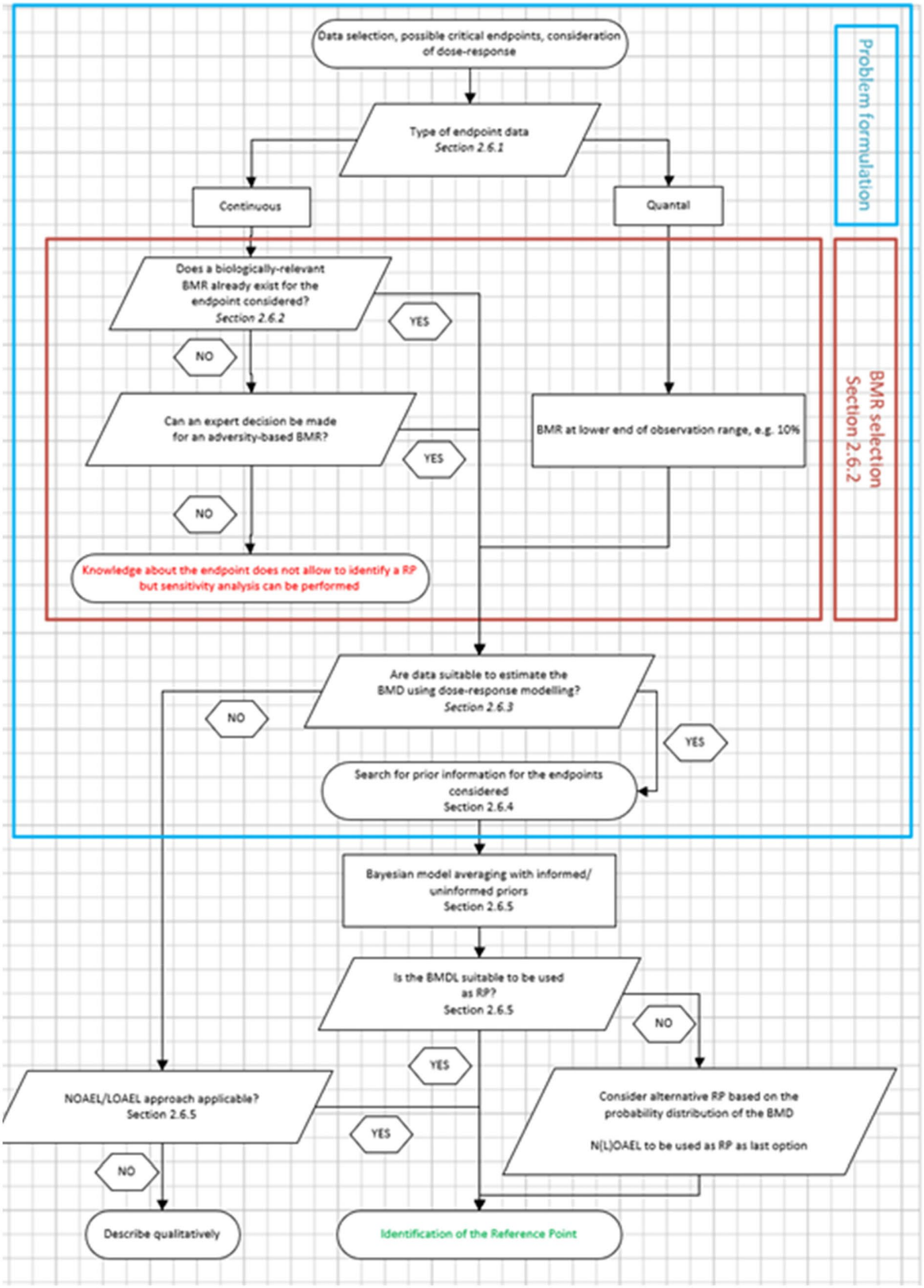
Extended dose range assumption was not applied in order to avoid confidence intervals falling outside of the dose range tested.

Default set of fitted models

		$y\|x \sim N(\mu(x), \sigma^2)$	$y\|x \sim LOGN(\mu(x), \sigma^2)$
Family	**Model**	**Dose–response function ($\mu(x)$)**	**Dose–response function ($e^{\mu(x)}$)**
1a	Exponential[(i)]	$a \cdot \left(1 + (c-1) \cdot \left(1 - e^{-b \cdot x^d}\right)\right)$	$e^{a \cdot \left(1+(c-1) \cdot \left(1-e^{-b \cdot x^d}\right)\right)}$
	Inverse Exponential	$a \cdot \left(1 + (c-1) \cdot \left(1 - e^{-b \cdot x^{-d}}\right)\right)$	$e^{a \cdot \left(1+(c-1) \cdot \left(1-e^{-b \cdot x^{-d}}\right)\right)}$
	Hill[(ii)]	$a \cdot \left(1 + (c-1) \cdot \left(1 - \frac{b}{b+x^d}\right)\right)$	$e^{a \cdot \left(1+(c-1) \cdot \left(1-\frac{b}{b+x^d}\right)\right)}$
	Log-Normal	$a \cdot (1 + (c-1) \cdot \Phi(\log(b) + d \cdot \log(x)))$	$e^{a \cdot (1+(c-1) \cdot \Phi(\log(b)+d \cdot \log(x)))}$
1b	Gamma[(iii)]	$a \cdot \left(1 + (c-1) \cdot \frac{\gamma(d, b \cdot x)}{\Gamma(d)}\right)$	$e^{a \cdot \left(1+(c-1) \cdot \frac{\gamma(d, b \cdot x)}{\Gamma(d)}\right)}$
	LMS-two stage	$a \cdot \left(1 + (c-1) \cdot \left(1 - e^{-b \cdot x - d \cdot x^2}\right)\right)$	$e^{a \cdot \left(1+(c-1) \cdot \left(1-e^{-b \cdot x - d \cdot x^2}\right)\right)}$
2	Probit increasing	$a \cdot \Phi\left(c + b \cdot x^d\right)$	$e^{a \cdot \Phi\left(c+b \cdot x^d\right)}$
	Probit decreasing	$a \cdot (1 + \Phi(c)) - a \cdot \Phi\left(c + b \cdot x^d\right)$	$e^{a \cdot (1+\Phi(c)) - a \cdot \Phi\left(c+b \cdot x^d\right)}$
	Logistic increasing	$a \cdot \frac{e^{c+b \cdot x^d}}{1+e^{c+b \cdot x^d}}$	$e^{a \cdot \frac{e^{c+b \cdot x^d}}{1+e^{c+b \cdot x^d}}}$
	Logistic decreasing	$a \cdot \left(1 + \frac{e^c}{1+e^c}\right) - a \cdot \frac{e^{c+b \cdot x^d}}{1+e^{c+b \cdot x^d}}$	$e^{a \cdot \left(1+\frac{e^c}{1+e^c}\right) - a \cdot \frac{e^{c+b \cdot x^d}}{1+e^{c+b \cdot x^d}}}$

Procedure for selection of BMDL

Flowchart to derive a Reference Point (RP) from a dose–response dataset of a specified endpoint, using Bayesian BMD analysis.



Selected studies

Dean (1942) – dental fluorosis prevalence and severity in children from 10 states in USA.

The detailed data for this study were obtained from the U.S. EPA 2010 assessment report on fluoride.

Data description

The endpoint to be analysed is prevalence of dental fluorosis in children in areas with different levels of fluoridated drinking water. Two subsets of the data were analysed: mild to severe and moderate to severe dental fluorosis, with BMR of 5% and 1%, respectively.

Data used for analysis

Concentration in drinking water (mg/L)	Mild to severe dental fluorosis	Moderate to severe dental fluorosis	*n*
0.0	0	0	423
0.1	0	0	236
0.2	0	0	459
0.3	0	0	454
0.4	2	0	263
0.5	3	0	403
0.6	2	0	614
0.9	2	0	123
1.2	7	0	633
1.3	14	0	447
1.8	17	2	170
1.9	20	3	273
2.2	65	16	138
2.6	128	42	404
2.9	52	26	97
3.9	217	136	289
4.0	40	21	59
4.4	162	121	189
5.7	38	34	38
7.6	59	45	65
8.0	21	19	21
14.1	25	24	26

Results for mild to severe dental fluorosis (BMR of 5%)

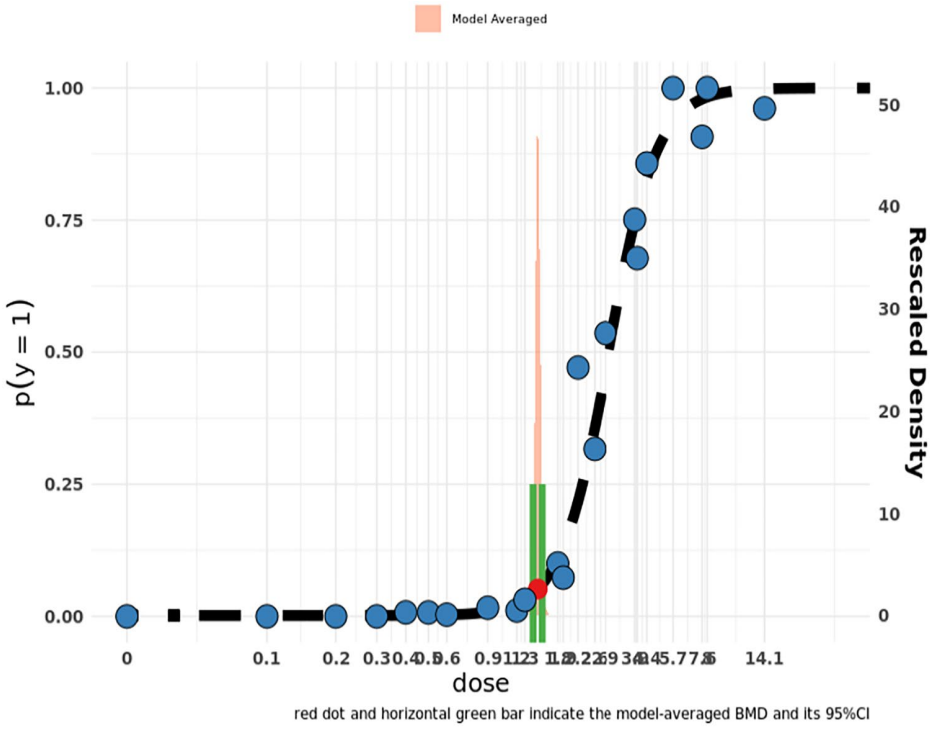
Estimated BMCs per model

Model	BMCL	BMC	BMCU	Model weights	Converged
E4_Q	1.213	1.273	1.335	0.000	1
IE4_Q	1.513	1.579	1.659	0.002	1
H4_Q	1.410	1.477	1.542	0.980	1
LN4_Q	1.436	1.495	1.554	0.017	1
QE4_Q	0.873	0.903	0.934	0.000	1
P4_Q	1.294	1.356	1.420	0.000	1
L4_Q	1.327	1.393	1.459	0.000	1

Model averaged BMC

Model	Type	BMCL	BMC	BMCU
Model Averaged	BS	1.409	1.477	1.543

Visualisation



Results moderate to severe fluorosis (BMR of 1%)

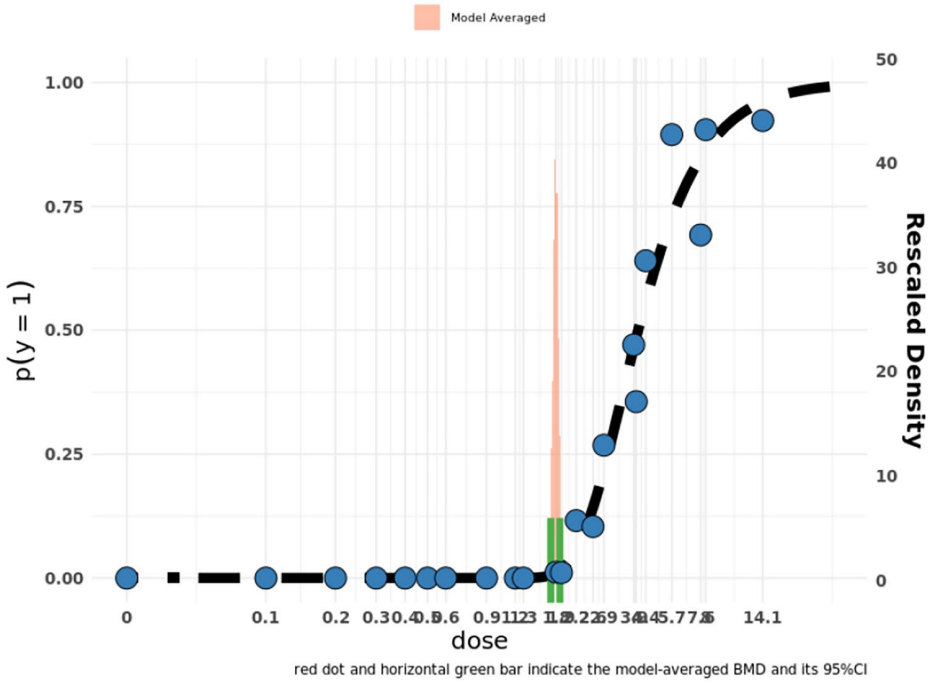
Estimated BMCs per model

Model	BMCL	BMC	BMCU	Model weights	Converged
E4_Q	0.977	1.079	1.167	0	1
IE4_Q	1.707	1.791	1.871	1	1
H4_Q	1.318	1.417	1.516	0	1
LN4_Q	1.447	1.537	1.624	0	1
G4_Q	1.293	1.391	1.490	0	1
QE4_Q	0.556	0.589	0.620	0	1
P4_Q	1.167	1.268	1.372	0	1
L4_Q	1.140	1.253	1.361	0	1

Model averaged BMC

Model	Type	BMCL	BMC	BMCU
Model Averaged	BS	1.708	1.791	1.871

Visualisation



D.2 | Benchmark dose (BMD) modelling illustration for animal data to assess the impact of the lack of a low/no fluoride control group for the RP derivation

Note – the analysis described here is presented for the purpose of assessing the impact of absence of a well-characterised control group in many animal studies. While the data from animal studies were not considered sufficient to be used for derivation of a reference point, quantitative data available were used for this illustration. It is noted that the data used in this illustration were not considered reliable (see Weight of Evidence Section 3.5.1, LoE4).

All but four rodent studies lack measurements of fluoride in the diet. Where no specific data were provided within the publications, a contribution of about 1.5 mg F/kg bw per day from chow (16–18 ppm in chow) was assumed. This introduces some uncertainty in the dose–response assessment. The brain weight data from Jiang et al. (2014) was used as an example dataset for BMD modelling, to assess whether this uncertainty on lack of data for fluoride content in rodent diet would strongly contribute to uncertainty in a RP derivation.

First, the BMDL, BMD and BMDU were estimated assuming that the control animals were exposed to 1.5 mg F/kg bw per day stemming from background fluoride in rodent diet. With this approach, a zero-fluoride exposure level is modelled based on the available dose–response relationship.

Thereby, a BMR of 5% lower brain weight in rodents results in BMDL/BMDU of 0.94/5.36 mg/kg bw per day.

			BMDL	BMD	BMDU
Reference	**Observed effect (BMR)**	**Dosing**	**mg/kg bw per day**		
Jiang et al. (2014)	Lower brain weight (5%)	2-months old M rats ($n = 6$) 1.5, 2.5, 3.5, 5.5 mg/kg bw	0.936	3.07	5.36

Second, the BMDL, BMD and BMDU were estimated without the addition of 1.5 mg F/kg bw per day to control and dose groups. With this approach, zero-fluoride exposure is assumed in the control group.

Using this assumption, a BMR of 5% lower brain weight in rodents results in BMDL/BMDU of 1.52/4.69 mg/kg bw per day.

			BMDL	BMD	BMDU
Reference	**Observed effect (BMR)**	**Dosing**	**mg/kg bw per day**		
Jiang et al. (2014)	Lower brain weight (5%)	2-months old M rats ($n = 6$) 0, 1, 2, 4 mg/kg bw	1.52	2.63	4.69

The difference in the BMDL/BMD/BMDU from the two approaches appears minor. These two BMD uncertainty distributions overlap. In the first approach, i.e. assuming intake of 1.5 mg/kg bw per day from baseline fluoride in the diet and modelling to zero exposure level, the brain weight is relatively higher at dose zero and the BMDL is reduced due to the increased uncertainty from the modelling.

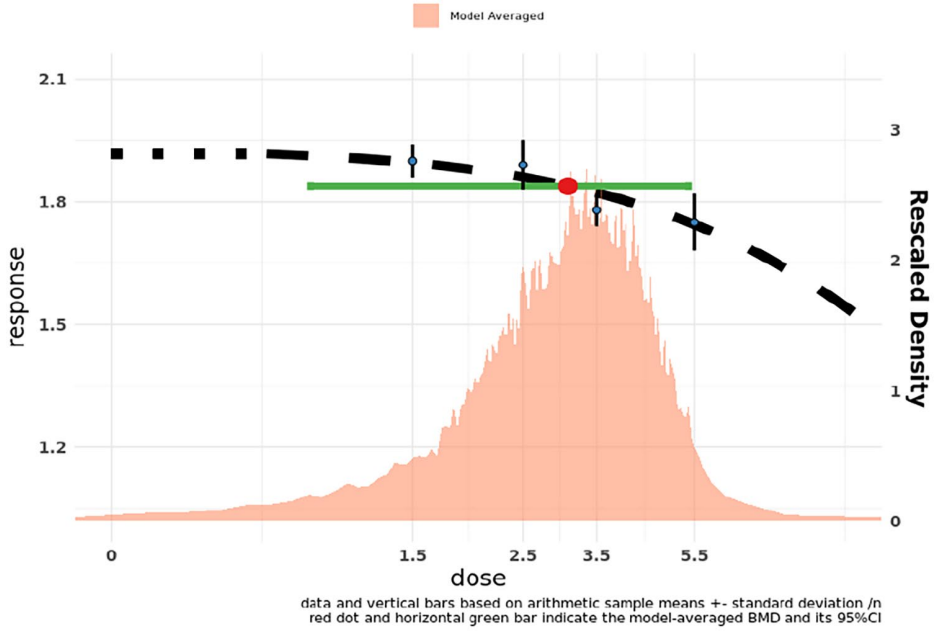


FIGURE D.1 Visualisation for BMD modelling using the assumption of 1.5 mg F/kg bw per day from background exposure to fluoride present in rodent diet.

The result from the modelling of the first approach is visualised in Figure D.1.

D.3 | Probabilistic extrapolation from animal BMDL to human HBGV

A probabilistic extrapolation from animal test NOAELs or BMDs to target human doses (HD) using the WHO 2017 Hazard Uncertainty Database and Approach,[80] was proposed by WG experts as an alternative to default interspecies and interindividual extrapolation factors.

Note: The Scientific Committee agreed to include this approach as an illustration only, acknowledging that it is not yet adopted in risk assessments of EFSA Scientific Opinions. It is also noted that the animal data were not used in this opinion as source of a reference point but only as supporting evidence. Therefore, the analysis described below is for illustration only.

In brief, the approach

1. allows a data-based extrapolation from NOAELs to BMDs based on historical test data in animals.
2. uses as a RP the probability distribution of the benchmark dose (GM & GSD) for a defined adverse effect (e.g. 5% lower brain weight) from a critical study.
3. uses the probability distribution for interspecies BMD ratios (between animal species and animal to human) available data from a large set of chemicals.
4. uses a probability distribution for human intra-species differences from available data from a large set of chemicals.
5. integrates these variabilities by dividing the probability distribution of BMD by the probability distributions for interspecies differences and the probability distribution for human intraspecies variability.
6. finally provides a probability distribution for a human reference dose, for a desired protection level in terms of effect size (e.g. not more than 5% lower brain weight; the effect for which the BMD was modelled in the animal experiment) and in terms of residual population under risk (e.g. not more than 1% or 0.1% of the human population). The 5th percentile of this distribution would represent a dose that meets the desired protection level with a probability of 95%. Further non-quantifiable uncertainties should be listed, e.g. in a tabular form.

The WHO 2017 approach for uncertainty extrapolation to human equivalent HBGV builds on:

- available probabilistic NOAEL to BMDL/BMDU ratios based on various historical animal study data.
- available inter-species NOAEL and BMD ratios between animal species (including data cleared for similar type of effects: body weight at necropsy, liver weight – absolute and relative, kidney weight – absolute and relative, erythrocyte count) and MTD ratios between animals and humans for chemotherapeutics.

[80] https://www.who.int/publications/i/item/9789241513548.

- available human intra-species ratios for TK (AUC), including some children data, and for TD of continuous (e.g. EEG changes, inhibition of enzymes, blood-pressure, diuretic efficiency) and quantal nature (e.g. deaths or ataxia or visual effects or psychomotor depression or anxiety – all based on comparisons of compound concentrations in blood).
- the understanding that these variabilities are log-normally distributed, which eases the mathematical approach.

This WHO 2017 database is currently limited in that it does not capture:

- the full human variability; so far, the majority of data stems from clinical trials with healthy human volunteers and some data from accidents like methylmercury contaminated food, Cd-worker exposure, Cu-exposure, carbamate; some data for children below 12 years are also included.
- DNT-specific animal to human extrapolation data.
- NOAEL/BMDL ratios for continuous endpoints other than liver weight, kidney weight, erythrocyte count and for quantal endpoints other than developmental.
- qualitative differences between species, in terms of effects on target organs such as dose–response relationships being present versus absent or increasing versus decreasing (about 23% of data sets).
- [and some aspects that are not relevant for the fluoride assessment, such as lack of interspecies uncertainty for inhalation (BMD ratios for pairs of species) and lack of uncertainty distributions for extrapolation between study types, such as sub-chronic to developmental; NOAEL to BMDU/BMDL uncertainty extrapolation should be amended to include more study types with continuous and quantal responses]

Until specific information on the inter- and intraspecies variability of fluoride is available, the breadth of the data sources used within this approach currently presents the best available data-based estimate for the uncertainty of health-based guidance values.

This WHO concept and tool may be amended by further specific data and/or expert judgement for other uncertainties, like extrapolations from LOAEL to NOAEL to other exposure durations.

The tool allows to visualise any deterministic HBGV derivation and/or deterministic exposure estimate. It may also provide a frame to include the fluoride specific human epidemiologic data.

Application to the (D)NT data reviewed for fluoride

DNT or NT effects were reported in different studies between 2.5 and 11.5 mg/kg bw per day in terms of different types of effects at cellular, organ and organism level (see Section 3.3.4.4). Notably, no behavioural effects were observed up to 2.1 mg/kg bw day within a relatively well-conducted study (McPherson et al., 2019). Considering the uncertainties in all the different studies and methods, a NOAEL or BMD from a single study may not represent an adequate point of departure. Rather, this range of 2.5–11.5 mg/kg bw per day may be appreciated as the magnitude where evidence of effects related to DNT or NT begins to appear. The related uncertainty for a 'true' BMD could be represented by a log-normal distribution with a BMDL of 2.5 and BMDU of 11.5.

Using this BMD including its uncertainty (BMDL/BMDU) as a surrogate for a RP for potential effects on the brain, the approach from WHO 2017 may be applied to extrapolation: Targeting a human protection level of 99%, 99.9% or 99.99% of the population against risk to be achieved with a probability of 5%–95%, the corresponding human equivalent dose range is 0.017–0.904, 0.005–0.624 or 0.002–0.472 mg/kg bw day, respectively (Figure D.2).

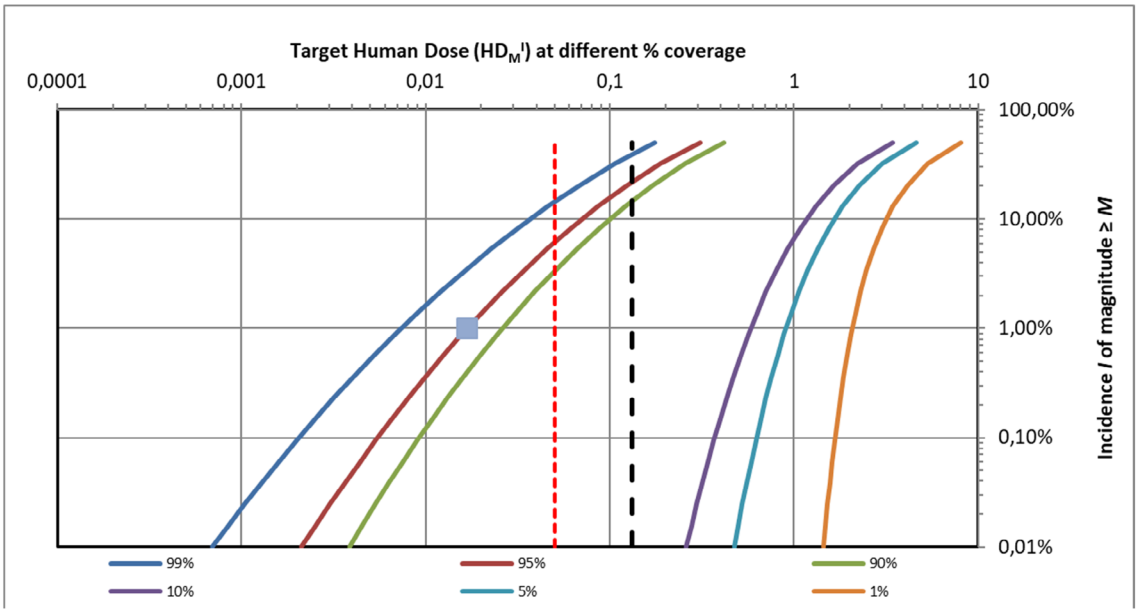


FIGURE D.2 Probabilistic extrapolation from animal derived BMDL to a human dose equivalent, HD_M^I for incidence *I* and magnitude *M*, using the APROBA approach (Appendix D.2). The credible confidence intervals of 10%–90%, 5–95% and 1%–99%, are shown as curved lines (colour code in the figure). The vertical axis indicates the target protection percentile of the population, as incidence, *I*. Effect magnitude, *M*, is defined in the BMD analysis as increased (D)NT effects. On the horizontal axis, the estimated human dose is in units of mg/kg bw per day. Vertical red dashed line: Safe level of intake for adults (0.05 mg/kg bw day, Reference Point) and Vertical blue dashed line Upper Intake Level (0.13 mg/kg bw day) for children, within this opinion both established mainly on the basis of human data. Square point: Lowest end of the range of human dose equivalent (0.002 mg/kg bw per day), protective of 99% of the population with a probability of 95%.

These ranges span two orders of magnitude and bracket both the 'safe level of intake' for adults (3.3 mg/day, equivalent to estimated 0.05 mg/kg bw per day, assuming 70 kg bw) and the tolerable upper intake levels (UL) for infants, toddlers and children (1, 1.6 and 2 mg/day, equivalent to 0.15, 0.13 and 0.1 mg/kg bw per day, respectively, assuming body weights of 6.7 kg, 12 kg and 20 kg), as established in this opinion based on human data.

Engaging the APROBA approach, the uncertainty in the human target dose (95th to 5th percentile) can be explained as dominated by the uncertainty about human variability: The uncertainty stems.

a. to 54% from human variability, 30 + 1% from interspecies extrapolation and 15% from BMD uncertainty (BMDL/BMDU) for the protection goal of 99.00% of population out of risk;
b. to 67% from human variability, 22 + 1% from interspecies extrapolation and 10% from BMD uncertainty (BMDL/BMDU) for the protection goal of 99.90% of population out of risk;
c. to 75% from human variability, 17 + 1% from interspecies extrapolation and 8% from BMD uncertainty (BMDL/BMDU) for the protection goal of 99.99% of population out of risk.

APPENDIX E

Literature Appraisal for Risk of Bias (Internal validity)

E.1 | Animal studies

Tier 1:

- All of the key questions are scored +/++

AND

- No more than one non-key question is scored -

AND

- No non-key question is scored --

Tier 2:

- All the other combinations not falling under tier 1 or 3

Tier 3:

- Any of the key questions is scored --

OR

- More than one key question is scored -

Number	Question	Domain of bias	Rating (++, +, -, --)
1*	Was administered dose or exposure level adequately randomised? (please apply the question also on F1 and F2 generation)	Selection	++ if the method is described and it is adequate + if the authors only indicate that randomisation was done but do not describe the method - no mention of randomisation -- direct evidence of no randomisation
2	Was allocation to study groups adequately concealed?	Selection	++ properly concealed and described how concealment was performed + mentioning that concealment was performed; + is also appropriate if non-concealment does not influence the outcome - if non-concealment does influence the outcome (measurements with a subjective part (e.g. preparation of fat pads, observation of behaviour) -- if non-concealment does influence the outcome to a very important part (subjective measurements)
3*	Were experimental conditions identical across study groups?	Performance	++ experimental conditions described and identical across study groups (feeding, water supply, bedding, day/night cycle; temperature; humidity) + incomplete description of experimental conditions; + is also appropriate if lack of information does not influence the outcome - if lack of information does influence the outcome -- if factors clearly indicate that treatment conditions were different does influence the outcome to a very important part
4*	Was the research personnel blinded to the study group?	Performance	
			++ if there is direct evidence that the research personnel did not know what group animals were allocated to, and it is unlikely that they could have broken the blinding of allocation
			+ if not reported and lack of adequate allocation concealment could not appreciably affect the handling/outcome of measurements of different study groups (e.g. methods used which do not have a subjective component)

(Continued)

Number	Question	Domain of bias	Rating (++, +, -, --)
			- if not reported and lack of adequate allocation concealment could appreciably affect the handling/measurement of different study groups (e.g. methods used which have a subjective component)
			-- if there is direct evidence that it was possible for the research personnel to know what group animals were allocated to, or it is likely that they could have broken the blinding of allocation
5	Were outcome data complete without attrition or exclusion from analysis?	Attrition/exclusion	
			++ There is direct evidence that loss of animals was adequately addressed and reasons were documented when animals were removed from a study. OR Missing data have been imputed using appropriate methods (ensuring that characteristics of animals are not significantly different from animals retained in the analysis).
			+ There is indirect evidence that loss of animals was adequately addressed, and reasons were documented when animals were removed from a study. OR It is deemed that the proportion lost would not appreciably bias results. This would include reports of no statistical differences in characteristics of animals removed from the study from those remaining in the study. OR There is insufficient information provided about loss of animals (record 'NR' as basis for answer) but it is considered that this does not have an impact on the validity of the study.
			- There is indirect evidence that loss of animals was unacceptably large and not adequately addressed (e.g. if unexplained loss is equal or more than 25%). OR There is insufficient information provided about loss of animals (record 'NR' as basis for answer) and it is suspected that this would have an impact on the validity of the study. Note: Unexplained inconsistencies between materials and methods and results sections (e.g. inconsistencies in the numbers of animals in different groups) could be an example of indirect evidence.
			-- There is direct evidence that loss of animals was unacceptably large and not adequately addressed.
6*	Can we be confident in the exposure characterisation?	Detection	
			++ There is direct evidence that the substance was sufficiently described and consistently administered (e.g. with the same method and timeframe) across treatment groups.
			+ There is indirect evidence that the substance was sufficiently described and consistently administered (i.e. with the same method and time-frame) across treatment groups. OR There is insufficient information provided about description and administration of the substance but it is considered that this does not have an impact on the validity of the study.

(Continued)

Number	Question	Domain of bias	Rating (++, +, -, --)
			- There is indirect evidence that the substance was not sufficiently described and was not consistently administered (e.g. with the same method and timeframes) across groups. OR There is insufficient information provided about description and administration of the substance and it is suspected that this has an impact on the validity of the study.
			-- There is direct evidence that the substance was not sufficiently described and/or was not consistently administered (e.g. with the same method and timeframes) across groups.
7*	Can we be confident in the outcome assessment?	Detection	
		Element 1: Was the outcome assessed at the same length of time (i.e. day and/or time of day) after initial exposure in all study groups? (remember to take into consideration the endpoints assignments) Element 2: Was a reliable and sensitive animal model used for investigating the test compound and selected endpoints? Element 3 Was the number of animals per dose group appropriate? Element 4: Was the number of animals per sex in each cage appropriate for the study type and animal model? Element 5 Was the timing and duration of administration of the test compound appropriate? Element 6: Were reliable and sensitive test methods used for investigating the selected endpoints? Element 7: Were the measurements collected at suitable time points in order to generate sensitive, valid and reliable data?	++ There is direct evidence + It is deemed that deviation would not appreciably bias results. OR There is insufficient information provided but it is considered that this does not have an impact on the validity of the study. - There is insufficient information provided (record 'NR' as basis for answer) and it is suspected that this has an impact on the validity of the study. -- There is direct evidence for a deviation
8	Were all outcomes measured according to the methodology section reported?	Selective reporting	
			++ There is direct evidence that all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract and/or introduction that are relevant for the evaluation have been reported. This would include outcomes reported with sufficient detail to be included in meta-analysis or fully tabulated during data extraction and analyses had been planned in advance.

(Continued)

Number	Question	Domain of bias	Rating (++, +, -, --)
			+ There is indirect evidence that all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract and/or introduction that are relevant for the evaluation have been reported. This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not). OR Analyses that had not been planned in advance (i.e. retrospective unplanned subgroup analyses) are clearly indicated as such and it is deemed that the unplanned analyses were appropriate and selective reporting would not appreciably bias results (e.g. appropriate analyses of an unexpected effect). OR There is insufficient information provided about selective outcome reporting (record 'NR' as basis for answer) but it is considered that this does not have an impact on the validity of the study.
			- There is indirect evidence that all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract and/or introduction that are relevant for the evaluation have not been reported. OR There is indirect evidence that unplanned analyses were included that may appreciably bias results. OR There is insufficient information provided about selective outcome reporting (record 'NR' as basis for answer) and it is suspected that this has an impact on the validity of the study. Note: Unexplained inconsistencies between materials and methods and results/abstract or summary sections (e.g. inconsistencies in the numbers of animals in different groups) could be an example of indirect evidence.
			-- There is direct evidence that not all of the study's measured outcomes (apical and intermediate) outlined in the protocol, methods, abstract and/or introduction that are relevant for the evaluation have not been reported. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.
9	Were statistical methods appropriate?	Other sources of bias	
			++ There is direct evidence that the statistical methods seem appropriate and were clearly reported (adequate treatment of multiple testing)
			+ Statistical methods were not clearly reported but it may be inferred from other information that they were appropriate. OR There is insufficient information provided about statistical methods (record 'NR' as basis for answer) but it is considered that this does not have an impact on the validity of the study.

(Continued)

Number	Question	Domain of bias	Rating (++, +, -, --)
			- Statistical methods were not clearly reported but it may be inferred from other information that they were not appropriate. OR There is insufficient information provided about statistical methods (record 'NR' as basis for answer) and it is suspected that this has an impact on the validity of the study.
			-- There is direct evidence that the statistical methods applied were inappropriate.

* Indicate the key-questions: **Q1, Q3, Q4, Q6, Q7**.

E.2 | Additional criteria for RoB appraisal of animal studies

Q1: Was administered dose or exposure level adequately randomised?	This is about randomised forming the groups. No additional considerations: ++ if randomisation mentioned and method for randomisation explained + if randomisation mentioned, but method for randomisation not explained - If randomisation is not mentioned -- if it is clear that there is no randomisation, e.g. control groups from other study or (e.g. in vitro) in timely sequential work-flows
Q2: Was allocation to study groups adequately concealed?	This is about blindly deciding which groups become control groups or one of the several dose groups.; usually this is not done and never mentioned and not critical for animal testing; handling of animals before/during the behavioural testing is critical and has to be counterbalanced between dose groups, but this is assessed in Q7. Therefore conclude by default '+ is also appropriate if non-concealment does not influence the outcome'
Q3: Were experimental conditions identical across study groups?	Practically never explicitly indicated that experimental conditions were identical across groups. ++, if all details of housing conditions are provided: temperature, humidity, bedding/enrichment, light/dark cycle, feed, drinking water +, if some aspects are missing. Conclude by default '+ is also appropriate if lack of information does not influence the outcome'
Q4: Was the research personnel blinded to the study group?	This is about the personnel handling the animals (and what we have earlier considered for Q2, and by default concluded with PLRoB, i.e. +) (The personnel doing the tests have to be blinded also, but this is part of Q7. Thus the following questions would be relevant for Q7: Quantitative tests, for which lack of (information for) blinding is less problematic (propose +) Motor activity with automated recording (unit is number of movements within time-blocks, subdivided in e.g. fine-movements, locomotion, vertical movement) Startle response testing (unit is time) Morris water maze (unit is time and distance) Cincinnati maze and letter mazes (unit is time and number of errors) Passive avoidance test (unit is time) Morphometrics (units is mm) Brain weight (unit is gram) Biochemical tests **However, for behavioural tests, automatic measure is important. Video tracking is considered as automatic measurement, also if not explicitly stated. Measurement by hand by stop watch needs blinding.)** Qualitative tests, for which lack of (information for) blinding is less problematic (propose +): Histology: analysis of baseline histopathology in the control group is often necessary and usually the analysis is started with control and high dose group (a necessary trade of between sensitivity and risk of bias) Qualitative tests, for which lack of (information for) blinding is more problematic: (propose -) Clinical signs Functional behavioural battery (except for some tests with quantitative read-outs, like limb strength, if results are given in newton measurements and foot splay given in cm, open arena – number of rearing)
Q5: Were outcome data complete without attrition or exclusion from analysis?	++, if number of animals is given in methods and results section and specific explanation is provided, if some animals were excluded +, if number of animals is given in methods and/or results section –, if number of animals is not given anywhere
Q6: Can we be confident in the exposure characterisation?	++, if source and purity of chemical is given and/or analytical confirmation is provided and route of exposure is clear and identical between groups +, if only source is given (but no purity and/or analytical confirmation) and route of exposure is clear and identical between groups –, if neither source, nor purity and/or analytical confirmation is given

(Continued)

Q7: Can we be confident in the outcome assessment?	Needs a careful analysis for behavioural tests, very often the method description, method performance and data assessment is not ideal. Nevertheless by default rather give + in the RoB screening phase and analyse more in detail later. –, if only one dose and few animals (e.g. 5)
Q8: Were all outcomes measured according to the methodology section reported?	++, if endpoints listed in methods section are also reported in results section 'data not shown', since negative and apparently not key, e.g. on bw, leads to +
Q9: Were statistical methods appropriate	Often the statistical analysis done for behavioural testing is not ideal, but this needs a careful review, which cannot be done in the fast RoB assessment phase Therefore by default rather give + in the RoB screening phase and analyse more in detail later. –, if no statistical analysis and/or no SD or CV is provided or if clear violations

E.3 | Human studies

Tier 1:

- All the key questions are scored +/++

AND

- No more than one non-key question is scored -

AND

- No non-key question is scored --

Tier 2:

- All the other combinations not falling under tier 1 or 3

Tier 3:

- Any question is scored --

OR

- More than one key question is scored -

*** Key question for HCT**

Key question for CaCo, Co and CrSe

	Question	Study type	Bias domain	Rating (++/+/-/--)
1*	*Were the participants adequately randomised to different treatments?*	**HCT**	**Selection**	**++** if the method is described **+** if the authors only say that randomisation was done **-** no mentioning randomisation **--** clearly not randomised
2	*Was allocation to study groups adequately concealed?*	**HCT**	**Selection**	**++** Allocation to study groups was concealed and the procedure is described and considered adequate **+** Concealment is mentioned but not described. Alternatively, it can be concluded that lack of concealment, due to the nature of the intervention, is not important. **-** Allocation to study groups was not concealed and it is concluded that lack of concealment here **may** be a source of bias. **--** Allocation to study groups was not concealed and it is concluded that lack of concealment here **will most likely have led to bias**.
3*	*Did the study population allow for appropriate comparisons?*	**Observational** **Cohort and cross-sectional studies**	**Selection**	**++** All participants were recruited from the same eligible population using the same inclusion exclusion criteria. There is clear evidence or data to suggest that that participant response rate is not related to exposure. **+** All participants were recruited from the same eligible population using the same inclusion exclusion criteria. It is concluded that participant response rate is unlikely to be related to exposure. **-** There were **some** differences in participant recruitment either in terms of different inclusion/exclusion criteria, time frame or differences in the (sub-)populations recruited. It is concluded that this **may be a source of bias**. **--** There were **important** differences in participant recruitment either in terms of different inclusion/exclusion criteria, time frame or differences in the (sub-)populations recruited. It is concluded that this **will most likely have led to bias**.

(Continued)

	Question	Study type	Bias domain	Rating (++/+/-/--)
		Case–Control studies		++ Cases and controls were recruited from the same eligible population using the same inclusion exclusion criteria (including time frame). **Data clearly shows that cases and controls are similar** (apart from their disease's status) and controls are described as having no history of the outcome. + Cases and controls were recruited from the same eligible population using the same inclusion exclusion criteria (including time frame). It is concluded that cases and controls are similar* (apart from their disease's status) and controls are described as having no history of the outcome. *Information on some relevant characteristics of cases and control may be absent or there are observed differences between cases and controls. In both cases this is juged that this will have led to bias - Cases and controls were not similarly recruited in terms of either source population or inclusion exclusion criteria (including time frame), **which may lead to some bias**; OR the information provided about the controls or the comparison between cases and controls is **not sufficient to exclude bias**. -- Cases and controls were not similarly recruited, and **this is judged to be a source of bias**; OR the information provided about the controls or the comparison between cases and controls is not sufficient and is **judged to be a source of bias**.
4*	*Did the study design or analysis account for important confounding and modifying variables?*	**Observational**	**Confounding**	++ It is judged that appropriate adjustments were made for all relevant confounders and/or covariates * and these were assessed using **valid and reliable measurements** *Acceptable considerations of confounder control include cases when the factor is not included in the final adjustment model because the author conducted analyses that indicated it did not need to be included. Adjustment for relevant co-exposures need to be considered on a case-by-case basis + It is judged that appropriate adjustments were made confounders and/or covariates and these were assessed using **acceptable methods**. Absence of control for known or possible confounders is not considered to appreciably bias the results*. *Either due to absence of such factors (e.g. co-exposure) or due to known or suspected weak confounding. - It is judged that one or more relevant confounders and/or covariates are not accounted for; and **this may lead to some bias**. Alternatively, the methods used to quantify important covariates or confounders were not appropriate which may, despite control, lead to some bias. -- It is judged that lack of control for important confounders and/or covariates **will most likely have led to bias**. Alternatively important covariates were assessed using i**nappropriate methods** which would are not suitable for confounder control.
5	*Were the research personnel and study participants blinded to the study group during the study?*	**HCT**	**Performance**	++ It is clearly stated that study participants and research personnel were blinded to the intervention status. How that was achieved is also explained and consisted appropriate. + Blinding of study participants and research personnel is mentioned but not described. Alternatively, it is judged that due to the nature of the intervention, departures from blinding of participants, research personnel or both is not important - There is insufficient information provided about blinding of participants and/or outcome assessors. Alternatively, there is indication that full blinding was not achieved. It is judged that this **may** be a source of bias. -- There is cleat lack of blinding of participants and/or research personnel (or insufficient information provided). This is judged taht lack of blinidn **is** a source of bias.

(Continued)

	Question	Study type	Bias domain	Rating (++/+/-/--)
6*	Were outcome data complete without attrition or exclusion from analysis?	**HCT, Co, CaCo, CrSe**	**Attrition/ exclusion**	**++** It is judged that that loss of subjects (i.e. incomplete outcome data) was adequately addressed and any removal or dropout of participants are documented and justified* *Acceptable attrition includes: very little missing outcome data; reasons for missing is unlikely to be related to outcome; missing outcome data is balanced across study groups with similar reasons for missing. Similarly imputing of missing data may be considered acceptable (but not % of missing is large). **+** Loss of subjects* (i.e. incomplete outcome data) could have been better explained and documented; or loss to follow-up is a cause of concern. Still it is judged that this dropout would not appreciably bias the results (see * for ++ above). **-** It is judged that that loss of subjects (i.e. incomplete outcome data) or loss to follow-up is unacceptably large and not adequately addressed. - It is judged that loss of subjects (i.e. incomplete outcome data) is unacceptably large and not adequately addressed, which is likely a source of bias** ** Reason for missing outcome is likely to be related to the outcome, with either imbalance in numbers or reasons for missing data across study groups; or potentially inappropriate application of imputation.
7*#	Can we be confident in the exposure characterisation?	**HCT, Co, CaCo, CrSe**	**Detection**	**++** It is judged that exposure was consistently assessed in all subjects (i.e. same method and time-frame); **AND** that well-established methods* were used that directly measure exposure at the individual level **OR** indirect methods which are validated against well-established methods *Direct methods: Blood and urine > hair and nails **+** It is judged that any inconsistent assessment of the exposure or any uncertainty regarding the method used to assess exposure (including use of **indirect methods**) would not considerably bias the results. ** **Indirect methods:** Water or water+diet OK if validated somehow against a biomarkers **-** It is judged that inconsistency in assessment of exposure or the method used for exposure assessment is somewhat inadequate and unreliable which **may lead to some bias**. **--** It is judged that that exposure the method for exposure assessment is inadequate and/or unreliable which **will most likely have led to bias**.
8*# Can we be confident in the outcome assessment (e.g. missing outcome)? **HCT, Co, CaCo, CrSe**			**Detection** *Element 1:* Was the outcome assessed using well-established methods?	**++** It is judged that the outcome was assessed using well-established methods that can be considered gold standard. **+** It is judged that that the outcome was assessed using well-established methods. **OR** it is deemed that any uncertainty in the method used to assess the outcome would not appreciably bias results. **-** It is judged that that there are flaws in the method used to assess the outcome which may bias the results **OR** There is insufficient information provided about the outcome assessment methods. **--** The outcome was assessed using a non-acceptable method.
			Element 2: Was the time-window between exposure and outcome assessment appropriate?	**++** The time window is considered *optimal* to capture any underlying relationship between exposure and outcome. **+** The time window is considered *sufficient* to capture any underlying relationship between exposure and outcome. **-** The time window (or lack of clarity on that window) *may not be sufficient* to capture any underlying relationship between exposure and outcome **but** may still provide useful information on that relationship. **--** The time window between exposure and outcome is considered insufficient to capture any underlying relationship between exposure and outcome. assessment was not appropriate for the endpoint of interest

(Continued)

	Question	Study type	Bias domain	Rating (++/+/-/--)
9	Were all measured outcomes reported?	**HCT, Co, CaCo, CrSe**	**Selective reporting**	**++** all outcomes relevant for the evaluation (as outlined in the protocol or manuscript) are reported in sufficient detail. **+** Lack of reporting for some outcomes or detail of reporting is not considered critical for the evaluation **OR** data driven analyses are clearly indicated as such and it is deemed that the unplanned analyses were appropriate and selective reporting would not appreciably bias results. **-** Not all of the measured outcomes are reported and their absence complicates or may lead to bias in interpretation of the study. **OR** there are indications that important findings may be a result for data driven analyses **--** Important outcomes are left unreported. **OR** there is strong suspicion that findings are based on data driven analyses (and biased) analyses.
10	Were statistical methods appropriate?	**HCT, Co, CaCo, CrSe**	**Other sources of bias**	**++** The statistical methods have been described in detail and are considered appropriate. **+** Some information on the statistical methods is lacking but there is enough information to conclude that the methods used are appropriate. **-** The statistical methods and underlying assumptions are not sufficiently well described making it difficult to o judge whether they are appropriate. **--** The statistical methods used are considered inappropriate making it likely that they could lead to biased results/conclusions

* Key question for HCT.

Key question for CaCo, Co and CrSe.

APPENDIX F

Food contribution to dietary intake of fluoride

TABLE F.1 Food contribution to adult exposure, in the Basic scenario (mg per day).

Country	Survey	Popclass	foodex2_L2	N_Subjects	Drinking water Mean_L2_LB	Drinking water P95_L2_LB	Mean TOT_LB	Perc_LB	FOOD: Total - drinking water
Austria	AT-NATIONAL-2016	Adults	Drinking water	2169	0.12	0.31	0.77	16.2	0.64
Belgium	NATIONAL-FCS-2014	Adults	Drinking water	1234	0.14	0.35	0.75	18.0	0.62
Cyprus	CY 2014-2017-LOT2	Adults	Drinking water	272	0.16	0.39	0.67	24.5	0.51
Czechia	SISP04	Adults	Drinking water	1666	0.12	0.30	0.74	16.8	0.62
Germany	NATIONAL NUTRITION SURVEY II	Adults	Drinking water	10419	0.19	0.49	0.90	20.7	0.71
Denmark	DANSDA2005-08	Adults	Drinking water	1739	0.08	0.24	0.83	9.9	0.75
Estonia	DIET-2014-EST-A	Adults	Drinking water	2124	0.06	0.19	0.73	8.6	0.66
Spain	ENALIA2	Adults	Drinking water	536	0.04	0.10	0.47	9.1	0.43
Finland	FINDIET2017	Adults	Drinking water	1196	0.09	0.26	0.75	12.7	0.65
France	INCA3	Adults	Drinking water	1773	0.10	0.25	0.82	11.7	0.73
Greece	GR-EFSA-LOT2 2014-2015	Adults	Drinking water	260	0.03	0.09	0.50	6.8	0.46
Croatia	NIPNOP-HAH-2011-2012	Adults	Drinking water	2002	0.08	0.18	0.55	14.0	0.47
Hungary	EU MENU DIETARY SURVEY OF HUNGARY	Adults	Drinking water	529	0.16	0.45	0.86	18.0	0.71
Ireland	NANS2012	Adults	Drinking water	1274	0.13	0.37	1.14	11.5	1.01
Italy	INRAN SCAI 2005-06	Adults	Drinking water	2313	0.11	0.26	0.64	16.4	0.54
Latvia	LATVIA_2014	Adults	Drinking water	1080	0.04	0.16	0.83	4.9	0.79
Netherlands	FCS2016_CORE	Adults	Drinking water	1478	0.05	0.15	1.02	5.0	0.97
Portugal	IAN-AF2015-2016	Adults	Drinking water	3102	0.13	0.33	0.72	18.2	0.59
Romania	RO-DIET-NATIONAL-STUDY-2019	Adults	Drinking water	740	0.17	0.40	0.69	24.6	0.52
Sweden	RIKSMATEN 2010	Adults	Drinking water	1430	0.05	0.16	0.70	7.0	0.65
Sweden	RIKSMATEN ADOLESCENTS 2016	Adults	Drinking water	215	0.05	0.14	0.73	6.4	0.68
Slovenia	SI.MENU-2018	Adults	Drinking water	385	0.06	0.18	0.54	11.5	0.48
Min					0.03	0.09	0.47		**0.43**
Mean					0.10	0.26	0.74		**0.64**
Max					0.19	0.49	1.14		**1.01**

TABLE F.2 Food contribution to exposure in toddlers (1–3 years), in the Basic scenario (mg/kg bw per day).

Country	Survey	Popclass	foodex2_L1	N_Subjects	Drinking water Mean_L2_LB	Drinking water P95_L2_LB	Mean TOT_LB	Perc_LB	FOOD: Total - drinking water
Cyprus	CY 2014-2017-LOT2	Toddlers	Drinking water	275	0.0066	0.0169	0.0310	21.2548	0.0244
Hungary	EU MENU DIETARY SURVEY OF HUNGARY	Toddlers	Drinking water	535	0.0042	0.0127	0.0304	13.7156	0.0262
Bulgaria	NUTRICHILD	Toddlers	Drinking water	428	0.0019	0.0058	0.0285	6.5735	0.0266
Spain	ENALIA	Toddlers	Drinking water	326	0.0016	0.0037	0.0284	5.7666	0.0267
Italy	INRAN SCAI 2005-06	Toddlers	Drinking water	36	0.0045	0.0121	0.0276	16.2940	0.0231
Belgium	REGIONAL	Toddlers	Drinking water	36	0.0014	0.0037	0.0269	5.1502	0.0255
Portugal	IAN-AF2015-2016	Toddlers	Drinking water	571	0.0027	0.0086	0.0267	9.9514	0.0240
Latvia	LATVIA_2014	Toddlers	Drinking water	242	0.0014	0.0041	0.0260	5.2708	0.0246
France	INCA3	Toddlers	Drinking water	139	0.0023	0.0066	0.0256	8.9144	0.0233
Netherland	FCS2016_CORE	Toddlers	Drinking water	440	0.0013	0.0031	0.0248	5.1612	0.0236
Estonia	DIET-2014-EST-A	Toddlers	Drinking water	268	0.0013	0.0033	0.0247	5.3570	0.0234
Finland	DIPP2001-2009	Toddlers	Drinking water	500	0.0022	0.0037	0.0246	8.7763	0.0225
Slovenia	SI.MENU-2018	Toddlers	Drinking water	343	0.0013	0.0032	0.0237	5.3098	0.0224
Denmark	IAT 2006-07	Toddlers	Drinking water	917	0.0016	0.0030	0.0237	6.7480	0.0221
Germany	VELS	Toddlers	Drinking water	348	0.0023	0.0063	0.0220	10.4406	0.0197
Min					0.0013	0.0030	0.0220		0.0197
Median					0.0019	0.0041	0.0260		0.0236
Max					0.0066	0.0169	0.0310		0.0267

TABLE F.3 Food contribution to exposure in children (4–8 years), in the Basic scenario (mg/kg bw per day).

Country	Survey	Popclass	foodex2_L1	N_Subjects	Drinking water Mean_L2_LB	Drinking water P95_L2_LB	Mean TOT_LB	Perc_LB	FOOD: Total - drinking water
Hungary	EU MENU DIETARY SURVEY OF HUNGARY	Other children	Drinking water	537	0.0028	0.0078	0.0258	10.7885	0.0230
Latvia	LATVIA_2014	Other children	Drinking water	782	0.0014	0.0042	0.0255	5.5026	0.0241
Bulgaria	NUTRICHILD	Other children	Drinking water	433	0.0018	0.0050	0.0246	7.1599	0.0228
Portugal	IAN-AF2015-2016	Other children	Drinking water	521	0.0022	0.0065	0.0219	10.1478	0.0197
Italy	INRAN SCAI 2005-06	Other children	Drinking water	193	0.0029	0.0062	0.0212	13.5903	0.0183
Cyprus	CY 2014-2017-LOT2	Other children	Drinking water	297	0.0043	0.0118	0.0200	21.6864	0.0156
Estonia	DIET-2014-EST-A	Other children	Drinking water	765	0.0010	0.0026	0.0199	4.9520	0.0189
Czechia	SISP04	Other children	Drinking water	389	0.0020	0.0049	0.0192	10.2577	0.0173
Germany	ESKIMO	Other children	Drinking water	835	0.0031	0.0089	0.0190	16.2352	0.0159
France	INCA3	Other children	Drinking water	852	0.0021	0.0058	0.0190	11.2213	0.0168
Germany	VELS	Other children	Drinking water	293	0.0017	0.0042	0.0189	8.7597	0.0173
Belgium	NATIONAL-FCS-2014	Other children	Drinking water	985	0.0030	0.0073	0.0187	16.2046	0.0157
Netherland	FCS2016_CORE	Other children	Drinking water	853	0.0010	0.0023	0.0186	5.2761	0.0176
Denmark	DANSDA2005-08	Other children	Drinking water	298	0.0016	0.0039	0.0182	8.7484	0.0166
Spain	ENALIA	Other children	Drinking water	556	0.0013	0.0030	0.0182	7.1079	0.0169
Greece	REGIONAL	Other children	Drinking water	838	0.0000	0.0000	0.0181	0.0176	0.0181
Austria	ASNS-CHI	Other children	Drinking water	128	0.0015	0.0043	0.0173	8.4389	0.0158
Finland	DIPP2001-2009	Other children	Drinking water	750	0.0009	0.0019	0.0168	5.4220	0.0159
Sweden	NFA	Other children	Drinking water	1473	0.0003	0.0011	0.0153	2.1731	0.0150
Min					0.0000	0.0000	0.0153		0.0150
Median					0.0017	0.0043	0.0190		0.0173
Max					0.0043	0.0118	0.0258		0.0241

TABLE F.4 Food contribution to exposure in infants in the Basic scenario (mg/kg bw per day).

Country	Survey	Popclass	foodex2_L1	N_Subjects	Drinking water Mean_L2_LB	Drinking water P95_L2_LB	Mean TOT_LB	Perc_LB	FOOD: Total - drinking water
France	INCA3	Infants	Drinking water	37	0.003	0.013	0.035	7.873	0.033
Latvia	LATVIA_2014	Infants	Drinking water	143	0.001	0.003	0.035	2.896	0.034
Italy	INRAN SCAI 2005-06	Infants	Drinking water	12	0.013	0.060	0.034	37.780	0.021
Cyprus	CY 2014-2017-LOT1	Infants	Drinking water	206	0.003	0.010	0.031	8.133	0.029
Portugal	IAN-AF2015-2016	Infants	Drinking water	234	0.002	0.009	0.026	8.682	0.024
Bulgaria	NUTRICHILD	Infants	Drinking water	659	0.003	0.009	0.026	10.593	0.023
Denmark	IAT2006-07	Infants	Drinking water	826	0.002	0.004	0.026	6.415	0.024
Slovenia	SI.MENU-2018	Infants	Drinking water	294	0.001	0.003	0.021	3.877	0.020
Finland	DIPP2001-2009	Infants	Drinking water	500	0.001	0.003	0.019	7.392	0.018
Germany	VELS	Infants	Drinking water	159	0.003	0.010	0.019	14.290	0.017
Estonia	DIET-2014-EST-C	Infants	Drinking water	504	0.0005	0.002	0.011	4.304	0.010
Min					0.000	0.002	0.011		0.0103
Median					0.002	0.009	0.026		0.0231
Max					0.013	0.060	0.035		0.0337

ANNEXES

All Annexes are available under the supporting information section on Wiley Online Library.

Annex A: Report of the Outcome of Public Consultation

Annex B: Protocol for human health risk assessment of fluoride in food and drinking water taking into account all sources of oral exposure

Annex C: Exposure assessment data

Annex D: Human studies on Fluoride reporting on neurodevelopment, thyroid and bone

Annex E: Animal Studies on Fluoride reporting on neurodevelopment, thyroid and bone

Annex F: Uncertainty Analysis

Annex G: Literature searches

Annex H: Data extraction tables of human and animal studies reporting on other endpoints