RISK SCIENCES INTERNATIONAL

REPORT

Critical Review of Potential Adverse Health Effects of Fluoride in Drinking Water

Prepared for:

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II. Glossary

The following list of abbreviations excludes one-time uses of common gene names and several in-text abbreviations found in tables of results, where the expanded term and abbreviation are described within the same section of the table.

25OHD	25-hydroxyvitamin D
aRR	Adjusted relative risk
ABP	Androgen binding protein
ADHD	Attention deficit hyperactivity disorder
ALT	Alanine aminotransferase
AST	Aspartate transaminase
ATPase	Adenosine triphosphate enzymes
BMD *	Bone mineral density
BMD *	Benchmark dose (NOTE: the same abbreviation is used in two different ways throughout the document. The context of the differing uses is always clear. This was intentionally done because both uses are established in the medical and statistical literature, respectively.)
BMC	Benchmark concentration
BMCL	Benchmark concentration lower bound
BMDL	Benchmark dose lower bound
BMI	Body mass index
BMR	Benchmark response
CADTH	Canadian Agency for Drugs and Technologies in Health
CFI	Community Fluoridation Index
CI	Confidence interval
CKD	Chronic kidney disease (CKDu, of uncertain etiology)

CVD	Cardiovascular disease
CWF	Community water fluoridation
DA	Dopamine
DDE	Developmental defects of enamel
DF	Dental fluorosis
DMA	Dimethylarsinic acid
D-R	Dose-response
DSM	Diagnostic and Statistical Manual of Mental Disorders
DW	Drinking water
DWL	Drinking water levels
E2	Estradiol
ER	Endoplasmic reticulum (i.e., ER stress)
ESRα	exposure and estrogen receptor alpha
FSH	Follicle stimulating hormone
FT	Free T4 index
GCI	General Cognitive Index
GIT	Gastrointestinal Tract Disorder
HBV	Health-based Value
HR	Hazard ratio
IARC	International Agency for Research on Cancer
IQ	Intelligence quotient
IQR	Interquartile range
LH	Luteinizing hormone
LOAEL	Lowest observed adverse effect level
MAC	Maximum acceptable concentration

MUFcr	Creatinine adjusted maternal urinary fluoride
NaF	Sodium fluoride
NASEM	National Academy of Sciences, Engineering, and Medicine
NHMRC	Australian National Health and Medical Research Council
NOAEL	No observed adverse effect level
NR	Not reported
NTP	National Toxicology Program
OCDO	Office of the Chief Dental Officer
OHAT	Office of Health Assessment and Translation (US National Toxicology Program)
OR	Odds ratio
P	Progesterone
PMI	Primary methylation index
POD	Point of departure
PPM	Parts per million
PTH	Parathyroid hormone
SD	Standard deviation
SDQ	Strengths and Difficulties Questionnaire
SE	Standard error
SHBG	Sex hormone-binding globulin
SMI	Secondary methylation index
SR	Systematic review
SUA	Serum uric acid
TSH	Thyroid-stimulating hormone
TT3	Total triiodothyronine
TT4	Total thyroxine

Tvol	Thyroid volumes
UF	Uncertainty factor
UF_{SG}	Urinary fluoride adjusted for specific gravity
WQP	Water Quality Program

III. Executive summary

Health Canada's current guideline technical document for fluoride in drinking water [1] derives a health-based value (HBV) of 0.9 mg/L (considered to be protective against all potential adverse health effects) and presents an optimal concentration for dental health of 0.7 mg/L for communities that choose to fluoridate their water. At present, the maximum acceptable concentration (MAC) for fluoride in drinking water of 1.5 mg/L is based on drinking water treatment considerations. The Water Quality Program (WQP) at Health Canada recently underwent a robust prioritization process for drinking water chemicals. Fluoride was identified as a high priority for review due to both new scientific publications and provincial and territorial needs. Moderate dental fluorosis is the key endpoint for Health Canada's current HBV; however, there is growing evidence that potential neurotoxic effects may occur at lower levels than previously thought. The mandate of the current review was to provide a critical evidence synthesis for the purposes of updating the current HBV for fluoride in drinking water.

This current review (the "RSI review") represents an update of several prior reviews on multiple health endpoints that were published by CADTH 2019 [2, 3] (human studies), Health Canada 2010 [1] (animal and in vitro studies), and NTP 2016 [4] (specifically on cognitive and neurotoxic endpoints over multiple streams of evidence). The RSI review consisted of a systematic literature search that was conducted between February 10 – 12, 2020 for all eligible types of publications. An update of this search for all potential adverse health effects was conducted on July 21 – 22, 2021, with the exception of reviews and in vitro evidence. A third update of the search was conducted on February 2, 2023 for studies on two endpoints: dental fluorosis and effects on IQ scores. The systematic search of human and animal evidence was conducted in accordance with the PRISMA guidelines, following the specific guidance provided by the Cochrane Collaboration, and covered 10 bibliographic databases, 6 clinical trial registries, and major grey literature sources. The quality of included human and animal studies was assessed using the US National Toxicology Program's OHAT risk of bias tool [5, 6], which includes seven domains of bias against which each study was assessed for quality of evidence. Mechanistic and in vitro evidence from systematic and authoritative reviews were critically assessed and summarized.

The RSI search for human evidence identified 89 studies that were conducted between 2016-2023, including cross-sectional (n=70), ecological (n=4), cohort (n=9), case-control (n=4)

studies, and abstracts (n=2). Studies were conducted in China (n=18), India (n=17), USA(n=9), Mexico (n=8), Canada (n=7), Brazil (n=5). Three studies were conducted in Pakistan and Sri Lanka, two in each of Ethiopia and Malaysia, and 1 study in each of Egypt, Indonesia, Iran, Ireland, Jordan, Lithuania, Peru, Saudi Arabia, Scotland, South Korea, Spain, Sudan, Sweden, Thailand, Ukraine. Most of the studies examined fluoride exposure in drinking water (n=57) or ground water (n=17). The examined populations were children and/or adolescents (n=54), adults (n=15), mixed (n=19 including 6 that enrolled mother-child pairs), and unreported population age (n=1).

NTP published updated draft reports in 2020 [328] and in 2022[420] after independent reviews conducted by the National Academy of Sciences, Engineering, and Medicine (NASEM)[7]. This update included a review of epidemiologic evidence on fluoride-related cognitive effects. The NTP results and conclusions are discussed in the context of evidence reviewed in the current report.

One hundred and ninety-nine original animal studies were retained for data abstraction and detailed analysis. Using a tiered approach of relevance and quality, 35 tier-1 studies were included in the evidence synthesis. These studies covered the effect of fluoride on several endpoints of concern over a range of drinking water fluoride concentrations for sub chronic to chronic durations; the majority of the studies investigated neurological, developmental, or reproductive outcomes. Ten reviews were identified that summarized effects of fluoride at cellular levels and its mechanism of action. These reviews indicated that fluoride caused changes in oxidative stress levels, gene expression levels, mitochondrial dysfunction, and eventual cell death through various molecular pathways and mechanisms, including ER stress, Na/K+ ATPase pathway, apoptosis, inflammatory pathways, or death receptor-mediated pathways. However, the evidence was considered too non-specific to necessarily support particular mechanisms that could be attributed to specific health outcomes.

Based on the entire body of evidence reported from human, animal, and in vitro streams to date, and relying predominantly on studies of high or acceptable quality, four endpoints were chosen as candidates for further assessment using the Bradford Hill considerations [8] on causality. These endpoints included cognitive (IQ) effects in children, thyroid dysfunction, kidney dysfunction, and sex hormone disruptions. The evidence supports a conclusion that fluoride exposure reduces IQ levels in children, likely at exposure levels close to those

currently seen in Canadian drinking water. The evidence also moderately supports the link with thyroid dysfunction, and weakly supports the link with kidney dysfunction. Evidence was considered limited to support a link between fluoride and sex hormone disruptions.

No points of departure (POD) were derived for thyroid dysfunction, kidney dysfunction, and sex hormone disruption because more evidence is needed to support the causal link of fluoride exposure and these health endpoints.

The US EPA, in its 2010 report on non-cancer effects of fluoride, used data from Dean (1942) to derive a benchmark concentration for severe dental fluorosis. In the current report, based on the selection of moderate dental fluorosis as an endpoint of concern, all epidemiologic studies published since Dean (1942) were considered as possible key studies for benchmark dose modelling. Based on several considerations, including risk of bias, sources of fluoride, and adequacy of data for modelling, Dean (1942) was still found to be a preferable key study. In the current work, several models were fit based on Dean's fluorosis index (DFI), using 3+ as the cutoff to combine moderate (DFI=3) and severe (DFI=4) categories of fluorosis. Bayesian model averaging was used to derive the lower bounds of the benchmark concentration (BMCL) of 1.56 mg/L for a benchmark response (BMR) of 1%, 2.13 mg/L for a BMR of 5%, and 2.46 mg/L for a BMR of 10%.

In considering cognitive effects in children, specifically for IQ reductions, there remains significant uncertainty as to the POD. While statements from draft NTP reports (2020,2022) concluded that evidence for associations below 1.5 mg/L was less consistent than above 1.5 mg/L, a recent benchmark dose analysis (Grandjean et al., 2022) using high-quality cohort data (from MIREC and ELEMENT) with individual-level measures provided maternal urinary fluoride benchmark concentrations far lower than 1.5 mg/L for a 1-point IQ reduction. For the pooled cohort studies, for both sexes at the youngest ages, the BMCL was 0.179 mg F/L in drinking water (when converted from maternal urinary fluoride) with a linear model, but various models produced estimates that varied by more than 9-fold (ranging from 0.077 mg F/L to 0.753 mg F/L drinking water). Considering the overall evidence for an effect of fluoride on IQ reduction in children, the POD for IQ may provisionally be considered as 1.5 mg F/L, while acknowledging that credible evidence exists to support that the POD may be lower than this concentration (based on cohort data). However, the majority of studies to date are cross-

sectional studies of low quality, and there is uncertainty in the shape of the dose-response curve at low levels of exposure to fluoride.

Since the POD of 1.56 mg F/L for moderate and severe dental fluorosis is based on high-quality population-based data in the target population (children), with less concern about other sources of ingested fluoride, a minimal adjustment factor could be entertained in deriving an HBV based on fluorosis. However, with currently available evidence suggesting that fluoride leads to reductions in children's IQ – arguably a more severe adverse health outcome than moderate dental fluorosis – the possibility of cognitive effects in children should be taken into account in setting an HBV for fluoride in drinking water. As the POD for IQ reduction is not yet well defined, the POD of 1.56 mg F/L for moderate dental fluorosis may be preferred as a starting point for deriving the HBV. To allow for protection against potential cognitive effects, an additional overall database uncertainty factor could be applied to this POD. As additional information on the association between fluoride in drinking water and reduction in children's IQ becomes available, the choice of the most appropriate endpoint on which to base the POD to serve as the starting point to deriving an HBV for fluoride can be revisited.

IV. Background

The Water Quality Program (WQP) at Health Canada recently underwent a robust prioritization process for drinking water chemicals. Fluoride was identified as a high priority for review due to both new scientific publications and provincial and territorial needs.

Moderate dental fluorosis is the key endpoint for Health Canada's current Health-based Value (HBV) in drinking water. Fluoride has been associated with various adverse health effects, including neurotoxicity. There is growing evidence that potential neurotoxic effects may occur at levels of exposure that are environmentally relevant and at lower levels than previously thought. Although Canada is not known to have wide geographic areas with elevated fluoride concentrations, fluoride may be found in Canadian water sources at levels of exposure cited in the new studies showing neurotoxic effects in children.

Health Canada's WQP published a review of the health effects of exposure to fluoride in drinking water in 2010, as part of the update to the Guideline for Canadian Drinking Water Quality for Fluoride [1]. This publication discussed all relevant scientific information available at the time of the review, as well as recommendations from an expert panel on fluoride. The panel was convened by HC in 2007 to provide recommendations related to fluoride in drinking water, including both potential adverse health effects and the public health benefit of preventing dental caries. Health Canada's Office of the Chief Dental Officer (OCDO) was part of the panel and reviewed dental effects of fluoride. The OCDO accepted the expert advice on the optimal level for fluoride in drinking water. The Health Canada guideline technical document [1] for fluoride includes a derivation of an HBV of 0.9 mg/L (considered to be protective against any potential adverse health effects), with a rationale provided by the Federal-Provincial-Territorial Committee on Drinking Water to risk manage at a maximum acceptable concentration (MAC) of 1.5 mg/L for fluoride in drinking water. Decisions to fluoridate drinking water are made at the municipal and/or provincial/territorial level.

The OCDO recently commissioned a third-party (the Canadian Agency for Drugs and Technologies in Health, CADTH) comprehensive review of the evidence and other considerations related to community water fluoridation (CWF), including the effectiveness and health impacts of CWF and ethical considerations. The results of the CADTH review [2, 3] were published on February 8th, 2019. The report acknowledges that dental fluorosis prevalence

may increase with increasing water fluoride levels, even though dental fluorosis of “aesthetic concern” among Canadian children is rare. Conclusions on the health impacts of CWF from this report are of qualitative nature and cannot be used in the context of a quantitative risk assessment, where the goal is to determine a “safe” level of exposure to a substance. The CADTH review was focused on community water fluoridation around 0.7 mg/L, and not the range of naturally occurring levels, which could be much higher (>1.5 mg/L). In order to determine an HBV and MAC, the WQP needed to build on the CADTH review to establish at which level in drinking water fluoride may be associated with various health outcomes, including neurotoxicity.

In recognition of recent research findings, the results of the prioritization exercise and the public interest in this issue, it was proposed that the state of the science of the health effects of fluoride in drinking water be re-examined. The current report consists of a critical review of the scientific evidence on potential health effects of fluoride in drinking water, complementary to the CADTH review.

The objective of this review was to assess the current body of evidence to support the update of Health Canada’s guideline for fluoride levels in drinking water, as well as inform any potential revision of the HBV. The work involved conducting a critical review of recently published evidence from human, animal, and in vitro studies.

V. Literature review methods

The current project (the “RSI review”) sought to update recent reviews with the following scope:

- For endpoints on dental fluorosis, cancer, bone/skeletal toxicity, developmental/reproductive toxicity, endocrine toxicity (including thyroid effects), immunotoxicity, genotoxicity and all other potential adverse effects), an update was undertaken of human evidence (CADTH 2019 [2]), animal and in vitro evidence (Health Canada 2010 [1]).
- For endpoints on neurotoxicity and other cognitive domains, an update was undertaken on human evidence (CADTH 2019a [3]), animal evidence (NTP 2016 [4]), and in vitro evidence (Health Canada 2010 [1]).

The eligibility criteria for inclusion in the RSI review included all original studies and reviews published between 2016 to present, which examined the association of exposure to fluoride in drinking water (fluoridation, or naturally occurring fluoride) with any adverse health outcomes. Exclusion criteria contained studies and/or reviews that were examined in CADTH 2019 reports [2, 3], published prior to 2016, examined other fluoride formulations or mixtures, assessed dental outcomes other than dental fluorosis, reported irrelevant assessments (e.g., hazard quotient), or published in a non-Latin language. Full-text references that could not be retrieved, or other irrelevant study types such as commentaries, editorials, case reports/case series, books and general informational materials were also excluded.

Human evidence

Previous reviews of human evidence

The RSI review is an update of two prior reports published by the Canadian Agency for Drugs and Technologies in Health (CADTH) in 2019 [2, 3]. The first review by CADTH [2] was an update of the systematic review conducted by the Australian National Health and Medical Research Council (NHMRC) in 2016 [9, 10], and investigated three research questions, where only the third was found to be relevant to the RSI review: “What are the negative effects of community water fluoridation (at a given fluoride level) compared with non-fluoridated drinking water (fluoride level < 0.4 ppm) or fluoridation at different levels on human health outcomes?”.

The literature search, which was developed using both controlled vocabulary and keywords, was peer-reviewed, and conducted by an information specialist. Multiple databases were searched to capture potentially relevant studies from 2014-2018. No search restrictions by language or methodology were applied. Search of grey literature sources, web-based materials, and reference lists of key articles were conducted. References captured by the first CADTH report [2] search underwent level 1 (title and abstract) and 2 (full-text) screening by two independent reviewers. In total, 41 studies were identified in addition to the 41 studies already included by the NHMRC 2016 report [9, 10].

The second report by CADTH 2019a [3] investigated a related but more focused research question: “What are the neurological or cognitive effects of community water fluoridation, compared with non-fluoridated or different fluoride levels in drinking water, in individuals less

than 18 years of age?". The literature search examined human studies from multiple databases between 2017-2019. No limitation by study type was applied. References identified by the second CADTH review [3] search were screened by a single reviewer. Studies already included in the prior CADTH 2019 report [2] were excluded from the review. In total, only a single prospective cohort study was identified as relevant. As part of the RSI review exclusion criteria, all references examined by either one of the 2 CADTH reports [2, 3] were not included in the RSI review.

The RSI search strategy: Identification of new human evidence

In conducting this updated review, RSI examined more recent human evidence on the association between fluoride in drinking water with a wide range of endpoints, which was published between 2016 – present and not included in either of the 2019 reports by the Canadian Agency for Drugs and Technologies in Health (CADTH) on neurocognitive [3], and dental and other endpoints [2].

A comprehensive, multi-step search strategy was implemented to identify review articles and original human studies that examined the association between exposure to fluoride in drinking water with the risk of adverse health outcomes. No filters were applied to limit the search output. The search was conducted in accordance with PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses), and following the specific guidance provided by the Cochrane Collaboration [11].

The search strategy for human studies was designed and implemented between February 10-12, 2020 by two reviewers. An update for original studies on all adverse health effects was conducted on July 21, 2021.

As requested by Health Canada, an updated literature search was conducted for intelligence quotient (IQ) and dental fluorosis adverse health effects. The search of bibliographic databases was performed on February 2, 2023. Search results were compiled in EndNote, and duplicates were removed. Level 1 (title and abstract) and 2 (full-text) screening were independently completed by two reviewers, and any conflicts were resolved via discussion. Data abstraction and quality assessment were conducted by a single investigator and verified by a second investigator.

RSI searched 10 bibliographic databases, 6 clinical trial registries to identify relevant literature using specific keywords without applying any filters. See Section 1 of the Supplementary Material for details on the searched sources, used terms, and search output. RSI also searched 18 major grey literature sources such as relevant national and international authoritative and technical health agencies, academic dissertations, major scientific hubs, and international conference proceedings. Additionally, bibliographies of examined studies were inspected for additional relevant studies not already identified via the original search.

Date of updates of literature search	Evidence streams and target endpoints
February 10, 2020	Summary of *human, animal and in vitro* evidence from reviews and original studies
July 21, 2021	Systematic review of animal evidence on all endpoints
July 21, 2021	Systematic review of epidemiologic evidence on endpoints: sex hormone dysfunction, ADHD, dementia, liver dysfunction, memory loss, preterm births, genotoxicity, ultrastructural kidney changes, BMI, childhood obesity, selected eye diseases, general health, trouble working, suicide, arsenic methylation, skeletal fluorosis, thyroid dysfunction, bone density and quality, bone cancer, hip fracture, atherosclerosis, myocardial infarction, kidney dysfunction, headache, diabetes mellitus, non-skeletal manifestations of fluoride toxicity, and sleep-related outcomes
February 2, 2023	Systematic review of epidemiologic evidence on endpoints: cognitive (IQ), dental fluorosis

Identified references from all sources were collated using the EndNote [12] reference management application. EndNote was used to identify potential duplicates, with manual resolution employed to remove additional actual duplicates. Screening of titles and abstracts (level 1) and full-text examination (level 2) were performed independently by two reviewers to

identify studies eligible for inclusion in the review. The review was completed through a multi-level assessment process, using the Distiller SR software [13].

Conflicts identified in each step were resolved via consensus prior to moving to the next level. In the event where multiple publications report on an original study or trial, only the primary publication was assessed in the review.

The RSI literature search considered original published reviews to assess whether they would suffice as updates to the reviews by CADTH, Health Canada, or NTP. Reviews were screened using three criteria:

1. Does the review have sufficient description of its methodology? (Yes, but only if 1 or more databases were searched)
2. Is the review peer-reviewed (e.g., journal publication) or prepared by an authoritative body (e.g., IARC) or high-profile research agency?
3. Does the review present a clear overall conclusion on the body of literature examined for each outcome of interest?

Reviews with a “Yes” response to all questions were considered potentially eligible.

Data abstraction

Data abstraction spreadsheets were developed using Microsoft Office Excel, and used to abstract the following information: study design, study population characteristics, exposure and outcome assessment, results and authors’ reported conclusion. Key characteristics of the included human studies are summarized in Table 3 in this report and provided in full details in Section 3 of the Supplementary Material.

Statements on the assessment of the evolving evidence

For each endpoint, RSI classified the evolving evidence into one of the following categories:

- Sufficient: Most of the evidence consistently supports no association or a confirmed association, based on several studies of high to acceptable quality that have been published on the relevant fluoride and health endpoint.

- Limited: Some evidence in support of an association, based on only a few studies of high to acceptable quality that have been published on the relevant fluoride and health endpoint.
- Inconsistent: Mixed evidence in support of an association, based on studies of high to acceptable quality that provided conflicting evidence on the relevant fluoride and health endpoint.
- Insufficient: Scarce or unclear evidence in support of an association, based on too few studies of high to acceptable quality that have been published on the relevant fluoride and health endpoint.

Animal evidence

In conducting this review, RSI examined animal evidence on the association between fluoride in drinking water with a wide range of endpoints, from original studies published after 2006 and not included in the 2010 report by Health Canada [1] on several endpoints including neurotoxicity and neurobehavioral effects.

RSI implemented a comprehensive, multi-step search strategy to identify review articles and original human studies that examined the association between exposure to fluoride in drinking water with the risk of multiple adverse health outcomes. No filters other than “animals only” were applied to limit the search output. The search was conducted in accordance with PRISMA guidelines, and following specific guidance provided by the Cochrane Collaboration [11].

The search strategy for animal studies was designed and implemented between March 14-18, 2020 by two reviewers. An update for original studies was conducted on July 21, 2021. RSI searched 10 bibliographic databases to identify relevant literature using specific keywords without applying any filters. See Section 1 of the Supplementary Material for details on the searched sources, used terms and search output.

RSI also searched 18 major grey literature sources such as relevant national and international authoritative and technical health agencies, academic dissertations, major scientific hubs, and international conference proceedings. Additionally, an inspection of the bibliographies of

examined studies was conducted for additional relevant studies not already identified via the original search.

Identified references from all sources were collated using the EndNote [12] reference management application. EndNote was used to identify potential duplicates, with manual resolution employed to remove additional actual duplicates. Screening of titles and abstracts (level 1) and full-text examination (level 2) were performed independently by 2 reviewers to identify studies eligible for inclusion in the review. The review was completed through a multi-level assessment process, using the Distiller SR software [13]. Conflicts identified in each step were resolved via consensus prior to moving to the next level.

Data abstraction spreadsheets were developed using Microsoft Office Excel, and used to abstract the following information: study design (animal model, age, sex, number of animals, chemical salt, guideline compliance), treatment (dose levels, route of administration, exposure duration, dosing frequency), endpoint information, statistical methods, outcomes assessed, effects levels (LOAEL, and NOAEL [no-observed-adverse-effect level]]), dose response trend, strengths and limitations, and authors conclusions.

Due to the large volume of potentially eligible animal studies (~200), a tiered approach was employed to determine and select studies with “key” information relevant for the current objectives. This approach categorized studies into three tiers with tier-1 containing all “key” information for the review, and tier-2 containing supporting information. Furthermore, studies in tier-1 underwent full data extraction and quality assessment. Tier-1 studies tended to be guideline studies (OECD (office of economic collaboration and development), GLP (good lab practices)) that assessed oral route of exposure at relevant concentrations (≤ 20ppm). A limited data extraction with no quality assessment was performed for studies placed in tier-2. No data abstraction or quality assessment was undertaken for tier-3 studies (See Section 4 of the Supplementary Material). In this approach, each study that passed level 2 screening was reviewed for the following “key” information and placed in the appropriate tier.

- whether the study tested more than one fluoride exposure concentration (to understand dose-response relationships)
- at least one exposure concentration tested was below 20 ppm (to examine effects at environmentally relevant exposures)

- whether primary objective was fluoride toxicity (to eliminate intervention studies such as studies with focus on exposures that may enhance or protect against fluoride toxicity)
- whether the study evaluated solely mechanistic endpoints (not purely a mechanistic study)
- whether the study had been already evaluated by an authoritative body

These considerations for the tiered approach are outlined in Figure 1.

Criteria	Response	
Is dose - response information available	Yes	No
At least one test conc ≤ 20 ppm	Yes	No
Is primary objective fluoride toxicity	Yes	No
Only mechanistic outcomes assessed	Yes	No
Included in an authoritative review	Yes	No

Assessment	Tier
All green	1
If not in Tier 1, but Yes for D-R information or fluoride conc ≤ 20 ppm	2
If No for D-R information AND No for fluoride conc ≤ 20 ppm	3

Figure 1: Considerations for tiered approach for animal studies

In vitro evidence

The in vitro evidence stream was comprised of a review of reviews. Authoritative reviews were first identified as those published after the 2010 Health Canada report and those having sections that pertained to mechanistic or in vitro evidence with no restriction on the endpoint being considered in the review. Narrative summaries were then developed for each key mechanism of action related to fluoride. A general description was provided for the mechanism of action and how it related to selected health endpoints. This was supplemented with a table of recent studies and a brief extraction of characteristics of those studies.

Risk of bias assessment

The quality of included human, animal, and in vitro studies was assessed using the OHAT risk of bias tool, designed by the US National Toxicology Program [5, 6]. This tool enlists 10 parameters across 7 domains against which each study is assessed for quality of provided evidence. For every parameter, each study is assessed into one of four levels based on their

risk of bias: definitely low risk of bias (++), probably low risk of bias (+), probably high risk of bias (–), and definitely high risk of bias (– –).

Based on the assessment, each study is assigned a score of 1, 2, or 3 corresponding to high, acceptable, or low level of quality, respectively. Score 1 implies that a study must be rated as “definitely low” or “probably low” risk of bias for key elements AND have most other applicable items answered “definitely low” or “probably low” risk of bias. Score 3 implies that a study must be rated as “definitely high” or “probably high” risk of bias for key elements AND have most other applicable items answered “definitely high” or “probably high” risk of bias. Score 2 is reserved for studies not meeting the requirements for scores 1 or 2 on the risk of bias assessment. A summary of the risk of bias assessment of the included studies is shown in Table 4 (human studies) and Table 10 (animal studies) in this report. Full details of the assessment of human studies provided in Section 3 of the Supplementary Material.

VI. Literature review results

Human evidence

The search strategy resulted in retrieval of 4,141 records, including 3.783 records from bibliographic databases and clinical trial registries, 358 records from major grey literature sources. Electronic de-duplication resulted in removal of 1,397 records studies, leaving 2,774 studies for title and abstract screening (level 1). Upon excluding 2,990 irrelevant studies, there were 409 studies left for full-text examination (level 2). This examination led to the exclusion of an additional 344 references for not matching our inclusion/exclusion criteria. RSI were finally able to retain 65 original studies for further analysis.

The 2023 search for new evidence for exposure to fluoride in drinking water and either effects on IQ or on dental fluorosis, identified 879 new studies since the last update in 2022. Upon removing duplicates and studies examined in the 2022 RSI report, a total of 343 studies underwent independent and duplicate title, abstract and full-text examination, leading to identification of 24 new studies, raising the number of retained studies to 89. A detailed PRISMA flow diagram [14] showing the selection process for human studies for the first and second searches only is shown in Figure 2. A summary of rationale for study exclusion for the first two searches as well is summarized in Table 1, and detailed in Section 2 of the

Supplementary Material. Studies retained for qualitative analysis with a listing of examined endpoints are shown in Table 2.

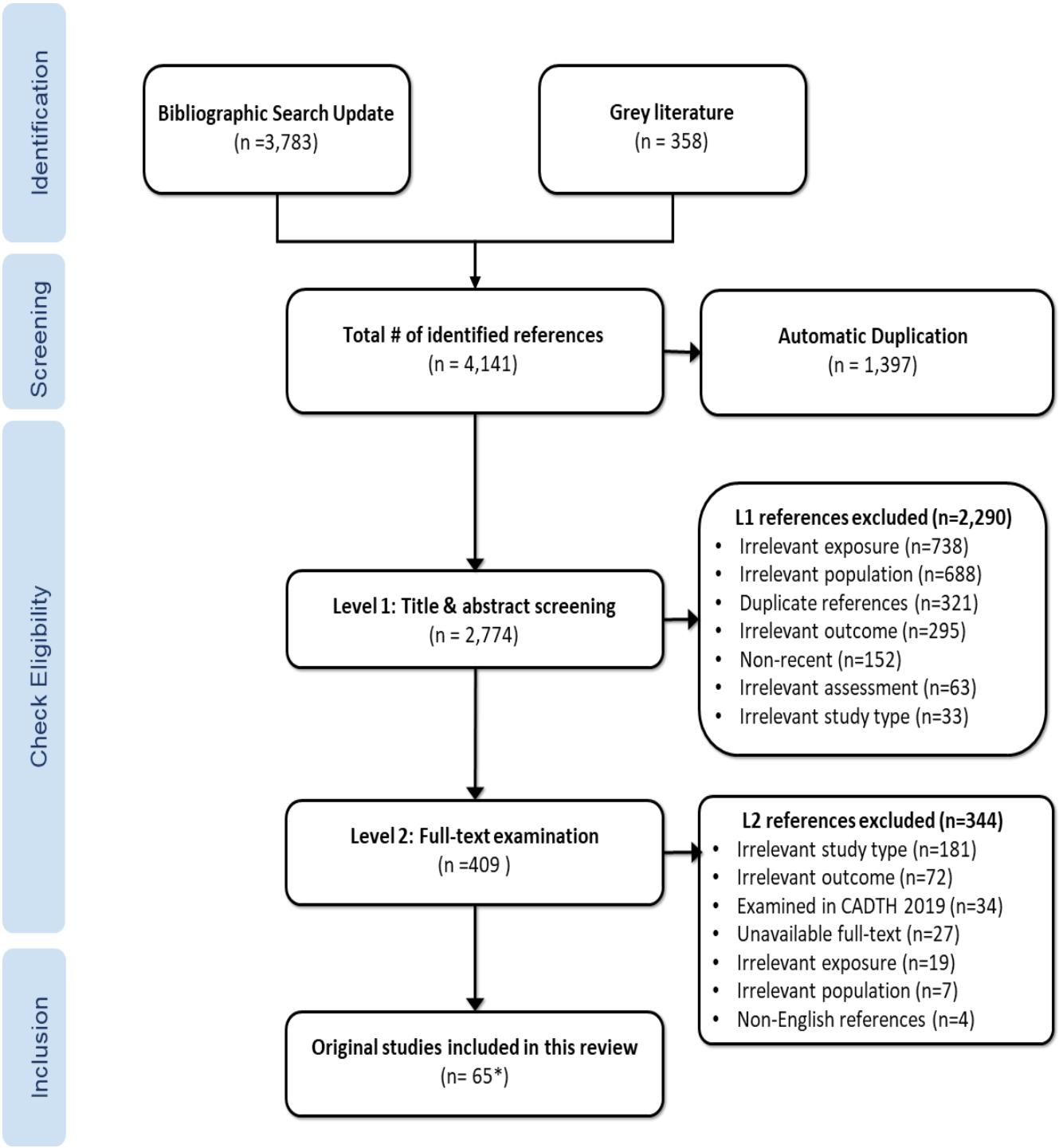

Figure 2: PRISMA flow diagram for human studies

** These 65 references include 2 relevant abstracts*

Table 1: Studies excluded at levels 1 and 2 by reason for exclusion

Level	Reason for exclusion	References
Level 1 *(Title and abstract screening)* *2,290*	Irrelevant exposure (other type of fluoride/water)	738
	Irrelevant population (non-human studies)	688
	Duplicate references	321
	Irrelevant outcome	295
	Non-recent (prior to 2016)	152
	Irrelevant assessment	63
	Irrelevant study type (non-original studies)	33
L2 *(Full-text examination)* *344*	Irrelevant study type (non-original studies)	181
	Irrelevant outcome	72
	Examined in CADTH 2019	34
	Unavailable full-text	27
	Irrelevant exposure (other type of fluoride/water)	19
	Irrelevant population (non-human studies)	7
	Non-English references	4

The 89 included original studies examined a wide range of adverse health effects due to exposure to fluoride in water, particularly dental fluorosis (33 studies) and neurocognitive (28 studies), bone and skeletal (9 studies), and endocrine and urogenital outcomes (8 studies, each). Table 2 provides a summary of all identified fluoride-related adverse health outcomes. Reviews identified during the RSI literature search are summarized in Table 5, but not evaluated further.

Table 2: List of original human studies retained for qualitative analysis, by endpoint

Study	Dental	Cancer	Bone / Skeletal	Neuro/ Cognitive	Development/ Reproductive	Endocrine	Urogenital	Cardio-vascular	Hepatic	Geno-toxicity	Others
Abstracts	**N= 0**	**N= 0**	**N= 0**	**N= 1**	**N= 1**	**N= 0**	**N= 0**	**N= 0**	**N= 0**	**N= 0**	**N= 0**
Chauhan 2017 [15]					✓						
Stephenson 2017 [16]				✓							
Original Studies	**N= 33**	**N= 4**	**N= 9**	**N= 28**	**N= 5**	**N= 8**	**N= 8**	**N= 4**	**N= 2**	**N= 3**	**N= 6**
Mercado 2023 [396]	✓										
Tang 2023 [397]	✓										
Ahmad 2022 [398]				✓							
Feng 2022 [417]				✓							
García-Escobar 2022 [399]	✓										
Goodman 2022 [418]				✓							
Gupta 2022 [400]	✓										
Ibarluzea 2022 [419]				✓							
Kaur 2022 [401]				✓							
Marques 2022 [402]	✓										
McLaren 2022 [403]	✓										

Study	Dental	Cancer	Bone / Skeletal	Neuro/ Cognitive	Development/ Reproductive	Endocrine	Urogenital	Cardio-vascular	Hepatic	Geno-toxicity	Others
Rani 2022 [404]	✓										
Saeed 2022 [405]	✓			✓							
Tawfik 2022 [406]	✓										
Thilakarathne 2022 [407]	✓										
Al-Omoush 2021 [17]	✓										
Ayele 2021 [18]			✓	✓				✓			GIT, fatigue
Cao 2021 [408]	✓										
Dong 2021 [19]	✓										
Du 2021 [20]						✓					
Farmus 2021 [409]	✓										
Fernandes 2021 [410]	✓										
Helte 2021 [21]			✓								
James 2021 [22]	✓										
Meghe 2021 [23]			✓								
Meng 2021 [24]										✓	
Mohd Nor 2021 [25]	✓										
Rojanaworarit 2021 [411]	✓										
Sharma 2021 [26]	✓		✓								Non-skeletal fluoride toxicity
Silva 2021 [412]	✓										
Tkachenko 2021 [27]								✓			

Study	Dental	Cancer	Bone / Skeletal	Neuro/ Cognitive	Development/ Reproductive	Endocrine	Urogenital	Cardio-vascular	Hepatic	Geno-toxicity	Others
Wang 2021 [413]	✓			✓							
Yani 2021 [414]	✓			✓							
Yu 2021 [415]	✓										
Zhao 2021 [416]	✓										
Bai 2020 [28]					✓						
Cui 2020 [29]				✓		✓					
Das 2020 [30]	✓										
Fernandes 2020 [31]	✓										
Godebo 2020 [32]			✓								
Kim 2020 [33]		✓									
Krishna 2020 [34]						✓					
Lee 2020 [35]		✓	✓								
Nanayakkara 2020 [36]							✓				
Russ 2020 [37]				✓							
Stangvaltaite-Mouhat 2020 [38]	✓										
Sun 2020 [39]			✓								
Till 2020 [40]				✓							
Wang 2020 [41]				✓		✓					
An 2019 [42]					✓						
Crnosija 2019 [43]		✓									

Study	Dental	Cancer	Bone / Skeletal	Neuro/ Cognitive	Development/ Reproductive	Endocrine	Urogenital	Cardio-vascular	Hepatic	Geno-toxicity	Others
Fernando 2019 [44]							✓				
Jimenez-Cordova 2019 [45]							✓	✓			
Jimenez-Cordova 2019a [46]											Arsenic metabolism
Khanoranga 2019 [47]		✓									
Liu 2019 [48]					✓						
Malin 2019 [49]							✓		✓		
Malin 2019a [50]				✓							
Pei 2019 [51]		✓	✓							✓	
Riddell 2019 [52]				✓							
Shaik 2019 [53]						✓					
Soto-barreras 2019 [54]		✓									
Zhang 2019 [55]					✓						
Zhou 2019 [56]											Select eye diseases
Zhou 2019a [57]	✓										
Bashash 2018 [58]				✓							
Cui 2018 [59]				✓							
Jimenez-Cordova 2018 [60]							✓				
Kumar, V 2018 [61]						✓					
Kumar, S 2018 [62]	✓										
Malin 2018 [63]						✓					

Study	Dental	Cancer	Bone / Skeletal	Neuro/ Cognitive	Development/ Reproductive	Endocrine	Urogenital	Cardio-vascular	Hepatic	Geno-toxicity	Others
Mohd Nor 2018 [25]	✓										
Mustafa 2018 [64]				✓							
Oweis 2018 [65]			✓								
Quadri 2018 [66]							✓				
Rathore 2018 [67]						✓					
Shruthi 2018 [68]											Non-skeletal fluoride toxicity (dyspepsia, muscle weakness, fatigue)
Yu 2018 [69]				✓							
Arulkumar 2017 [70]				✓				✓	✓		
Bashash 2017 [71]				✓							
Verma 2017 [72]	✓										
Cardenas-Gonzalez 2016 [73]							✓				
de Moura 2016 [74]	✓										
Heck 2016 [75]				✓							General health, trouble working
Kousik 2016 [76]				✓	✓						
Sabokseir 2016 [77]	✓										
Xiang 2016 [78]	✓										

Characteristics of included human studies

Out of 89 original studies retained for qualitative analysis, 70 (79%) were cross-sectional in design, 9 were cohort studies [21, 37, 40, 58, 65, 71, 409, 419], 4 were case-control studies [33, 34, 44, 70], 4 were ecological [35, 43, 64, 76], and 2 were abstracts only [15, 16]. All of the retrieved studies were published between 2016 and 2023. The sampling time frame included variable time intervals between 1992-2021, with 29% of studies that did not report a time frame.

Eighteen studies (20%) were carried out in China and 17 in India (19%), with USA, Mexico and Canada involved in 9 (10%), 8 (9%) and 7 (8%) studies, respectively. Sixty-five percent of studies examined fluoride exposure in drinking water (n=57), or 19% in ground water (n=17), compared to urine/MUF or serum with 11% (n=10) and 1% (1), respectively. Three studies reported no source of fluoride exposure.

The examined population were comprised of children and/or adolescents in 54 studies (61%), adults in 15 studies (17%), mixed populations in 13 studies (15%), and mother-child pairs in 6 studies (7%). The number of study participants ranged from as low as n=83 to as high as 6,914,124 persons. The majority of studies included both men and women (n=78), whereas 3 studies involved only men, 2 involved only women, and 6 studies examined mother-child pairs.

A summary of major study characteristics is shown in Table 3. The table also provides summaries of findings for each study, where positive association refers to increased adverse effects with increasing fluoride exposure, and negative association refers to decreased adverse effects with increasing fluoride exposure. More detailed characteristics of individual studies are provided in Section 3 of the Supplementary Material.

Table 3: Characteristics of included human studies[1]

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Mercado 2023 [396]				
Reference type: Original study **Study design:** Cross-sectional **Country:** Peru **Participants:** 12-15 years old students **Sampling time frame:** 2012 **Sample size:** 504 **Sex:** Girls: 34.52% **Source of funding / support:** NR **Author declaration of interest:** NR	**Exposures:** Fluoride levels in: Ground water **Exposure level(s):** • Ground water (mg/L) 0.22-0.98 mg/L **Outcome(s):** • Dental fluorosis	Fluoride in water/Dean's fluorosis index: Panchacutes I: 0.98mg/L/2.08 Tiabaya Pampas Nuevas: 0.79 mg/L/1.90 Tiabaya El Cural: 0.73 mg/L/1.72 La Bedoya: 0.43 mg/L/1.54 Panchacutes II: 0.32 mg/L/1.42 La Tomialla: 0.22 mg/L/1.26 Dental fluorosis for Panchacutes I: Severe: 10.71% Moderate: 23.81% Mild: 32.14% Very Mild: 26.19% Questionable: 7.143 % Normal: 0% Tiabaya Pampas Nuevas: Severe: 8.33% Moderate: 21.43% Mild: 30.95% Very Mild: 26.19% Questionable: 9.52 % Normal: 3.57% Tiabaya "El Cural": Severe: 5.95% Moderate: 19.05% Mild: 29.76% Very Mild: 26.19% Questionable: 10.71 % Normal: 8.33% La Bedoya: Severe: 3.57% Moderate: 15.48%	"The higher concentration of fluoride in drinking water is directly related to the higher degree of fluorosis."	2

[1] Information and data in this table was taken directly from the original publications

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Mild: 29.76% Very Mild: 27.38% Questionable: 13.10 % Normal: 10.71% <u>Panchacutes II:</u> Severe: 2.38% Moderate: 13.10% Mild: 28.57% Very Mild: 28.57% Questionable: 15.48 % Normal: 11.90% <u>La Tomialla:</u> Severe: 0% Moderate: 10.71% Mild: 27.38% Very Mild: 30.95% Questionable: 16.69% Normal: 14.29 % Relationship between fluoridation and DF: ($p<0,05$; $\chi 2<0,05$) Relationship between "Never" Fluoridation and DF Normal: 7.5% Questionable: 12.5% Very Mild: 27.5% Mild: 30% Moderate: 17.5% Severe: 5% Relationship between "One" Fluoridation and DF Normal: 8.26% Questionable: 11.98% Very Mild: 27.69% Mild: 29.75% Moderate: 17.36% Severe: 4.96%		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Relationship between "Two" Fluoridation and DF Normal: 8.14% Questionable: 12.21% Very Mild: 27.33% Mild: 29.65% Moderate: 17.44% Severe: 5.23% Relationship between "Three" Fluoridation and DF Normal: 8.0% Questionable: 12.0% Very Mild: 28.0% Mild: 30.0% Moderate: 16.0% Severe: 6.0%		
Tang 2023 [397]				
Reference type: Original study **Study design:** Cross-sectional **Country:** China **Participants:** 7-14 years old children residing since birth in study area that is supplied by groundwater **Sampling time frame:** NR **Sample size:** 593 **Sex: N (%):** Girls: 300 (50.6%) **Source of funding / support:** • National Natural Science Foundation of China (Grants No. 82073515, and No. 81773388) • The State Key Program of National Natural Science Foundation of China (Grant No. 81430076)	**Exposures:** Fluoride levels in: • Ground water • Urine samples **Exposure level(s):** (Chinese standard fluoride limit in water = 1.0mg/L) • Water fluoride: 0.20 to 3.90, mean 1.42 (SD 1.00), median 1.20 (IQR 0.70–2.20) mg/L • Urinary fluoride: 0.01 to 5.54, mean 1.36 (SD 1.31), median 0.56 (IQR 0.16-2.29) mg/L • Fluoride concentration.: Mean ± SD (>1mg/L):	• Water fluoride concentration >1mg/L and DF prevalence: Normal: 17 (5.6%) Very mild: 47 (15.5%) Mild: 210 (69.3%) Moderate:29(9.6%) • Water fluoride concentration 1mg/L and DF prevalence: Normal: 216 (74.5%) Very mild: 22 (15.2%) Mild: 30 (10.3%) Moderate:0(0.00%) • Water fluoride and DF (*PR* (95% *CI*), increase per 1ml/L): Overall DF: 1.50 (1.42, 1.57) Very mild DF: 1.85 (1.64, 2.07) Moderate DF: 3.92 (3.03, 5.06) *P* < 0.001	• Since "stratified analysis indicated a weaker association between fluoride concentration and DF prevalence in boys than in girls.", "the DF prevalence may be sex-specific." • "Inflammatory factors may partially mediate the increased prevalence of mild DF in school-aged children with low-to-moderate fluoride exposure." • "The study demonstrates that the risk of DF has an upward trend when the fluoride gradually in	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Author declaration of interest: No COI	Higher exposure gp.: Water: 2.19 ±0.81 Urine: 2.48 ±0.88 <u>Lower exposure gp.:</u> Water: 0.61 ±0.24 Urine: 0.18 ±0.12 **Outcome(s):** • Dental fluorosis	• <u>Urinary fluoride DF (*PR* (95% *CI*), increase per 1ml/L):</u> Overall DF: 1.42 (1.35, 1.50) Very mild DF: 1.67 (1.48, 1.88) Mild DF:1.72 (1.61, 1.84) moderate DF: 3.02 (2.50, 4.13) P < 0.001 • <u>Association between fluoride content and DF by sex: PR (95%CI)</u> *Water Fluoride* Overall: 1.33 (1.29, 1.36), P-interaction=0.325 Very Mild: 1.31 (1.23, 1.39) P-interaction=0.485 Mild: 1.39 (1.35, 1.44) P-interaction=0.431 Moderate: 1.33 (1.25, 1.42) P-interaction=0.852 *Urinary Fluoride:* Overall: 1.27 (1.23, 1.30) P-interaction=0.013 Very Mild: 1.25 (1.17, 1.32) P-interaction=0.025 Mild: 1.32 (1.28, 1.36) P-interaction=0.014 Moderate: 1.27 (1.20, 1.36) P-interaction=0.170 <u>Sensitivity analysis for effect of fluoride exposure on DF: [PR (95%CI) for every 1mg/L increment of water fluoride]</u> *Adjusted for age and sex, water fluoride (mg/L)* Overall: 1.50 (1.42, 1.57) WHO Guideline: 0.78 (0.66, 0.89) * Very Mild: 1.83 (1.62, 2.06) WHO Guideline: 1.25 (0.98, 1.52) *	increases, in water and urine."	

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Mild: 1.72 (1.61, 1.83) WHO Guideline: 1.10 (0.93, 1.27) * Moderate: 3.18 (2.54, 3.98) WHO Guideline: 3.13 (2.35, 3.90) * *Adjusted for BMI, water fluoride (mg/L)* Overall: 1.50 (1.42, 1.58) WHO Guideline: 0.79 (0.67, 0.91) * Very Mild: 1.82 (1.62, 2.05) WHO Guideline: 1.23 (0.95, 1.51) * Mild: 1.72 (1.61, 1.83) WHO Guideline: 1.11 (0.94, 1.28) * Moderate: 3.27 (2.73, 3.92) WHO Guideline: 3.15 (2.40, 3.90) * *Adjusted for parental education, and family income, water fluoride (mg/L)* Overall: 1.50 (1.43, 1.58) WHO Guideline: 0.79 (0.67, 0.91) * Very Mild: 1.83 (1.63, 2.06) WHO Guideline: 1.22 (0.95, 1.50) * Mild: 1.73 (1.62, 1.84) WHO Guideline: 1.11 (0.94,1.28) * Moderate: 3.78 (2.93, 4.88) WHO Guideline: 3.12 (2.29, 3.95) * *Adjusted for LBW, water fluoride (mg/L)* Overall: 1.50 (1.42, 1.57) WHO Guideline: 0.79 (0.67, 0.91) * Very Mild: 1.83 (1.62, 2.06) WHO Guideline: 1.21 (0.92, 1.50) * Mild: 1.72 (1.61, 1.83) WHO Guideline: 1.11 (0.94, 1.28) * Moderate: 3.384 (2.82, 4.07) WHO Guideline: 3.13 (2.37, 3.89) *		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Adjusted for age, sex, BMI, parental education, family income, and LBW, water fluoride (mg/L) Overall: 1.50 (1.42, 1.58) WHO Guideline: 0.78 (0.66, 0.90) * Very Mild: 1.85 (1.64, 2.07) WHO Guideline: 1.24 (0.95, 1.52) * Mild: 1.723 (1.61, 1.84) WHO Guideline: 1.10 (0.93,1.27) * Moderate: 3.92 (3.03, 5.06) WHO Guideline: 3.13 (2.32, 3.94) * *Water fluoride ≤ 1.5 is reference. P=0.001 <u>Sensitivity analysis for effect of fluoride exposure on DF: [PR (95%CI) for every 1mg/L increment of urinary fluoride]</u> *Adjusted for age and sex, urinary fluoride (mg/L)* Overall: 1.41 (1.34, 1.48) Very Mild: 1.66 (1.48, 1.87) Mild: 1.57 (1.48, 1.68) Moderate: 2.68 (2.26, 3.19) *Adjusted for BMI, urinary fluoride (mg/L)* Overall: 1.41 (1.34, 1.48) Very Mild: 1.63 (1.44, 1.85) Mild: 1.57 (1.47, 1.67) Moderate: 2.59 (2.18, 3.08) *Adjusted for parental education, and family income, urinary fluoride (mg/L)* Overall: 1.41 (1.34, 1.48) Very Mild: 1.65 (1.47, 1.85) Mild: 1.57 (1.47, 1.67) Moderate: 2.98 (2.37, 3.75)		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Adjusted for low birth weight, urinary fluoride (mg/L) Overall: 1.41 (1.34, 1.48) Very Mild: 1.64 (1.45 1.86) Mild: 1.57 (1.47, 1.67) Moderate: 2.57 (2.14, 3.08) *Adjusted for urinary creatinine, urinary fluoride (mg/L)* Overall: 1.42 (1.35, 1.50) Very Mild: 1.63 (1.43, 1.86) Mild: 1.59 (1.48, 1.71) Moderate: 2.76 (2.19, 3.48) *Adjusted for age, urine creatinine, sex, BMI, parental education, family income and low birth weight, urinary fluoride (mg/L)* Overall: 1.42 (1.35, 1.50) Very Mild: 1.67 (1.48, 1.88) Mild: 1.59 (1.48, 1.72) Moderate: 3.20 (2.49, 4.13)		
Ahmad 2022 [398]				
Study design: Cross-sectional **Country:** Pakistan **Participants:** Students (9 – 11 years of age) of madrassa (Islamic religious school) in urban and rural locations within the province of Sindh **Sampling time frame:** NR **Sample size:** N = 120 **Sex N:** Girls: 34 (28.3%) **Source of funding / support:** NR **Author declaration of interest:** NR	**Exposures:** Fluoride levels in • Drinking water • Urine Exposure level: Mean fluoride levels in urban madrassas (Karachi Central) • Drinking water: 2.04 mg/L • Urine: 5.99 (±3.57) mg/L	N (%) of IQ scores by high (urban) and low (rural) fluoride areas IQ <70 retarded (low) • High fluoride: 2 (3.33) • Low fluoride: 5 (8.33) IQ 70 – 79 borderline (below average) • High fluoride: 4 (6.67) • Low fluoride: 6 (10) IQ 80 – 89 dull normal (low average) • High fluoride: 10 (16.67) • Low fluoride: 9 (15) IQ 90 – 109 normal (average) • High fluoride: 20 (33.33) • Low fluoride: 19 (31.67)	"The significantly higher IQ, 99.95±15.50, of boys in the urban area madrassas with a high drinking water fluoride level compared to the IQ, 92.30±14.97, of boys in the rural area madrassas with a low drinking water fluoride level contradicts the previous reports of higher fluoride levels being associated with a lower IQ. However, several confounding factors were not controlled for in	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	Mean fluoride levels in rural madrassas (Umerkot) • Drinking water: 1.07 mg/L • Urine: 3.53 (±1.09 mg/L) **Outcome(s):** • Intelligence quotient (IQ)	<u>IQ 110 – 119 bright normal (high average)</u> • High fluoride: 16 (26.67) • Low fluoride: 15 (25) <u>IQ 120 – 129 superior (good)</u> • High fluoride: 7 (11.67) • Low fluoride: 6 (10) <u>IQ >129 very superior (excellent)</u> • High fluoride: 1 (1.66) • Low fluoride: 0 (0.0) "No significant difference was present between the IQ distribution in the high and low fluoride areas on chi-square testing after combining the groups IQ <70 and IQ 70–79, and the groups IQ 120–129 and IQ >129, so that the cells had an n of 5 or more" (p. 56) IQ scores by high (urban) and low (rural) fluoride areas stratified by gender <u>Boys</u> • High fluoride: 99.95 (± 15.50) • Low fluoride: 92.30 (± 14.97) <u>Girls</u> • High fluoride: 96.90 (± 16.31) • Low fluoride: 90.30 (± 15.49) "comparing IQ of high fluoride boys and low fluoride boys p<0.05" (p. 57)	the present study, including the level of parental education, socio-economic status, and the levels of arsenic, lead, and iodine." (p. 57)	
Feng 2022 [417] [2]				

[2] This publication cites and refers to methods from an earlier publication [Feng et al., 2022], partly using the same study population. That earlier publication has been retracted with the following statement [from BMC Public Health. 2022; 22: 2044]: "The Editor has retracted this article. After publication, concerns were raised regarding the data analysis and conclusions in the paper. The authors have provided raw data, and post-publication review found inconsistencies in methodology and major misinterpretation of the primary result. None of the authors agree to this retraction." Although Feng et al. (2022) was included in the current systematic review, the link between the two publications suggests that caution is warranted in interpreting any results.

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Reference type: Original study **Study design:** Cross-sectional **Country:** China **Participants:** Children aged 8-12 years **Sampling time frame:** April-May 2017 **Sample size:** 683 **Sex: N (%):** Boys: 324 (47.44%) **Source of funding / support:** • The National Natural Science Foundation of China (Nos. 81972981, 82003401, and 81673116) • Key Projects of Colleges and Universities of Henan Education Department (21A330006) **Author declaration of interest:** no COI	**Exposures:** Fluoride level(s) in: • Urine **Exposure level(s):** Water fluoride: fluoride concentration in drinking water >1.0 mg/L.[3] Median UFcr (mg/L): 1.33 Children were divided into two groups, high fluoride group (HFG, UFcr>1.33 mg/L) and control group (CG, UFcr≤1.33 mg/L). Mean urinary fluoride [UF, unadjusted for creatinine] (mg/L): • HFG:1.56±0.82 • CG: 0.98±0.62 • P<0.001 • Total: 1.27±0.79 Mean UFcr (mg/L) • HFG: 2.15±0.91 • CG: 0.83±0.30 • P<0.001 • Total: 1.49±0.95 **Outcome(s):** Intelligence quotient (IQ).	Mean IQ scores • HFG: 122.61±11.61 • CG: 121.50±12.14 • P=0.290 • Total: 122.05±11.88 Distribution by intelligence level in HFG and CG • Normal: (IQ 90-109): 15.25% (HFG); 17.54% (CG) • High-normal (IQ 110-119): 25.81% (HFG); 24.85% (CG) • Superior (IQ 120-129): 30.21% (HFG); 33.04% (CG) • Excellent (IQ≥130): 28.74% (HFG); 24.56% (CG) • P=0.539 High fluoride group (HFG) • Change in IQ score per 1.0 mg/L increase in UFcr level: β=-2.502 (95% CI: -4.411, -0.593); p=0.010 • Change in the probability of "excellent" intelligence (IQ≥130) per 1.0 mg/L increase in UFcr level: OR=0.537 (95% CI: 0.290, 0.994); p=0.048 • No significant trend in IQ scores by tertile of UFcr (≤1.63, 1.64-2.14, >2.14 mg/L); p=0.116 Control group • No significant change in IQ score per 1.0 mg/L increase in UFcr level: p=0.181	"Excessive fluoride exposure may have adverse effects on children's intelligence, and changes in children's intelligence may be associated with the interaction between fluoride and MTHFD1 polymorphisms." • Note: significant trends in IQ with increasing creatinine-adjusted urinary fluoride were found only in high fluoride group; no significant trends were seen in the total population.	2

[3] The water fluoride concentration was reported by another study (reported by Feng et al 2022) that was done by the same research group, used the same population/data, and provided more information on methods and exposure assessment.

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• •No significant change in the probability of "excellent" intelligence (IQ≥130) per 1.0 mg/L increase in UFcr level: p=0.659 • No significant trend in IQ scores by tertile of UFcr (≤0.66, 0.67-1.02, >1.02 mg/L); p=0.343 Total • No significant change in IQ score per 1.0 mg/L increase in UFcr level: p=0.376 • No significant change in the probability of "excellent" intelligence (IQ≥130) per 1.0 mg/L increase in UFcr level: p=0.396 • No significant trend in IQ scores by tertile of UFcr (≤1.02, 1.03-1.63, >1.63 mg/L); p=0.426 <u>Statistically significant</u> gene-environmental interaction on the IQ scores *[Polymorphisms in 4 loci of MTHFD1 related to neurodevelopment (rs11627387, rs1076991, rs2236224, and rs2236225) were analyzed]* • UFcr x rs11627387 x rs1076991 x rs2236224: F=1.669; p=0.021 • UFcr x rs11627387 x rs1076991 x rs2236225: F=1.764; p=0.012 UFcr x rs11627387 x rs1076991 x rs2236224 x rs2236225: F=1.614; p=0.012		
Garcia-Escobar 2022 [399]				
Study design: Cross-sectional **Country:** India	**Exposures:** <u>Fluoride levels in</u>	<u>Overall prevalence</u> • 94.6% (DI)	• "Patients from rural communities of the	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Participants: 785 subjects aged 10-60 years **Sampling time frame:** NR **Sample size:** 785 **Sex: N (%):** Men: 322 (41.3%) **Source of funding / support:** No external funding **Author declaration of interest:** No COI	Drinking water <u>Exposure level(s):</u> • Water fluoride (ppm): 1.1 to 2.92 (mean 1.71, median 1.5) **Outcome(s):** • Dental fluorosis	• 94.4 (TFI) <u>Prevalence of Moderate–Severe (MS) cases (DI) and TFI score 4–9 cases</u> [DI MS group corresponds to TFI 4–9] • 62.8% (DI MS) • 73.1% (TFI 4-9) <u>Prevalence of fluorosis among those consuming water with water fluoride ≤1.5 ppm</u> • 54.3% (DI) • 54.5% (TFI) <u>Prevalence of DI MS and TFI 4-9 among those consuming water with water fluoride ≤1.5 ppm</u> • 33.2% (DI MS) • 39.9% (TFI 4-9) <u>OR (95% CI)</u> DI MS • ≤1.5 ppm: reference • >1.5 ppm: 1.81 (1.34–2.45) • P=0.000 TFI 4-9 • ≤1.5 ppm: reference • >1.5 ppm: 1.79 (1.28–2.5) • P=0.000 Spearman's rank order correlation between water fluoride and moderate-severe fluorosis • DI MS: R_S=0.527; p=0.064 TFI 4-9: R_S=0.610; p=0.027	Anantapur district showed a high prevalence (over 90%) of dental fluorosis. Moreover, the Anantapur population presents a high number of moderate and severe cases (over 60%), while other populations showed less severe forms of fluorosis, despite reporting superior fluoride levels to those found in the Anantapur drinking water." • "The severity of fluorosis concerning fluoride concentration levels in drinking water in Anantapur suggests that other factors are involved in the severity of the dental fluorosis observed. A potential change in the biological susceptibility of the population to the toxin, due to the long-term exposition (including several generations) could explain the phenomenon…"	
Goodman 2022 [418]				

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Reference type: Original study **Study design:** Cohort (ELEMENT) **Country:** Mexico **Participants:** • Women who were planning to conceive or were pregnant at <14 weeks gestation (Cohorts 2A and Cohort 3 of the ELEMENT project). • Children examined at ages 4, 5, and 6–12 years **Sampling time frame:** Recruitment: Cohort 2A in 1997-1999; Cohort 3 in 2001-2003 **Sample size:** • Primary sample with complete covariate, maternal urinary fluoride, and outcome data for at least two time points: 348 mother-child dyads • Examined at age 4 years: 386 • Examined at age 5: 308 • Examined at age 6-12: 278 **Sex: N (%):** Boys: • Primary sample: 167 (47.99%) • Age 4: 183 (47.41%) • Age 5: 151 (49.03%) • Age 6-12: 132 (47.48%) **Source of funding / support:** • The American British Cowdray Hospital provided facilities for the ELEMENT research. • U.S. National Institutes of Health (NIH; grants R01ES021446 and R01-ES007821) • The National Institute of Environmental Health Sciences/the U.S. Environmental	**Exposures:** Fluoride level in Maternal urine collected during one or more trimesters of pregnancy **Exposure level(s):** • Creatinine-adjusted maternal urinary fluoride (MUFcre, µg/L): 0.14 to 3.01; mean 0.90 (SD 0.39), median 0.83; IQR 0.64-1.11 **Outcome(s):** Children's IQ	Changes in cognitive score per 0.5 mg/L increase in MUFcre GEE population-averaged models • FSIQ/GCI: B=-2.12 (95% CI: -3.49, -0.75); p=0.002 • PIQ: B=-2.63 (95% CI: -3.87, -1.40); p<0.001 • VIQ: B=-1.29 (95% CI: -2.60, 0.01); p=0.053 • No interactions were between MUFcre and time (p>0.10). • No interaction between MUFcre and child sex (p>0.10) Linear regression analysis Age 4 • GCI: B=-2.12 (95% CI: -3.83, -0.41); p=0.015 • PIQ: B=-3.08 (95% CI: -4.69, -1.47); p<0.001) • VIQ: B=-0.81 (95% CI: -2.30, 0.69); p>0.05 Age 5 • GCI: B=-1.97 (95% CI: -3.64, -0.30); p=0.021 • PIQ: B=-2.46 (95% CI: 4.04, -0.87); p=0.003 • VIQ: B=-1.24 (95% CI: -2.97, 0.49); p>0.05 Age 6-12 • FSIQ: B=-2.01 (95% CI: -3.66, -0.46); p=0.012 • PIQ: B=-1.80 (95% CI: -3.39, -0.21); p=0.027	• "… prenatal exposure to fluoride is associated with sustained impacts on IQ." • "… an increment of 0.5 mg/L in maternal urinary fluoride concentration was associated with a 2-point decrement in children's Full-Scale IQ scores". • "Non-verbal abilities may be more susceptible to impairment from prenatal fluoride exposure as compared to verbal abilities." • "These results were found among mother-child pairs living in a region of Mexico in which fluoride is added to salt."	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Protection Agency (NIEHS/EPA; grant P01ES022844, 83543601) • The NIEHS (grant P42-ES05947, P20ES018171) • NIEHS Center Grant P30ES017885) • National Institute of Public Health/Ministry of Health of Mexico **Author declaration of interest:** No COI		• VIQ: B=-1.93 (95% CI: -3.67, -0.18); p=0.031 • No interaction between MUFcre and child sex <u>Sensitivity analyses (GEE models), B (95% CI)</u> FSIQ/GCI. • Model A[4]: -2.10 (-3.47, -0.73) • Model A + number/timing of urine samples[5]: -2.12 (-3.49, -0.75) • Model A – IQ score<70[6]: -1.67 (-2.93, -0.41) • Model A – Cohort 3 Ca[7]: -1.98 (-3.70, -0.27) • Model A – Maternal IQ[8]: -2.40 (-3.79, -1.01) • Model A + Maternal IQ[9]: -2.09 (-3.44, -0.73) • Model A – HOME[10]: -2.33 (-4.46, -0.20) • Model A + HOME[11]: -2.11 (-4.06, -0.16) • Model A – Patella Lead[12]: -2.42 (-3.98, -0.86)		

[4] GEE models adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, time of testing, smoking history (ever smoked during the pregnancy vs. non-smoker), marital status (married vs. others), maternal age at delivery, maternal education, and cohort/calcium treatment.
[5] Number/timing of urine samples included as a covariate
[6] Excluding cases with FSIQ/GCI, PIQ, or VIQ scores less than 70
[7] Subset of cases who received calcium supplementation
[8] Subset of cases who have data on maternal IQ
[9] Subset of cases who have data on maternal IQ, adjusted for maternal IQ
[10] Subset of cases who have data on Home Observation for the Measurement of the Environment (HOME) scores
[11] Subset of cases with HOME score, adjusted for HOME score
[12] Subset of cases who have data on maternal patella lead

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• Model A + Patella Lead[13]: -2.41 (-3.98, -0.85) • Model A – Tibia Lead[14]: -2.75 (-4.61, -0.89) • Model A + Tibia Lead[15]: -2.23 (-4.09, -0.38) • Model A – Tibia and Patella Lead[16]: -2.73 (-4.71, -0.76) • Model A + Tibia and Patella Lead[17]: -2.20 (-4.18, -0.22) PIQ • Model A: 2.61 (-3.85, -1.38) • Model A + number/timing of urine samples: -2.63 (-3.86, -1.39) • Model A – IQ score<70: -2.61 (-3.81, -1.42) • Model A – Cohort 3 Ca: -3.13 (-4.67, -1.58) • Model A – Maternal IQ: -2.78 (4.04, -1.52) • Model A + Maternal IQ: -2.46 (-3.68, -1.24) • Model A – HOME: -3.67 (-5.52, -1.82) • Model A + HOME: -3.44 (-5.15, -1.72) • Model A – Patella Lead: -2.66 (-4.05, -1.27) • Model A + Patella Lead: -2.65 (-4.04, -1.27) • Model A – Tibia Lead: -2.81 (-4.46, -1.16)		

[13] Subset of cases with data on maternal patella lead, adjusted for maternal patella lead
[14] Subset of cases who have data on maternal tibia lead
[15] Subset of cases with data on maternal tibia lead, adjusted for maternal tibia lead
[16] Subset of cases who have data on maternal tibia and patella lead
[17] Subset of cases with data on maternal tibia and patella lead, adjusted for maternal tibia and patella lead

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• Model A + Tibia Lead: -2.41 (-4.07, -0.76) • Model A – Tibia and Patella Lead: -2.75 (-4.50, -1.00) • Model A + Tibia and Patella Lead: -2.32 (-4.08, -0.56) VIQ • Model A: -1.28 (-2.58, 0.03) • Model A + number/timing of urine samples: -1.30 (-2.60, 0.01) • Model A – IQ score<70: -1.05 (-2.31, 0.21) • Model A – Cohort 3 Ca: -0.69 (-2.31, 0.94) • Model A – Maternal IQ: -1.55 (-2.86, -0.24) • Model A + Maternal IQ: -1.33 (-2.62, -0.04) • Model A – HOME: -0.71 (-2.72, 1.30) • Model A + HOME: -0.54 (-2.43, 1.35) • Model A – Patella Lead: -1.62 (-3.12, -0.11) • Model A + Patella Lead: -1.62 (-3.13, -0.11) • Model A – Tibia Lead: -2.09 (-3.88, -0.31) • Model A + Tibia Lead: -1.65 (-3.44, 0.14) • Model A – Tibia and Patella Lead: -2.09 (-3.99, -0.19) Model A + Tibia and Patella Lead: -1.63 (-3.55, 0.28)		
Gupta 2022 [400]				
Reference type: Original study **Study design:** Case-Control **Country:** India	**Exposures:** <u>Fluoride levels in:</u> • Drinking water	Water fluoride concentration associated with: • Dental fluorosis: 0.67-0.83 ppm	• "Besides high concentrations of fluoride in potable water, poor	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Participants: from all ages, with subjects from endemic villages, and controls from non-endemic villages **Sampling time frame:** 2014-2015 **Sample size:** 180 **Sex: N (%):** NR **Source of funding / support:** • UGC, New Delhi • Chhattisgarh Council of Science and Technology **Author declaration of interest:** No COI	• Serum **Exposure level(s):** • Mean drinking water fluoride levels 1.16-7.56 ppm **Outcome(s):** • Dental fluorosis • Skeletal fluorosis	Skeletal fluorosis: 0.43-0.83 ppm	socio-economic status and nutritional deficiency also contribute to fluorosis in exposed individuals from endemic regions." • For the individuals residing in an endemic area and consuming the same high fluoride containing drinking water which doesn't have visible symptoms of dental or skeletal fluorosis, individuals might be considered in a preclinical stage of fluorosis and may develop symptoms of fluorosis in subsequent years. The finding of this study might be a preliminary screening for those individuals. However, urine and blood fluoride analyses of the subjects are also needed for further confirmation."	
Ibarluzea 2022 [419]				
Reference type: Original study **Study design:** Cohort (INMA project) **Country:** Spain **Participants:** Mother-child pairs from a spanish cohort. Children were examined at ages 1 and 4 years old	**Exposures:** Fluoride level in • Maternal urine collected in the first and third trimesters of pregnancy **Exposure level(s):**	**Changes in cognitive score per unit (mg/g) increase in maternal creatinine-adjusted urinary fluoride (MUFcr), β (95% CI)**[21] Bayley Mental Development Index (MDI) ***Both trimesters MUFcr*** • All: 1.48 (-4.2, 7.16) • Boys: 3.84 (-5.04, 12.72)	• "We observed no negative effects on children's cognition and even found positive associations for verbal, performance, numeric, memory scores and GCI, in boys at the age of 4 years, although	1

[21] Adjusted for child's age at testing (only for McCarthy), order of the child (between siblings), nursery at 14 months, breastfeeding, maternal social class, IQ and smoking

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Sampling time frame: Recruitment of pregnant women: 1997- 2008 in different study areas (**Guxen et al. 2012**)[18] **Sample size:** • Assessed at age 1 year: 316 mother-child pairs • Assessed at ages 1 and 4 years: 248 mother-child pairs **Sex: N (%):** Boys: • Assessed at age 1 year: 146 (46.2%) • Assessed at age 4 years: 125 (50.4%) **Exclusions:** <u>At recruitment</u> • Maternal age <16 years • Multiple pregnancy • Pregnancy achieved with assisted reproduction techniques • Not planning birth in the referral hospital • Communication problems in Spanish or Basque <u>Analytical sample</u>	**Fluoride levels in drinking water** • Community fluoridated drinking water systems: mean (SD): 0.81 (0.15) mg/L • Community non-fluoridated drinking water systems: <0.1 mg/L **Mean (95% CI) maternal creatinine-adjusted urinary fluoride levels (mg/g creatinine)[20]** <u>Assessed at age 1 year</u> • Both trimesters: 0.66 (0.61; 0.70) • Week 12 of pregnancy: 0.57 (0.52; 0.62) • Week 32 of pregnancy: 0.74 (0.69; 0.79) • P<0.001 [1st vs. 3rd trimester] <u>Assessed at age 4 years</u> • Both trimesters: 0.64 (0.59; 0.68) • Week 12 of pregnancy: 0.55 (0.50;0.60) • Week 32 of pregnancy: 0.73 (0.67;0.79)	• Girls: 0.75 (-6.92, 8.43) ***Week 12 MUFcr*** • All: 0.55 (- 4.64, 5.74) • Boys: 2.96 (-5.09, 11.01) • Girls: -1 (-8.07, 6.07) ***Week 32 MUFcr*** • All: 1.52 (-2.92, 5.97) • Boys: 2.50 (-4.46, 9.46) • Girls: 1.7 (-4.30, 7.71) <u>McCarthy, verbal</u> ***Both trimesters MUFcr*** • All: 13.86 (3.91, 23.82) • Boys: 13.38 (2.81, 23.95) • Girls: -1.31 (-9.35, 6.74) • P<0.05 ***Week 12 MUFcr*** • All: 1.11 (-4.86, 7.07) • Boys: 3.78 (-6.16, 13.71) • Girls: -0.91 (-8.78, 6.96) ***Week 32 MUFcr*** • All: 12.01 (4.82, 19.19) • Boys: 11.79 (4.22, 19.36) • Girls: -0.93 (-7.01, 5.15) • P<0.01 <u>McCarthy, performance</u> ***Both trimesters MUFcr*** • All: 5.86 (0.32, 11.39) • Boys: 12.24 (2.87, 21.61) • Girls: 2.03 (-4.77, 8.83) • P<0.05 ***Week 12 MUFcr***	when Hg levels were included in the model only verbal and GCI at week 32 and whole pregnancy remained significant or marginally significant." • "The positive associations between MUFcr and cognitive functions seemed to be more evident in children of mothers who lived their pregnancy in the nonfluoridated zones." • "The associations have been seen with MUFcr of the third trimester and not with those of the first one." • "As there is not information of MUFcr of the second trimester of pregnancy, it is difficult to identify a window of exposure related to the effect, but the lack of associations in the first trimester indicate that the effects are associated with later periods in pregnancy."	

[18] Guxens M, Ballester F, Espada M, Fernández MF, Grimalt JO, Ibarluzea J, Olea N, Rebagliato M, Tardón A, Torrent M, Vioque J, Vrijheid M, Sunyer J; INMA Project. Cohort Profile: the INMA--INfancia y Medio Ambiente--(Environment and Childhood) Project. Int J Epidemiol. 2012 Aug;41(4):930-40. doi: 10.1093/ije/dyr054. Epub 2011 Apr 5. PMID: 21471022

[20] Detailed data on maternal creatinine-adjusted urinary fluoride levels by maternal and children's characteristics are reported in Supplementary tables S2, S3 and S5

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
• Incomplete data [To be included, participants had to have 1) data on neuropsychological assessment at 1 year of age; 2) data on neuropsychological assessment at 4 years of age provided they also had assessment data at 1 year; 3) maternal urinary creatinine adjusted fluoride levels at the first and third trimesters of pregnancy.] **Source of funding / support[19]:** • The Instituto de Salud Carlos III, Red de Centros de investigación en Epidemiología y Salud Pública (RCESP) • CIBER Epidemiología y Salud Pública (CIBERESP) • The Fondo de Investigación Sanitaria • The European Union's 6th and 7th Framework Programmes (Hiwate, Escape, Hitea and Contamed projects) • The Ministerio de Educación y Ciencia, the Generalitat de Catalunya • The Centre for Research in Environmental Epidemiology (CREAL) of Barcelona • The Fundació La Caixa, the Fundació Roger Torné • The Consejería de Salud de Andalucía	• P<0.001 [1st vs. 3rd trimester] **Whole pregnancy mean (SD) maternal urinary fluoride (mg/L)** <u>Assessed at age 1 year</u> • Non-fluoridated zone: 0.36 (0.21) • Fluoridated zone: 0.65 (0.29) • P<0.001 <u>Assessed at age 4 years</u> • Non-fluoridated zone: 0.35 (0.20) • Fluoridated zone: 0.62 (0.26) • P<0.001 **Both trimesters mean (SD) creatinine-adjusted maternal urinary fluoride (mg/g creatinine)** <u>Assessed at age 1 year</u> • Non-fluoridated zone: 0.46 (0.25) • Fluoridated zone: 0.84 (0.40) • P<0.001 <u>Assessed at age 4 years</u> • Non-fluoridated zone: 0.45 (0.26) • Fluoridated zone: 0.82 (0.39) • P<0.001	• All: 4.63 (-0.57, 9.82) • Boys: 9.11 (0.47, 17.75) • Girls: 1.10 (-5.53, 7.73) ***Week 32 MUFcr*** • All: 3.68 (-0.49, 7.85) • Boys: 7.17 (0.24, 14.09) • Girls: 1.69 (-3.44, 6.83) • P<0.05 <u>McCarthy, numeric</u> ***Both trimesters MUFcr*** • All: 6.22 (0.65, 11.79 • Boys: 11.09 (1.79, 20.4) • Girls: 3.03 (-3.96, 10.03) • P<0.05 ***Week 12 MUFcr*** • All: 4.47 (-0.79, 9.73) • Boys: 5.03 (-3.65, 13.7) • Girls: 2.92 (-3.95, 9.78) ***Week 32 MUFcr*** • All: 4.13 (-0.07, 8.32) • Boys: 8.56 (1.81, 15.31) • Girls: 1.55 (-3.74, 6.85) • P<0.05 <u>McCarthy, memory</u> ***Both trimesters MUFcr*** • All: 11.63 (2.62, 20.63) • Boys: 11.3 (1.90, 20.7) • Girls: -2.12 (-9.32, 5.09) • P<0.05 ***Week 12 MUFcr*** • All: 1.71 (-3.66, 7.09) • Boys: 4.28 (-4.51, 13.06) • Girls: -1.40 (-8.46, 5.67) ***Week 32 MUFcr*** • All: 9.2 (2.67, 15.73)	• "A positive association between MUF and GCI scores and other measures of cognitive functions at 4 years of age is observed among boys in a prospective birth cohort in Spain." • " The current findings contradict, with a few exceptions, results obtained previously in cross-sectional and prospective studies."	

[19] Information from Guxen et al. 2012.

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
• The Junta the Andalucía • The Conselleria de Sanitat de la Generalitat Valenciana • The CAJASTUR—Caja Asturias • The Spanish Association against the Cancer (AECC) (Delegación Provincial Asturias) • The Departamento de Sanidad-Gobierno Vasco • The Diputación Floral de Gipuzkoa • The University of Oviedo, the KUTXA – Caja Gipuzkoa San Sebastián • The city councils of Zumarraga, Urretxu, Legazpi, Azpeitia, Beasain and Azkoitia in Gipuzkoa **Author declaration of interest:** no COI	**Outcome(s):** Children's cognition/ intelligence	• Boys: 9.26 (2.47, 16.05) • Girls: -1.72 (-7.17, 3.72) • P<0.01 <u>McCarthy, general cognitive</u> ***Both trimesters MUFcr*** • All: 15.4 (6.32, 24.48) • Boys: 15.03 (5.3, 24.75) • Girls: -0.02 (-7.16, 7.12) • P<0.01 ***Week 12 MUFcr*** • All: 3.37 (-2.09, 8.83) • Boys: 7.14 (-2.06, 16.33) • Girls: 0.21 (-6.77, 7.19) ***Week 32 MUFcr*** • All: 11.48 (4.88, 18.08) • Boys: 11.39 (4.33, 18.44) • Girls: -0.16 (-5.55, 5.23) • P<0.01 **Changes in cognitive score per unit (mg/g) increase in MUFcr, β (95% CI) <u>additionally adjusted for cord blood Hg levels</u>.** <u>Bayley Mental Development Index (MDI)</u> ***Both trimesters MUFcr*** • All: 2.67 (-3.46, 8.81) • No significant interaction by sex ***Week 12 MUFcr*** • All: 0.89 (-4.55, 6.32) • No significant interaction by sex ***Week 32 MUFcr*** • All: 2.65 (-2.14, 7.45) • No significant interaction by sex <u>McCarthy, verbal</u> ***Both trimesters MUFcr*** • All: 9.4 (-1.78, 20.57) • Boys: --		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• Girls: -2.07 (-10, 5.87) • P<0.1 ***Week 12 MUFcr*** • All: -1.5 (-7.53, 4.54) • No significant interaction by sex ***Week 32 MUFcr*** • All: 9.74 (1.75, 17.74) • Boys: -- • Girls: -0.74 (-6.72, 5.25) • P<0.05 McCarthy, performance ***Both trimesters MUFcr*** • All: 4.41 (-1.59, 10.41) • No significant interaction by sex ***Week 12 MUFcr*** • All: 3.85 (-1.62, 9.33) • No significant interaction by sex ***Week 32 MUFcr*** • All: 2.33 (-2.15, 6.82) • No significant interaction by sex McCarthy, numeric ***Both trimesters MUFcr*** • All: 5.28 (-0.54, 11.1) • No significant interaction by sex ***Week 12 MUFcr*** • All: 3.38 (-1.96, 8.71) • No significant interaction by sex ***Week 32 MUFcr*** • All: 3.47 (-0.88, 7.82) • No significant interaction by sex McCarthy, memory ***Both trimesters MUFcr*** • All: 0.8 (-5.3, 6.9) • No significant interaction by sex ***Week 12 MUFcr*** • All: -0.52 (-6.06, 5.02) • No significant interaction by sex		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Week 32 MUFcr • All: 1.15 (-3.4, 5.69) • No significant interaction by sex McCarthy, general cognitive ***Both trimesters MUFcr*** • All: 10.54 (0.19, 20.89) • Boys: -- • Girls: -0.83 (-8.18, 6.52) • P<0.05 ***Week 12 MUFcr*** • All: 1 (-4.61, 6.61) • No significant interaction by sex: ***Week 32 MUFcr*** • All: 8.15 (0.69, 15.61) • Boys: -- • Girls: -0.46 (-6.04, 5.12) • P<0.05 **Changes in cognitive score per unit (mg/g) increase in MUFcr, β (95% CI), stratified by fluoridated and non-fluoridated zone** Bayley Mental Development Index (MDI) ***Both trimesters MUFcr*** • Both zones/non-fluoridated: -0.52 (-7, 5.95) • No significant interaction by zone ***Week 12 MUFcr*** • Both zones/non-fluoridated: -1 (-6.66, 4.65) • No significant interaction by zone ***Week 32 MUFcr*** • Both zones/non-fluoridated: 0.33 (-4.52, 5.19) • No significant interaction by zone McCarthy, verbal ***Both trimesters MUFcr***		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• Both zones/non-fluoridated: 15.58 (3.71, 27.45) • Fluoridated zone: -2.4 (-11.17, 6.37) • P<0.01 ***Week 12 MUFcr*** • Both zones/non-fluoridated: 0.27 (-6.12, 6.65) • No significant interaction by zone ***Week 32 MUFcr*** • Both zones/non-fluoridated: 16.11 (7.4, 24.81) • Fluoridated zone: -2.3 (-8.6 , 3.99) • P<0.01 McCarthy, performance ***Both trimesters MUFcr*** • Both zones/non-fluoridated: 7.82 (1.58, 14.07) • Fluoridated zone: not reported • P<0.05 ***Week 12 MUFcr*** • Both zones/non-fluoridated: 5.5 (-0.07, 11.07) • No significant interaction by zone ***Week 32 MUFcr*** • Both zones/non-fluoridated: 4.67 (0.08, 9.26) • Fluoridated zone: not reported • P<0.05 McCarthy, numeric ***Both trimesters MUFcr*** • Both zones/non-fluoridated: 4.08 (-2.21, 10.36) • No significant interaction by zone ***Week 12 MUFcr*** • Both zones/non-fluoridated: 2.63 (-2.96, 8.23) • No significant interaction by zone		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Week 32 MUFcr • Both zones/non-fluoridated: 2.53 (-2.06, 7.13) • No significant interaction by zone <u>McCarthy, memory</u> ***Both trimesters MUFcr*** • Both zones/non-fluoridated: 2.71 (-3.77 , 9.18) • No significant interaction by zone ***Week 12 MUFcr*** • Both zones/non-fluoridated: 1.01 (-4.74, 6.77) • No significant interaction by zone ***Week 32 MUFcr*** • Both zones/non-fluoridated: 2.17 (-2.56, 6.9) • No significant interaction by zone: <u>McCarthy, general cognitive</u> ***Both trimesters MUFcr*** • Both zones/non-fluoridated: 15.46 (4.55, 26.36) • Fluoridated zone: 1.96 (-6.09, 10.02) • P<0.01 ***Week 12 MUFcr*** • Both zones/non-fluoridated: 3.5 (-2.36, 9.36) • No significant interaction by zone ***Week 32 MUFcr*** • Both zones/non-fluoridated: 12.88 (4.82, 20.94) • Fluoridated zone: 0.11 (-5.73, 5.95) • P<0.01 **Analyses stratified by fluoridated and non-fluoridated zone, <u>boys only</u>**		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• Significant associations only in non-fluoridated zones [see supplementary table S21 for details.] **Analyses stratified by maternal social class** • "more positive and significant associations were observed in children of mothers with a better social position" [see supplementary table S22] **Analyses stratified by quality of the family context; <u>boys only</u>** • Statistically significant associations only in families with a lower quality of the family context (supplementary table S23) **Other analyses** • Inclusion of other variables, such as other neurotoxicants (As, Mn, Pb, As x Pb), iodine, quality child's family context (HES), deprivation index did not substantially change the results. Analyses including women with only one sample of urine available (first or third trimester), adjustment for zone (fluoridated vs non-fluoridated), or excluding extreme low scores of cognitive functions (less than 2 SD) did not substantially change the results		
Kaur 2022 [401]				
Study design: Cross-sectional **Country:** India **Participants:** School children (12-13 years of age) residing in Dhand of Amer Tehsil, Mohanpura, or Muhana of Sanganer Tehsil. **Sampling time frame:** September 2011 – October 2011	**Exposures:** <u>Fluoride levels in</u> • Water • Urine <u>Exposure level:</u> Water fluoride concentration by group	Correlation between IQ and urinary fluoride level • Group A: r = -0.161 p = > 0.05 • Group B: r = -0.485 p = < 0.01 • Group C: r = -0.334 p = < 0.05	• "No statistically significant correlation (p> 0.05) existed between fluoride excretion and IQ in Group A children. But there was a statistically significant correlation between fluoride excretion and IQ	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Sample size: N = 90 **Sex N (%):** NR **Source of funding / support:** None **Author declaration of interest:** No COI	• Group A: 2 ppm • Group B: 5 ppm • Group C: 2 – 5 ppm Urinary fluoride concentration by group • Group A: 1.60ppm • Group B: 6.82 ppm • Group C: 2.69 ppm **Outcome(s):** • IQ		level in Group B (p<0.01) and Group C (p< 0.05). As the level of fluoride ion concentration in urine increased, there was a significant decrease in IQ level" (p. 3) • "The results indicated that there was a positive correlation between excess fluoride in drinking water and IQ." (p. 1)	
Marques 2022 [402]				
Study design: Cross-sectional **Country:** Brazil **Participants:** High school students aged 17–20 years **Sampling time frame:** January to September 2017 **Sample size:** 660 **Sex: N (%):** Boys: 275 (41.7%) **Source of funding / support:** NR **Author declaration of interest:** NR	**Exposures:** Fluoride levels in Drinking water Exposure level(s): • Fluoridated water: 0.50 to 0.90 ppm • Non-fluoridated water: <0.05 ppm **Outcome(s):** • Dental fluorosis	Fluorosis prevalence and severity (n, %) Fluorosis absent • Exposed: 195 (58.9%) • Unexposed: 260 (79.0%) Very mild or mild fluorosis: • Exposed: 96 (29.0%) • Unexposed: 55 (16.7%) Moderate fluorosis: • Exposed: 40 (12.1%) • Unexposed: 14 (4.3%) P<0.001 Multivariate logistic regression Reference: unexposed, aOR (95% CI) • Very mild or mild fluorosis Exposed: 2.26 (1.54–3.32), P<0.001 • Moderate fluorosis Exposed: 3.66 (1.93–6.95), P<0.001	"The prevalence of dental fluorosis at all levels was higher in fluoridated areas, however, in both groups, there were few cases with esthetic implications."	1
McLaren 2022 [403]				
Study design: Cross-sectional ["pre-post cross-sectional design with comparison group"]	**Exposures:** Water fluoridation Fluoride levels in	Fluorosis prevalence (95% CI), %	"Although estimates of fluorosis were higher in Edmonton than in Calgary,	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Country: Canada **Participants:** Children aged ~7 years old (grade 2 schoolchildren) **Sampling time frame:** • 2018-2019 school year • Pre-cessation data (2004/2005 and 2009/2010 [Calgary only]), early post-cessation data (2013/2014) from previous studies **Sample size:** 2018-2019 • Calgary: 1620 • Edmonton: 1402 2004-2005 • Calgary: 380 • Edmonton: 41,749497 2009-2010 • Calgary: 365 • Edmonton: -- 2013-2014 • Calgary: 2084 • Edmonton: 1749 Fingernail clippings (2018/2019) • Calgary: 34 • Edmonton: 31 **Sex: N (%):** NR **Source of funding / support:** • Research grant from the Canadian Institutes of Health Research (CIHR) (PJT-156258) • Dr McLaren was supported by an Applied Public Health Chair research award funded by CIHR (Institute of Population & Public Health and Institute of	• Fingernails • Water (in water treatment plants) Exposure level(s): Fluoride in water: range (average, if available), µg/L[22] **Calgary** • Bearspaw plant: 2005: 0.6-0.8 2006: 0.7-0.7 2007: 0.6-0.7 2008: 0.7-0.7 2009: 0.7-0.7 2010: 0.7-0.7 **2011: 0.1-0.7** 2012: 0.1-0.1 2013: 0.1-0.2 2014: 0.1-0.3 2015: 0.1-0.1 (0.1) 2016: 0.1-0.1 (0.1) 2017: 0.1-0.2 (0.1) 2018: 0.1-0.2 (0.1) 2019: 0.1-0.3 (0.2) • Glenmore plant: 2005: 0.7-0.8 2006: 0.6-0.8 2007: 0.7-0.7 2008: 0.6-0.7 2009: 0.6-0.8 2010: 0.6-0.9 **2011: 0.1-0.7** 2012: 0.2-0.3 2013: 0.1-0.3 2014: 0.1-0.3	[Note: crude - weighted estimate for the full samples; adjusted - weighted estimate adjusted for covariates; subset - crude weighted estimate for lifelong residents of Calgary or Edmonton who reported usually drinking tap water.] **Years 2018-2019** Calgary (water fluoridation ceased in 2011) • Crude: 8.3 (6.6-10.3)* • Adjusted: 7.7 (5.9-9.6)* • Subset: 6.2 (4.3-8.9)* Edmonton (water fluoridation continues) • Crude: 19.4 (16.3-22.9) • Adjusted: 18.3 (14.9-21.6) • Subset: 18.8 (14.4-24.2) *Calgary vs. Edmonton: P<0.05 **Changes over time (crude estimates)** Calgary (water fluoridation ceased in 2011) • 2004-2005: 22.6 (18.8, 26.9) • 2009-2010: 29.1 (24.6, 34.1) • 2013-2014: 19.9 (17.8, 22.2) • 2018-2019: 8.3 (6.6-10.3) Edmonton (water fluoridation continues) • 2004-2005: 39.8 (37.0, 42.7) • 2009-2010: no data • 2013-2014: 14.1 (11.4, 17.4) • 2018-2019: 19.4 (16.3-22.9) Coefficient (95% CI) for difference of changes: −0.1 [−0.2 to −0.1], P<0.001.	it is important to note that nearly all cases (>99%) in both cities were mild, which is in line with national estimates."	

[22] Fluoridation of drinking water in Calgary ceased on May 19, 2011. Water fluoride values for year 2011 in Calgary are underlined.

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Musculoskeletal Health & Arthritis), the Public Health Agency of Canada, and Alberta Innovates—Health Solutions (CIHR ID CPP-137907) • Dr Weijs was supported by a CIHR Health System Impact Fellowship, 2017-2020 (Award # 403867). **Author declaration of interest:** No COI	2015: 0.2-0.3 (0.3) 2016: 0.2-0.3 (0.2) 2017: <0.1-0.3 (0.2) 2018: 0.2-0.3 (0.2) 2019: 0.1-0.3 (0.2) **Edmonton** • Rossdale plant: 2005: 0.7-1.0 (0.8) 2006: 0.8-0.9 (0.8) 2007: 0.5-0.9 (0.7) 2008: 0.1-0.9 (0.8) 2009: 0.7-0.9 (0.8) 2010: 0.6-0.8 (0.7) 2011: 0.6-0.8 (0.7) 2012: 0.0-0.8 (0.5) 2013: 0.6-0.8 (0.7) 2014: 0.6-0.9 (0.7) 2015: 0.6-0.8 (0.7) 2016: 0.6-0.8 (0.7) 2017: 0.6-0.8 (0.7) 2018: 0.6-0.8 (0.7) 2019: 0.6-0.8 (0.7) • EL Smith plant: 2005: 0.7-0.9 (0.8) 2006: 0.7-0.9 (0.8) 2007: 0.1-0.9 (0.8) 2008: 0.0-0.8 (0.4) 2009: 0.7-0.8 (0.7) 2010: 0.7-0.8 (0.7) 2011: 0.1-0.8 (0.6) 2012: 0.6-0.8 (0.7) 2013: 0.6-0.8 (0.7) 2014: 0.5-0.9 (0.7) 2015: 0.6-0.8 (0.7) 2016: 0.6-0.8 (0.7) 2017: 0.6-0.8 (0.7) 2018: 0.5-0.8 (0.7) 2019: <0.1-0.8 (0.5)			

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	Outcome(s): • Dental fluorosis			
Rani 2022 [404]				
Study design: Cross-sectional **Country:** India **Participants:** Children aged 6-12 years **Sampling time frame:** NR **Sample size:** 1262 **Sex: N (%):** Boys: 615 (48.7%) **Source of funding / support:** None **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in Groundwater used for drinking Exposure level(s): • Fluoride in groundwater (ppm): 0.532–8.802 **Outcome(s):** • Dental fluorosis	Dean's fluorosis index (mean) by level of groundwater fluoride: • Low (<0.7 ppm): 0.62 [1 village] • Optimum (0.7–1.5 ppm): 0.72 to 1.33 [5 villages] • High (1.5-4 ppm): 1.32 to 2.31 [19 villages] • Very high (>4 ppm): 2.62 to 3.34 [5 villages] Correlation between groundwater fluoride and Dean's fluorosis index r=0.922; p<0.01	• "The risk of dental fluorosis was significantly higher in the areas showing more fluoride content in drinking water." • "There is an urgent need to improve the quality of water and institute de-fluoridation of drinking water in affected areas to lower the burden of dental fluorosis in the community either by making alternative sources available or providing water with an optimal concentration of fluoride."	2
Saeed 2022 [405]				
Study design: Cross-sectional **Country:** Pakistan **Participants:** Children aged 5-16 years **Sampling time frame:** NR **Sample size:** 148 (118 exposed; 30 controls) **Sex: N (%):** Boys: 112 **Source of funding / support:** None **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Urine • Groundwater used for drinking Exposure level: Water fluoride (mg/L) • Control group: 0–0.5, mean 0.15 (SD 0.13) • Exposed group: 0.10–15.80, mean 5.64 (SD 3.52) • P=0.000 Urinary fluoride (mg/L)	**Dental fluorosis** Frequency and severity of dental fluorosis, n (%) Control group • Normal: 28 (94.0) • Questionable: 2 (6.0) Exposed group • Normal: 0 • Questionable: 16 (13.55) • Very mild: 22 (18.65) • Mild: 21 (17.80) • Moderate: 25 (21.19) • Severe: 34 (28.81) Correlation analysis	• "Mean urinary concentrations of As … and F- … as well as the frequency of dental fluorosis were found elevated among the exposed group." • "The cases of children with lower IQ were observed high in the exposed group." • "… it was revealed that variations in dental fluorosis and IQ levels were more significantly associated with F-	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	• Control group: 0.40–0.75, mean 0.24 (SD 0.15) • Exposed group: 0.47–14.56, mean 3.27 (SD 2.60) • P=0.000 **Outcome(s):** Dental fluorosis Non-verbal intelligence quotient (IQ)	Water fluoride and urinary fluoride: R^2=0.224; p=0.006 Water fluoride and dental fluorosis: R^2=0.380; p=0.000 Urinary fluoride and dental fluorosis: R^2=0.721; p=0.000 Linear regression analysis Fluoride in urine as an independent variable: • β=0.38 (SE 0.03) [unstandardized] • β=0.66 [standardized]; p=0.00 Other independent variables in the model: gender, family economic status, arsenic in urine. Model summary: F = 49.00; adjusted R^2=0.57; p=0.000 **Non-verbal intelligence quotient (IQ)** IQ score Control group: 80.25–127.75; mean 100.93 (SD 13.1) Exposed group: 63.97–127.31; mean 97.26 (SD 15.39) P=0.233 Correlation analysis Water fluoride and urinary fluoride: R^2=0.224; p=0.006 Water fluoride and IQ score: R^2=-0.034; p=0.683 Urinary fluoride and IQ score: R^2=-0.655; p=0.000 Dental fluorosis and IQ score: R^2=-0.552; p=0.000 Note: Levels of fluoride significantly correlated with arsenic levels.	exposure compared to As."	

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		<u>Linear regression analysis</u> Fluoride in urine as an independent variable: • β=-3.45 (SE 0.50) [unstandardized] • β=-0.60 [standardized] • P=0.00 Other independent variables in the model: age, gender, parental education, dental fluorosis. Model summary: F = 29.64; adjusted R^2=0.49; p=0.000 <u>Intelligence level vs mean (SD) water fluoride (WF), urinary fluoride (UF), water arsenic (WA) and urinary arsenic (UA)</u> Superior (IQ score ≥130): no participants with this level Above average (IQ score 120-129) • WF: 1.96±2.77 mg/L • UF: 0.54±0.59 mg/L • WA: 0.02±0.05 mg/L • UA: 0.68±1.54 mg/L High Average (IQ score 111-119) • WF: 4.60±4.40 mg/L • UF: 1.20±0.80 mg/L • WA: 0.12±0.15 mg/L • UA: 2.71±1.78 mg/L Average (QI score 90-100) • WF: 4.3±3.99 mg/L • UF: 1.99±1.28 mg/L • WA: 0.16±0.22 mg/L • UA: 3.13±2.29 mg/L Low average (IQ score 80-89) • WF: 3.84±3.63 mg/L • UF: 3.61±2.84 mg/L • WA: 0.14±0.16 mg/L		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• UA: 2.65±1.80 mg/L Borderline (IQ score 70-79) • WF: 6.19±4.59 mg/L • UF: 7.13±2.62 mg/L • WA: 0.15±0.09 mg/L • UA: 3.75±1.26 mg/L Retarded (IQ score <70) • WF: 4.92±3.46 mg/L • UF: 8.10±5.84 mg/L • WA: 0.17±0.28 mg/L UA: 3.50±0.81 mg/L		
Tawfik 2022 [406]				
Reference type: Original study **Study design:** Cross-sectional **Country:** Egypt **Participants:** 7-14 years old children with no tooth fillings or braces, who live in the same region since birth **Sampling time frame:** December 2020- March 2021 **Sample size:** 202 **Sex: N (%):** NR **Source of funding / support:** Self-funded **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in: • Groundwater **Exposure level(s):** • Fluoride Levels in drinking water: • 7.5-9.5, mean 8mg/L **Outcome(s):** • Dental fluorosis	• Dental Fluorosis – Modified Dean's Index: Mean ± SD: 2.31 ±0.94 • Dental Fluorosis (%) Normal: 0% Questionable: 0% Very Mild: 19.8% Mild: 40% Moderate: 30% Severe:9.9%	• "Correlation between fluorosis status and fluoride level in drinking water was performed by using Pearson`s correlation coefficient and revealed strong, positive, significant correlation." • "Nubian children recorded moderate and severe fluorosis status score because on analysis of their drinking water, their result showed that mean fluoride level was 8 mg/L."	1
Thilakarathne 2022 [407]				
Study design: Cross-sectional **Country:** Sri Lanka **Participants:** Children aged 15 years **Sampling time frame:** NR **Sample size:** 1040 **Sex: N (%):** Boys: 45.2%	**Routes of exposures:** Fluoride level in • Drinking water Exposure level(s): Fluoride levels in water: 0.0-1.9 mg/L	Prevalence of dental fluorosis • TF score > 0: 51.7% • TF score > 1: 41.5% • TF score > 2: 20.5% Prevalence of dental fluorosis by TF score • TF0 [normal]: 48.3%	"The prevalence of dental fluorosis was high and it increased with the increase in the fluoride content in the drinking water source."	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Source of funding / support: Research Grant (RG/2016/84/D) from the University of Peradeniya **Author declaration of interest:** NR	**Outcome(s):** • Dental fluorosis	• TF1: 10.2% • TF2: 20.9% • TF3: 11.8% • TF4: 5.9% • TF5: 2.3% • TF6: 0.5% <u>Association between fluoride level in drinking water and prevalence of dental fluorosis (TF score>0)</u> • Water fluoride <0.3 mg/L: 42.3% • Water fluoride 0.31-0.6 mg/L: 62.8% • Water fluoride 0.61-0.9 mg/L: 70.1% • Water fluoride >0.9 mg/L: 88.9 • p (Chi sq for trend) <0.001		
Al-Omoush 2021 [17]				
Study design: Cross-sectional **Country:** Jordan **Participants:** Schoolchildren residing in Ruwaished (age 15.3 +/- 1.4 years) and Kuraymah (age 16.1 +/- 1.3 years) **Sampling time frame:** NR **Sample size:** • Ruwaished: 100 • Kuraymah: 141 **Sex:** • Ruwaished: Men: 60% • Kuraymah: Men: 39.7% **Source of funding:** NR **Author declaration of interest:** No COI	**Exposures:** <u>Fluoride level in</u> • Drinking water samples from wells <u>Exposure level:</u> Average fluoride level in water (ppm) • Ruwaished: 1.38 • Kuraymah: 1.10 **Outcome(s):** Dental fluorosis prevalence and severity	Frequency (%) distribution of dental fluorosis by Dean's Fluorosis Index in: • Kuraymah ○ Normal: 10 / 141 (7.1%) ○ Very mild: 13 / 141 (9.2%) ○ Mild: 21 / 141 (14.9%) ○ Moderate: 51 / 141 (36.2) ○ Severe: 46 / 141 (32.6) • Ruwaished ○ Normal: 0 / 100 (0%) ○ Very Mild: 9 / 100 (9%) ○ Mild: 19 / 100 (19%) ○ Moderate: 22/100 (22%) Severe: 50 / 100 (50%)	"This study concluded that higher fluorosis incidence and severity were present in the higher-altitude location (Ruwaished). Moreover, this study also indicated that … the preventive management of dental fluorosis should be directed to de-fluoridation of drinking water in endemic areas." (p. 707 – 708)	2
Ayele 2021 [18]				

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Study design: Cross-sectional **Country:** Ethiopia **Participants:** Persons aged 10–70 years old, selected at random from those who lived and used water wells from 23 rural villages **Sampling time frame:** Two sampling periods (between 2018 and 2019) **Sample size:** 316 **Sex:** Men: 55.7% **Source of funding:** NIEHS's career development grant **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Ground water (community wells) Exposure level: • Mean concentration: 6.8 ± 4.3 mg/L • Range: 0.3–15.5 mg/L **Outcome(s):** • Skeletal fluorosis • Joint pain • Neurological manifestations (headache, paresthesia, loss of appetite, constipation, and fatigue)	• At least one clinical sign of skeletal fluorosis was observed in 54.4% of the study participants. • For every 1 mg/L increment of fluoride in drinking water, the odds of skeletal fluorosis increased by 1.15 upon adjustment for age and selected clinical variables [Adjusted OR 1.15, 95%CI (1.04–1.27); p = 0.006]. • Signs of crippling fluorosis were observed in small proportion (1.6%) of participants. • Fluoride concentration in drinking water and joint pain were found to be independent predictors of skeletal fluorosis. • Headache and joint pain reported by 67.1% and 56.3% of participants as the most common neurological manifestation, and skeletal fluorosis symptom, respectively. • The mean fluoride level was higher for those individuals who reported paresthesia compared to those with no-paresthesia. • Loss of appetite, constipation, and fatigue were reported by 48.0%, 45.6%, and 56.6% of the participants, respectively. Individuals who reported headache are most likely exposed to higher fluoride concentrations in drinking water compared to those reported no-headache (p<0.001).	"The study demonstrates high prevalence of neuro-medical manifestations of fluorosis in population living in the Main Ethiopian Rift valley. Fluoride concentration in drinking water and joint pain were independent predictors of fluorosis."	2[23]
Cao 2021 [408]				

[23] *Quality was assessed as tier 1 for the skeletal fluorosis outcome, and tier 2 for the neurological symptoms' outcome. A conservative assessment of the overall quality was set to tier 2.*

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Reference type: Original study **Study design:** Cross-sectional **Country:** China **Participants:** Dental fluorosis: Children aged 8-<13 years Urinary fluoride: Age 25 and over **Sampling time frame:** June 2017-June 2019 **Sample size:** Dental fluorosis: 1346 Urinary fluoride: 450 **Sex:** Boys: 50% **Source of funding / support:** NR **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in: • Drinking water • Urine **Exposure level(s):** • Drinking water Fluoride range: 0.05-0.76 mg/L • Urinary Fluoride 0.04 - 3.76 mg/L (Geometric Mean: 0.8 mg/L) • Upper limit of normal value is ≤1.60 mg/L. **Outcome(s):** • Dental fluorosis	**Results: CHI SQURE tests add** • Detection rates for dental fluorosis: (P:0.357) 2017: 1.75% (7/401) 2018: 1.40% (7/500) 2019: 0.67% (3/445) .062, P=0.357 Overall, 2017-2019: 1.26% (17/1 346) Total DF Index: 0.03 • Dental fluorosis cases: Suspicious: 35(2.60%) Very Mild: 12 (0.89%) Mild: 5 (0.37%) Moderate: 0 Severe:0 • Highest DF in Minhou County Detection rates/years: 2017: 21.21% (7/33) 2018: 17.95% (7/39) 2019: 13.04% (3/23) P=0.7	• "The prevalence rate of dental fluorosis among children in each diseased area is <30%." • "Results indicate reduction of fluoride in Fuzhou county, concluded in reduction of endemic dental fluorosis (with very mild and mild cases)." "There is no statistically significant difference in the detection rate of dental fluorosis among children in each year and among children of different age. groups"	1
Dong 2021 [19]				
Study design: Cross-sectional **Country:** United States **Participants:** US children and adolescents 6–19 years old (NHANES survey) **Sampling time frame:** 2015-2016 **Sample size:** 2098 children and adolescents **Sex:** Men: 50.24% **Source of funding:** • Fundamental Research Funds for the Central Universities (No. 3332019030) • Youth Program of Peking Union Medical College Hospital	**Exposures:** Fluoride levels in • Drinking water • Serum Exposure level: Mean (SD) water fluoride (mg/L): • All: 0.46 (0.40) • Men: 0.48 (0.41) • Women: 0.47 (0.38) • Children: 0.52 (0.44) • Adolescents 0.43 (0.35)	• The rate of fluoride concentration in water above the recommended level of 0.7 mg/L was 25%, but the prevalence of dental fluorosis was 70%. • Binary logistic regression adjusted for covariates showed that higher water fluoride concentrations (0.31–0.50, 0.51–0.70, > 0.70 compared 0.00–0.30) were associated with higher odds of dental fluorosis ○ *0.31–0.50: OR=1.48 (1.13–1.96), p = 0.005* ○ *0.51–0.70: OR=1.92, (1.44–2.58, p < 0.001*	"Even low level of water or plasma fluoride exposure was associated with increased risk of dental fluorosis."	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Foundation (No. PUMCH 201910847), • National Natural Science Foundation of China (81703198). **Author declaration of interest:** No COI	Mean (SD) plasma fluoride (µmol/L): • All: 0.35 (0.22) • Men: 0.36 (0.19) • Women: 0.34 (0.25) • Children: 0.38 (0.24) • Adolescents: 0.32 (0.20) **Outcome(s):** • Dental fluorosis	○ <u>> 0.70:</u> *OR=2.30 (1.75–3.07), p < 0.001* • The pattern of regression between plasma fluoride and dental fluorosis was similar.		
Farmus 2021 [409]				
Study design: Cohort study **Country:** Canada **Participants:** Mother-child pairs in the Maternal-Infant Research on Environmental Chemicals (MIREC) study **Sampling time frame:** 2008-2011 **Sample size:** N = 596 **Sex (%):** Females: 51.2% **Source of funding / support:** • National Institute of Environmental Sciences (NIEHS) • Chemicals Management Plan at Health Canada, the Ontario Ministry of the Environment, and the Canadian Institutes for Health Research **Author declaration of interest:** No COI	**Exposures:** <u>Fluoride levels in</u> • Maternal urine (MUF): prenatal exposure • Children urine (CUF): Childhood exposure <u>Exposure level:</u> Median (range) fluoride levels • MUF T1 (mg/L): 0.31 (0.01 – 4.29) • MUF T2 (mg/L): 0.37 (0.03 – 5.28) • MUF T3 (mg/L): 0.49 (0.08 – 5.56) • IFI (mg F): 0.09 (0.00 – 0.61) • CUF (mg/L): 0.39 (0.05, 2.89) **Outcome(s):** • Intelligence at 3 to 4 years of age	Change (95% CI) in age-normed in FSIQ scores per unit increase in standardized fluoride exposure <u>Males</u> • MUF: -1.86 (-3.22, -0.49) • IFI: -0.01 (-1.67, 1.65) • CUF: 0.07 (-1.66, 1.80) • Pint: .012 <u>Females</u> • MUF: -0.23 (-2.06, 1.60) • IFI: -0.72 (-2.34, 0.89) • CUF: -0.41 (-2.07, 1.24) • Pint: 0.77 <u>Overall</u> • MUF: -1.28 (-2.37, -0.18) • IFI: -0.38 (-1.53, 0.78) • CUF: -0.18 (-1.38, 1.02) • Pint: -0.23 Change (95% CI) in age-normed in PIQ scores per unit increase in standardized fluoride exposure <u>Males</u> • MUF: -3.01 • IFI: -1.45 (-3.40, 0.49)	"Our results suggest the associations of prenatal and postnatal fluoride exposure with cognitive development may be modified by sex, though further replication of this finding is needed. These results indicate that it is important to balance the risks of fluoride exposure during early brain development with its potential to prevent caries, especially for pregnant women and infants." (p. 7)	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• CUF: -1.49 (-3.50, 0.53) • Pint: 0.01 <u>Females</u> • MUF: -1.18 (-3.32, 0.96) • IFI: -2.71 (-4.59, -0.83) • CUF: -1.53 (-3.45, 0.39) • Pint: 0.01 <u>Overall</u> • MUF: -2.36 (-3.63, -1.08) • IFI: -2.11 (-3.45, -0.76) • CUF: -1.51 (-2.90, -0.12) • Pint: <0.001 Change (95% CI) in age-normed in VIQ scores per unit increase in standardized fluoride exposure <u>Males</u> • MUF: -0.25 (-1.57, 1.07) • IFI: 1.22 (-0.39, 2.83) • CUF: 1.61 (-0.06, 3.29) • Pint: 0.12 <u>Females</u> • MUF: 0.87 (-0.91, 2.64) • IFI: 1.31 (-0.25, 2.87) • CUF: 0.63 (-0.98, 2.23) • Pint: 0.30 <u>Overall</u> • MUF: 0.15 (-0.91, 1.20) • IFI: 1.27 (0.15, 2.39) • CUF: 1.10 (-0.06, 2.26) • Pint: 0.04 Change (95% CI) in FSIQ scores per unit increase (0.5 mg/L MUF; 0.1 mg/day IFI; 0.5 mg/L CUF) in fluoride exposure <u>Males</u>		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• MUF: -2.48 (-4.30, -0.66) • IFI: -0.01 (-1.25, 1.24) • CUF: 0.09 (-2.10, 2.28) • Pint: 0.12 <u>Females</u> • MUF: -0.31 (-2.76, 2.14) • IFI: -0.54 (-1.75, 0.66) • CUF: -0.52 (-2.62, 1.58) • Pint: 0.77 <u>Overall</u> • MUF: -1.71 (-3.17, -0.24) • IFI: -0.28 (-1.15, 0.58) • CUF: -0.23 (-1.75, 1.29) • Pint: 0.23 Change (95% CI) in PIQ scores per unit increase (0.5 mg/L MUF; 0.1 mg/day IFI; 0.5 mg/L CUF) in fluoride exposure <u>Males</u> • MUF: -4.02 (-6.15, -1.89) • IFI: -1.09 (-2.54, 0.37) • CUF: -1.89 (-4.44, 0.67) • Pint: 0.01 <u>Females</u> • MUF: -1.58 (-4.43, 1.28) • IFI: -2.03 (-3.43, -0.63) • CUF: -1.94 (-4.37, 0.50) • Pint: 0.01 <u>Overall</u> • MUF: -3.15 (-4.85, -1.44) • IFI: -1.58 (-2.59, -0.57) • CUF: -1.91 (-3.68, -0.15) • Pint: <0.001 Change (95% CI) in VIQ scores per unit increase (0.5 mg/L MUF; 0.1 mg/day IFI; 0.5 mg/L CUF) in fluoride exposure		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		<u>Males</u> • MUF: -0.34 (-2.10, 1.43) • IFI: 0.92 (-0.29, 2.12) • CUF: 2.05 (-0.08, 4.16) • Pint: 0.12 <u>Females</u> • MUF: 1.16 (-1.22, 3.53) • IFI: 0.98 (-0.19, 2.15) • CUF: 0.79 (-1.24, 2.82) • Pint: 0.30 <u>Overall</u> • MUF: 0.20 (-1.22, 1.61) • IFI: 0.95 (0.11, 1.79) • CUF: 1.39 (-0.08, 2.86) • Pint: 0.04 Sensitivity analysis where influential mother-child dyads were removed was conducted • Association of MUF and FSIQ in boys became weaker and not statistically significant • No change in status of statistical significance for other associations tested		
Fernandes 2021 [410]				
Study design: Cross-sectional **Country:** Brazil **Participants:** Children 6-12 years old **Sampling time frame:** April-September 2019 **Sample size:** 610 **Sex: N (%):** Boys: 329 (53.9%) **Source of funding / support:** Federal University of Paraiba, Pro-Reitoria de Pesquisa	**Exposures:** <u>Fluoride levels in</u> • Drinking water (school water fountains) <u>Exposure level(s):</u> Water fluoride (ppm): 0.06-1.98 Group I (≤0.7): 485 children	Group I (water fluoride ≤0.7 ppm): • Fluorosis absent: 306 (63.1%) children. • Fluorosis present: 179 (36.9%) children Group II (water fluoride >0.7 ppm): • Fluorosis absent: 69 (55.2%) children. • Fluorosis present: 56 (44.8%) children P=0.10	The authors pointed to the high prevalence of dental fluorosis among children exposed to water fluoride ≤0.7 ppm, which may be "an indication of other sources of fluoride (F-toothpaste 1500 ppm) in this region, which was previously observed in other studies".	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Author declaration of interest: No COI	Group II (>0.7): 125 children, including: • 0.7-1.0: 14 children • >1.0-1.98: 111 children **Outcome(s):** • Dental fluorosis	Fluorosis absent: OR=1.02 (95% CI: 0.983-1.168) Fluorosis present: 0.77 (0.565-1.055)		
Du 2021 [20]				
Study design: Cross-sectional **Country:** China **Participants:** Children aged 7–12 years old **Sampling time frame:** 2017 **Sample size:** 446 **Sex:** Boys: 237 (53.1%) **Source of funding:** • National Natural Science Foundation of China • The Henan Department of Science and Technology, China • Zhengzhou University **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Urine Exposure level: Urinary fluoride (mg/l) • All: 1.45 ± 0.88 • Boys: 1.43 ± 0.89 • Girls: 1.48 ± 0.87 • t/x2: 0.490 • P-value: 0.624 **Outcome(s):** Thyroid hormone dysfunction: • Total triiodothyronine (TT3) • Total thyroxine (TT4) • Thyroid-stimulating hormone (TSH) • Tvols (thyroid volumes)	• No significant difference between boys and girls in age, maternal education, urinary creatinine, urinary fluoride, urinary iodine, Tvol, TT4, and TT3. • BMI in boys was significantly higher than that in girls (P < 0.05), • TSI concentration was significantly lower in boys than girls (P < 0.001) • Tvols increased by 0.22 (95% CI: 0.14, 0.31) cm^3 with each standard deviation increment of UF. • Tvols in boys were more susceptible to fluoride exposure than those in girls • Tvols of children with high urinary iodine are less susceptible to fluoride exposure (P for interaction < 0.05). TT3 levels were negatively related to UF concentrations at moderate urinary iodine levels (≤ 300 μg/l).	"Fluoride exposure can elevate the Tvols of school-age children, especially in boys, and high levels of iodine may alleviate this effect to some extent"	1
Helte 2021 [21]				
Study design: Cohort **Country:** Sweden **Participants:** All SMC participants who were <85 years of age and residing in the city of Uppsala or nearby surrounding areas	**Exposures:** Fluoride levels in • Water • Diet • Urine Exposure level:	• At baseline: o Mean urinary fluoride: 1.2 mg/g creatinine (± 1.9) o mean dietary intake was 2:2 mg/d (± 0.9) • During follow-up:	"In this cohort of postmenopausal women, the risk of fractures was increased in association with two separate indicators of fluoride exposure. Our findings are consistent with	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Sampling time frame: Baseline: 2004-2009 Follow-up: 2017 **Sample size:** 4,306 **Sex:** Women: 100% **Source of funding:** • Formas, the Swedish Research Council for Environment • Agricultural Sciences and Spatial Planning • Swedish Research Council **Author declaration of interest:** No COI	• Water: ≤1 mg/L • Mean urinary fluoride at baseline: 1.2 mg/g creatinine (0.1–7.3 mg/g creatinine) • Mean estimated dietary fluoride intake: 2.2 mg/d (0.3–8.4 mg/d) **Outcome(s):** Bone mineral density and fracture incidence in postmenopausal women	○ 850, 529, and 187 cases of any fractures, osteoporotic fractures, and hip fractures, respectively, were ascertained. • Baseline BMD was slightly higher among women in the highest vs. lowest tertiles of exposure. • Fluoride exposures were positively associated with incident hip fractures, with multivariable-adjusted hazard ratios of 1.50 (95% CI: 1.04, 2.17) and 1.59 (95% CI: 1.10, 2.30), for the highest vs. lowest tertiles of urine fluoride and dietary fluoride, respectively. • Associations with other fractures were less pronounced for urine fluoride, and null for dietary fluoride. • Restricting the analyses to women with consistent long-term drinking water exposures prior to baseline strengthened associations between fractures and urinary fluoride.	RCTs and suggest that high consumption of drinking water with a fluoride concentration of ~1 mg/L may increase both BMD and skeletal fragility in older women"	
James 2021 [22]				
Study design: Cross-sectional **Country:** Ireland **Participants:** Children (7 to 9 years of age) from Dublin and Cork-Kerry in the year 2002 and 2017 **Sampling time frame:** 2002 and 2014 **Sample size (N):** • Year 2000 ○ Dublin= 679 ○ Cork-Kerry = 565 • Year 2017 ○ Dublin= 707 ○ Cork-Kerry = 1,148	**Exposures:** Community water fluoridation (CWF) Exposure level: CWF before and after introduction of policy measures • Before in 2002: ○ 0.8 to 1.0 ppm • After in 2007: ○ 0.6 to 0.8 ppm **Outcome(s):**	*Odds (95% CI) of fluorosis prevalence in the year 2017 compared to 2002* • Dublin Full CWF OR = 16 (-13, 56), p = 0.312 • Cork-Kerry Full CWF OR = -7 (-41, 48), p = 0.771 • Cork-Kerry No CWF OR = 97 (-18, 373), p = 0.129 "Among children with full CWF in Dublin, fluorosis prevalence was 18% in 2017 and 15% in 2002, and in Cork-Kerry, it was 12% in 2017 and 13% in 2002... Fluorosis prevalence among children with no CWF in Cork-Kerry was 5% in	"In 2017, fluorosis prevalence was 18% in Dublin (full CWF) and 12% in Cork-Kerry (full CWF). Fluorosis was predominantly "very mild" with no statistically significant difference between 2017 and 2002." (p. 507)	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Sex: (2002) • Dublin Full CWF: boys: 53% • Cork-Kerry Full CWF: boys: 45% • Cork-Kerry No CWF: boys: 44% (2017) • Dublin Full CWF: Men: 46% • Cork-Kerry Full CWF: Men: 47% • Cork-Kerry No CWF: Men: 49% **Source of funding:** • Health Research Board • Department of Health and the National Oral Health Office of the Health Services Executive **Author declaration of interest:** No COI	Dental fluorosis	2017 and 3% in 2002. None of the differences were statistically Significant..." *Fluorosis prevalence (N, %) in year 2002 and 2017 by location* • Dublin Full CWF *2002* ○ Normal/Questionable: 567 (85%) ○ Very mild or higher: 104 (15%) *2017* ○ Normal/Questionable: 576 (82%) ○ Very mild or higher: 127 (18%) • Cork-Kerry Full CWF *2002* • Normal/Questionable: 283 (87%) • Very mild or higher: 42 (13%) *2017* ○ Normal/Questionable: 328 (88%) ○ Very mild or higher: 43 (12%) • Cork-Kerry No CWF *2002* ○ Normal/Questionable: 222 (97%) ○ Very mild or higher: 6 (3%) *2017* ○ Normal/Questionnaire: 732 (95%) ○ Very mild or higher: 40 (5%) *Dean's index score (N, %) in year 2002 and 2017 by location* • Dublin Full CWF *2002* ○ Normal: 488 (73%) ○ Questionable: 79 (12%) ○ Very mild: 75 (11%) ○ Mild: 24 (4%) ○ Moderate: 5 (<1%) *2017* ○ Normal: 441 (63%) ○ Questionable: 135 (19%)		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		○ Very mild: 99 (14%) ○ Mild: 26 (4%) ○ Moderate: 2 (<1%) • Cork-Kerry Full CWF *2002* ○ Normal: 245 (75%) ○ Questionable: 38 (12%) ○ Very mild: 25 (8%) ○ Mild: 17 (5%) ○ Moderate: 0 (0%) *2017* ○ Normal: 232 (63%) ○ Questionable: 96 (26%) ○ Very mild: 34 (9%) ○ Mild: 8 (2%) ○ Moderate: 1 (<1%) • Cork-Kerry No CWF *2002* ○ Normal:196 (86%) ○ Questionable: 26 (11%) ○ Very mild: 6 (3%) ○ Mild: 0 (0%) ○ Moderate: 0 (0%) *2017* ○ Normal: 613 (79%) ○ Questionable: 119 (15%) ○ Very mild: 38 (5%) ○ Mild: 2 (<1%) ○ Moderate: 0 (0%)		
Meghe 2021 [23]				
Study design: Cross-sectional **Country:** India **Participants:** Residents with no evidence of skeletal fluorosis **Sampling time frame:** NR **Sample size:** 3,268 **Sex (N):** Men: 1,760 (53.86%)	**Exposures:** <u>Fluoride levels in</u> • Ground water <u>Exposure level:</u> • ≤1mg/L • 1.01-2.0 mg/L • 2.01-4.0 mg/L	**Relation of skeletal fluorosis with F-level in drinking water** • **Normal (74.8%):** ○ ≤1 ppm: 29.73% ○ 1.01–2.00: 28.14% ○ 2.01–4.00: 24.21% ○ >4.00: 17.92% • **Mild (13.2%):**	• "Out of the total 3268 subjects 2445 subjects included in the 'normal' grade, which does not show indications of skeletal fluorosis." • "… as the concentration of fluoride increases the	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Source of funding: Datta Meghe Institute of Medical Sciences **Author declaration of interest:** No COI	• >4.0 mg/L **Outcome(s):** Skeletal fluorosis	○ ≤1 ppm: 13.9% ○ 1.01–2.00: 16.47% ○ 2.01–4.00: 22.7% ○ >4.00: 46.87% • **Moderate (6.0%):** ○ ≤1 ppm: – ○ 1.01–2.00: 18.46% ○ 2.01–4.00: 25.13% ○ >4.00: 56.41% • **Severe (4.1%):** ○ ≤1 ppm: – ○ 1.01–2.00: 15.55% ○ 2.01–4.00: 31.11% ○ >4.00: 53.34% • **Very severe (1.9%):** ○ ≤1 ppm: – ○ 1.01–2.00: 17.74% ○ 2.01–4.00: 25.81% ○ > 4.00: 56.45%	cases of 'normal' grade decreases."	
Meng 2021 [24]				
Study design: Cross-sectional **Country:** China **Participants:** Adults (> 18 years of age) born in one of five villages (Hongguang, Xiaoshan, Fushan, Wanfa, and Leye) **Sampling time frame:** April – September 2016 **Sample size:** 281 **Sex:** Men: 32% **Source of funding:** • National Natural Science Foundation of China • The Wu Liande Science Foundation of Harbin Medical University	**Exposures:** <u>Fluoride levels in</u> • Drinking water • Urine <u>Exposure level:</u> Fluoride quartiles in drinking water: • Q1 (≤ P25): ○ 1.4559 mg/L • Q2 (P25 ~ P50): ○ 1.4559 ~ 2.2434 mg/L • Q3 (P50 ~ P75): ○ 2.2434 ~ 3.2342 mg/L • Q4 (>P75): ○ 3.2342 mg/L	*Mean (SD) of 5-mC by water quartile groups in mg/L* • Q1: 0.15 (0.09) • Q2: 0.11 (0.08) • Q3: 0.11 (0.08) • Q4: 0.14 (0.07) • p = 0.001 *Association between fluoride and 5-mC with cubic curve fitted* • R^2 = 0.061 • F = 6.045 • p = 0.001	"…fluoride could impact 5-mC level in human and rat. The U-shaped relationship was found between fluoride and 5-mC in the population and in the rats with 3 months fluoride treatments. These results clued that the disruption of DNA methylation in mammals may has a certain association with fluoride in natural exposures." (p. 5 – 6)	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
• Post-doctoral Scientific Research Developmental Fund of Heilongjiang Province **Author declaration of interest:** No COI	Median levels of fluoride in drinking water • 2.2434 mg/L P50 (P25, P75) levels of fluoride in water by quartile (mg/L) • Q1 (N = 70) ○ 1.100 (0.767, 1.414) • Q2 (N = 71) ○ 1.853 (1.629, 2.069) • Q3 (N = 70) ○ 2.691 (2.400, 2.949) • Q4 (N = 70) ○ 4.123 (3.600, 5.200) P50 (P25, P75) levels of fluoride in urine by quartile (mg/L) • Q1 (N = 70) ○ 2.040 (1.612, 3.331) • Q2 (N = 71) ○ 2.432 (1.981, 3.083) • Q3 (N = 70) ○ 2.432 (1.788, 3.169) • Q4 (N = 70) ○ 3.780 (2.940, 5.692) **Outcome(s):** Genotoxicity (5-methylcytosine (5-mC) level of genome in human blood)			
Mohd Nor 2021 [25]				
Study design: Cross-sectional **Country:** Malaysia **Participants:** Lifelong residents aged 9- and 12-year-olds	**Exposures:** <u>Fluoride levels in</u> • Public drinking water supply	"Fluorosis prevalence was lower (31.9 percent) among the younger children born after the reduction of fluoride concentration in the water, compared to	• "Fluorosis was lower among children born after the adjustment of	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Sampling time frame: 2015 (calculated using the following information reported by the authors) • 9-year-old children (born between 1 January and 31 December 2006 • 12-year-old children (born between 1 January and 31 December 2003) **Sample size:** 1,143 **Sex:** Boys: 43% **Source of funding:** Ministry of Higher Education, Malaysia **Author declaration of interest:** No COI	Exposure level: • Original: 0.7 ppm • Reduced: 0.5 ppm **Outcome(s):** Dental fluorosis	a prevalence of (38.4 percent) in the older cohort." **Simple logistic regression of fluorosis and infant feeding (n=830)** *Fluorosis (Deans ≥ 2),* *Type of water used to prepare formula* Bottled water • Fluorosis: 3 (9.4%) • No fluorosis: 29 (90.6%) • Reference Tap water • Fluorosis: 162 (25.7%) • No fluorosis: 469 (74.3%) • OR (95% CI): 3.34 (1.0–11.11) • P-value: 0.049* Filtered tap water • Fluorosis: 47 (28.1%) • No fluorosis: 120 (71.9%) • OR (95% CI): 3.79 (1.1–13.03) • P-value: 0.035* **Simple logistic regression of fluorosis and water fluoride (n=1,143)** *Fluorosis (Deans ≥ 2),* 0 lifetime • Fluorosis: 30 (12.30%) • No fluorosis: 517 (57.4%) • Reference 0.5 ppm lifetime • Fluorosis: 100 (41.2%) • No fluorosis: 204 (22.7%) • OR (95% CI): 8.45 (5.45–13.10) • P-value: 0.001 0.7 ppm for first 2 years then 0.5 ppm • Fluorosis: 113 (46.5%) • No fluorosis: 179 (19.9%) • OR (95% CI): 10.88 (7.03–16.84) • P-value: 0.001	fluoride concentration in the water." • "Fluoridated water remained as a strong risk factor for fluorosis after downward adjustment of its fluoride concentration." • "Early tooth brushing practices and fluoridated toothpaste were not statistically associated with fluorosis status." • "However, the prevalence of fluorosis was significantly associated with parents' education level, parents' income, fluoridated water, type of infant feeding method, age breast feeding ceased, use of formula milk, duration of formula milk intake, and type of water used to reconstitute formula milk"	

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Multiple logistic regression of fluorosis (n=830) *Fluorosis (Deans ≥ 2),* *Type of water used to prepare formula* Bottled water • Reference Tap water • OR (95% CI): 9.90 (1.28–76.38) • P-value: 0.028 Filtered tap water • OR (95% CI): 8.78 (1.11–69.71) 0.040 • P-value: 0.040 **Multiple logistic regression of fluorosis and water fluoride (n=1,143)** 0 lifetime • Reference 0.5 ppm lifetime • Adj. OR (95% CI): 5.97 (3.32–10.72) • P-value: <0.001 0.7 ppm for first 2 years then 0.5 ppm • Adj. OR (95% CI): 9.12 (5.15–16.14) • P-value: <0.001		
Rojanaworarit 2021 [411]				
Study design: Cross-sectional **Country:** Thailand **Participants:** Children aged 6-10 years **Sampling time frame:** 2015 **Sample size:** 289 **Sex: N (%):** Boys: 153 (52.9%) **Source of funding / support:** Fogarty International Center of the National Institutes of Health under Award Number U2RTW010088. **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Groundwater used for household water supply. Exposure level(s): Time-averaged fluoride concentration (ppm) by dental fluorosis status Normal (no fluorosis) • Mean (SD): 2.0±1.6 • Median (IQR): 1.6 (1.1) • Range: 0.4-9.4 Questionable fluorosis • Mean (SD): 1.7±0.6	Prevalence of dental fluorosis (%) by subdistrict • Sai Ngam: 50.77 • Bang Sai Pa: 42.50 • Hin Mun: 64.18 • Bang Luang: 59.43 • Nin Phet: 9.09 Prevalence of dental fluorosis (%) by water fluoride level • <0.7 ppm: 23.3% • 0.7–1.49 ppm: 37.7% • ≥1.5 ppm: 64.1% • Exact probability test; P < 0.001	• "In fluoride endemic areas, groundwater containing natural fluoride utilized for household consumption resulted in high dental fluorosis prevalence, particularly in the groundwater with fluoride concentrations of ≥ 1.5 ppm." • "The finding of 23.3% prevalence with only the very mild dental fluorosis among children with time-averaged fluoride	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	• Median (IQR): 1.7 (0.6) • Range: 0.6-3.0 <u>Very mild fluorosis</u> • Mean (SD): 2.8±2.2 • Median (IQR): 2.0 (1.4) • Range: 0.4-9.4 <u>Mild fluorosis</u> • Mean (SD): 2.8±2.3 • Median (IQR): 2.1 (1.4) • Range: 1.1-9.4 <u>Moderate fluorosis</u> • Mean (SD): 4.1±3.5 • Median (IQR): 2.0 (7.1) • Range: 1.2-9.4 <u>All</u> • Mean (SD): 2.4±2.1 • Median (IQR): 1.9 (0.9) • Range: 0.4-9.4 Time-averaged fluoride concentration (ppm) by subdistrict <u>Sai Ngam</u> • Mean (SD): 3.72 (3.71) • Median (IQR): 1.40 (8.20) • Range: 0.39-9.38 <u>Bang Sai Pa</u> • Mean (SD): 3.06 (1.00) • Median (IQR): 3.35 (0.95) • Range: 1.07-3.94 <u>Hin Mun</u> • Mean (SD): 2.31 (1.20) • Median (IQR): 1.97 (0.58) • Range: 1.13-5.94	<u>Severity of dental fluorosis by water fluoride level (number of cases; prevalence)</u> • <0.7 ppm: 1 (3.4%) questionable; 7 (23.3%) very mild • 0.7-1.49 ppm: 5 (8.2%) questionable; 14 (23.0%) very mild; 6 (9.8%) mild; 3 (4.9%) moderate • ≥1.5 ppm: 8 (4.1%) questionable; 96 (48.4%) very mild; 21 (10.6%) mild; 10 (5.1%) moderate <u>PR (95% CI) by time-averaged water fluoride concentrations</u> Univariable analysis • <0.7 ppm: reference • 0.7–1.49 ppm: 1.62 (0.78; 3.34); p=0.195 • ≥1.5 ppm: 2.75 (1.42; 5.31); p=0.003 Multivariable analysis; adjusted for child's demographic factors • <0.7 ppm: reference • 0.7–1.49 ppm: 1.62 (0.79; 3.32); p=0.190 • ≥1.5 ppm: 2.78 (1.45; 5.32); p=0.002 Multivariable analysis; adjusted for caregiver factors • <0.7 ppm: reference • 0.7–1.49 ppm: 1.61 (0.28; 9.21); p=0.592 • ≥1.5 ppm: 2.81 (0.51; 15.51); p=0.235 Multivariable analysis; adjusted for breastfeeding • <0.7 ppm: reference • 0.7–1.49 ppm: 3.08 (0.47; 20.04); p=0.238 • ≥1.5 ppm: 5.30 (0.84; 33.45); p=0.076	concentrations of < 0.7 ppm (the referent category) was evidence that reassured the safety of this recommended optimal fluoride level …" • "When the fluoride concentrations increased to the range of 0.7–1.49 ppm …, the prevalence among children in this group also increased to 37.7%, with the additional higher levels of mild and moderate severity. Although the fluoride concentrations in this range did not surpass the WHO's recommended limit of 1.5 ppm …, the results of this study were concerning as the prevalence exceeded one-third of the children and 14.7% of the severity was beyond the very mild level." • "In the extreme group with the fluoride ≥ 1.5 ppm … the prevalence further rose to 64.1% or approximately 2.8 times the prevalence of those in the reference group. The severity beyond the very mild level also grew to 15.7%."	

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	Bang Luang • Mean (SD): 1.76 (0.36) • Median (IQR): 1.82 (0.51) • Range: 0.84-2.20 Nin Phet • Mean (SD): 0.44 (0.05) • Median (IQR): 0.46 (0.10) • Range: 0.37-0.51 **Outcome(s):** Dental fluorosis	Multivariable analysis; adjusted for oral health behaviors • <0.7 ppm: reference • 0.7–1.49 ppm: 3.44 (0.48; 24.62); p=0.218 • ≥1.5 ppm: 6.46 (0.94; 44.48); p=0.058 Multivariable analysis; adjusted for all covariates • <0.7 ppm: reference • 0.7–1.49 ppm: 1.64 (0.24; 11.24); p=0.615 • ≥1.5 ppm: 2.85 (0.44; 18.52); p=0.273		
Sharma 2021 [26]				
Study design: Cross-sectional **Country:** India **Participants:** Children (age 6 – 19 years) residing in 12 villages from the Rudraprayag District **Sampling time frame:** NR **Sample size:** 558 **Sex:** NR **Source of funding:** Self **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Ground water samples Exposure level: • Low-risk area: ○ <0.6ppm • Intermediate risk area ○ 0.6 – 1.5 ppm • High-risk area ○ >1.5ppm **Outcome(s):** Dental fluorosis	• Positive association between drinking water fluoride levels and dental fluorosis prevalence • Percent of children with dental fluorosis by drinking water fluoride levels ○ <0.7mg/L: 1% ○ > 1mg/L: 92% ○ p-value: <0.001 • Prevalence of dental fluorosis by geological categories (fluoride level) Low-risk area (< 0.6ppm) ○ No fluorosis Intermediate risk area (0.6 – 1.5ppm) ○ Dental fluorosis: 59.9% ○ Severe grade: 3.2% ○ Community fluorosis index: 1.05 High-risk area (>1.5ppm) ○ Dental fluorosis: 93% ○ Severe grade: 25.9% ○ Community fluorosis index: 2.59	"This study confirms the positive association between the presence of fluoride-rich rocks around the water source and the prevalence of fluorosis in the population of the area." (p. 126)	2
Silva 2021 [412]				

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Reference type: Original study **Study design:** Cross-sectional **Country:** Brazil **Participants:** 5 and 12 years old **Sampling time frame:** NR **Sample size:** 692 5 years old: 330 (47.6%) 12 years old: 362 (52.4%) **Sex: N (%):** Girls: 342 (49.4%) **Source of funding / support:** Coordination of Improvement of Higher Education Personnel (Capes) **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in: • Drinking water (water fountains of schools/ daycares) **Exposure level(s):** Fluoridated Water (FW) Conc:<0.05 µg/mL Non- Fluoridated Water (NFW) Conc: 0.5-0.6 µg/mL **Outcome(s):** • Dental fluorosis	Data for 12-year-old children [No dental fluorosis was observed in 5-year-old children in either group] • Dental Fluorosis in FW n(%)/NW n(%): Absent: 72 (40.4)/150(81.5) Very Mild/Mild: 74(41.6)/28(15.2) Moderate: 32(18.0)/6(3.3) P<0.001 Kappa index: 0.90 • Logistic regression Very mild/mild DF vs. FW (Desviance Test: p=0,088): OR:5.45 CI 95%: 3.23-9.19 P: <0.001 Moderate DF vs. FW (Desviance Test: p=0,088): OR:11.11 CI 95%: 4.43-27.87 P: <0.001 Reference: NFW for both Mild and moderate fluorosis Multiple analysis controlled by socioeconomic and demographics.	"Adolescents consuming fluoridated water were 5 to 11 times more likely than those of consuming non-fluoridated water to develop very mild/ mild and moderate fluorosis."	1
Tkachenko 2021 [27]				
Study design: Cross-sectional **Country:** Ukraine **Participants:** Children aged 7–10 years old with clinically diagnosed fluorosis from endemic fluorosis areas (exposed to drinking water fluoride (> 1.5 ppm) for >5 years.) **Sampling time frame:** 2014 (date of the project's ethics approval)	**Exposures:** Fluoride levels in • Drinking water Exposure level: • >1.5 ppm **Outcome(s):**	• Children with chronic fluorosis had by 25% higher blood TBARS levels ($p < 0.05$) than the healthy subjects living in the non-fluorosis areas • There was a non-significant 17.5% increase ($p > 0.05$) in the primary products of lipid peroxidation (acyl hydroperoxides) in the blood of children from the endemic fluorosis	"The children had higher blood TBARS levels, while the acyl hydroperoxide levels were non-significantly increased in comparison with healthy children living in the non-fluorosis area."	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Sample size: 31 **Sex:** Boys: 48.4% **Source of funding:** NR **Author declaration of interest:** No COI	Blood level of the lipid peroxidation biomarkers (lipid acyl hydroperoxides, 2-thiobarbituric acid reactive substances (TBARS)) in the blood of children with chronic fluorosis	areas, compared with the values obtained in the blood of the healthy children from the non-fluorosis area		
Wang 2021 [413]				
Reference type: Original study **Study design:** Cross-sectional **Country:** China **Participants:** 6.7–13 years old school children from Tianjin, China **Sampling time frame:** 2015 **Sample size:** 709 **Sex: N (%):** Girls: 328 (46.26%) **Source of funding / support:** • National Natural Science Foundation of China (Grants No. 82073515 and No. 81773388) • The State Key Program of National Natural Science of China (Grant No. 81430076) **Author declaration of interest:** No COI	**Exposure:** • Drinking *water fluoride:* 0.20–3.90 mg/L • Urinary fluoride: 0.02–5.41 mg/L • Urine creatinine: 0.30–2.99 mg/L **Exposure level(s):** • Normal fluoride-exposure group: water fluoride ≤1.0 mg/L • High-fluoride-exposure group: water fluoride >1.0 mg/L **Outcome(s):** • IQ • Dental fluorosis (DF)	**Results:** *IQ, Linear regression* • Water fluoride (mg/L): IQ scores, *β (95% CI)* ○ Q1 (≤ 0.30): Reference ○ Q2 (0.30−1.00) All: 1.77 (−0.73, 4.27) Boys: 1.40 (−2.29, 5.08) Girls: 2.51 (−1.42, 6.45) ○ Q3 (1.00−1.60) All: −2.77 (−5.44, −0.10) Boys: −4.45 (−8.41, −0.50) Girls: −1.72 (−5.91, 2.47) ○ Q4 (> 1.60) All: −4.10 (−6.71, −1.48) Boys: −5.74 (−9.57, −1.91) Girls: −5.27 (−9.32, −1.22) • Urinary fluoride (mg/L): IQ scores, *β (95% CI)* ○ Q1 (≤ 0.20): Reference ○ Q2 (0.20−0.48) All: −1.99 (−4.64, 0.66) Boys: −1.62 (−5.65, 2.42) Girls: −3.29 (−7.34, 0.77) ○ Q3 (0.48−0.90) All: −3.02 (−5.71, −0.33) Boys: −3.54 (−7.60, 0.52) Girls: −1.86 (−6.01, 2.29) ○ Q4 (> 0.90)	• "low-to-moderate fluoride exposure was associated with the alteration of cholinergic system, DF and IQ" • "AChE partly mediated the elevated prevalence of DF and the lower probability of developing superior and above intelligence caused by fluoride."	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		All: −4.49 (−7.21, −1.77) Boys: −6.09 (−10.29, −1.90) Girls: −5.98 (−9.99, −1.96) *IQ, Logistic regression* • Water fluoride (mg/L) and IQ scores [OR (95% CI)] ○ Superior and above (≥120): 0.69 (0.54, 0.90) ○ High normal (110-119): 0.86 (0.70, 1.06) ○ Normal (90-109): 1 (control) ○ Dull normal and below (≤89): 1.42 (1.08, 1.88) • Urinary fluoride (mg/L) and IQ scores [OR (95% CI)] ○ Superior and above (≥120): 0.67 (0.46, 0.97) ○ High normal (110-119): 0.90 (0.68, 1.18) ○ Normal (90-109): 1 (control) ○ Dull normal and below (≤89): 1.39 (0.97, 2.00) • AChE (nmol/L) and IQ scores [OR (95% CI)] ○ Q1 (≤0.30): Reference ○ Q2 (0.30−1.00) Superior and above (≥ 120): 1.67 (0.92, 3.02) High normal (110-119): 1.22 (0.73, 2.04) Normal (90-109): 1 (control) Dull normal and below (≤ 89): 0.96 (0.40, 2.27) ○ Q3 (1.00−1.60) Superior and above (≥ 120): 0.47 (0.24, 0.94) High normal (110-119): 0.78 (0.47, 1.30)		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Normal (90-109): 1 (control) Dull normal and below (≤ 89): 0.63 (0.27, 1.47) ○ Q4 (>1.60) Superior and above (≥ 120): 0.54 (0.29, 1.00) High normal (110-119): 0.92 (0.53, 1.57) Normal (90-109): 1 (control) Dull normal and below (≤ 89): 1.68 (0.77, 3.64) *DF, Prevalence* • Water fluoride (mg/L): dental fluorosis, PR *(95% CI)* ○ Q1 (≤ 0.30): Reference ○ Q2 (0.30−1.00) Crude: 1.21 (0.86, 1.70) Adjusted: 1.20 (0.85, 1.69) ○ Q3 (1.00−1.60) Crude: 3.78 (2.90, 4.94) Adjusted: 3.79 (2.90, 4.95) ○ Q4 (>1.60) Crude: 3.90 (3.00, 5.08) Adjusted: 3.97 (3.04, 5.17) • Urinary fluoride (mg/L): dental fluorosis, PR *(95% CI)* ○ Q1 (≤0.20): Reference ○ Q2 (0.20−0.48) Crude: 1.42 (1.09, 1.86) Adjusted: 1.66 (1.28, 2.14) ○ Q3 (0.48−0.90) Crude: 2.18 (1.72, 2.75) Adjusted: 2.73 (2.17, 3.44) ○ Q4 (>0.90) Crude: 2.56 (2.04, 3.21) Adjusted: 3.24 (2.58, 4.07)		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• Cholinergic system AChE (nmol/L) and DF/IQ [PR (95% CI)] *Either DF or IQ <120* ○ Q1 (≤ 133.66): Reference ○ Q2 (133.66–157.97) Crude: 1.09 (0.94,1.26) Adjusted: 1.06 (0.92,1.22) ○ Q3 (157.97–184.03): Crude: 1.14 (1.00,1.31) Adjusted: 1.12 (0.97,1.28) ○ Q4 (>184.03) Crude: 1.21 (1.06,1.38) Adjusted: 1.22 (1.07,1.38) *DF and IQ <120* ○ Q1 (≤ 133.66): Reference ○ Q2 (133.66–157.97) Crude: 1.29 (1.08,1.54) Adjusted: 1.27 (1.07,1.50) ○ Q3 (157.97–184.03): Crude: 1.37 (1.16,1.62) Adjusted: 1.37 (1.17,1.62) ○ Q4 (>184.03) Crude: 1.46 (1.25,1.72) Adjusted: 1.44 (1.23,1.68) • "Sensitivity analyses were conducted for the association between fluoride exposure, DF, IQ, and cholinergic system by adjusting for the covariates among demographics, development, socioeconomics, and delivery conditions. We obtained similar results to what we found in the present analyses."		
Yani 2021 [414]				
Reference type: Original study **Study design:** Cross-sectional	**Exposure:** • Ground water	Dental fluorosis • High-fluoride area:	• "There is a relationship between Fluoride level in	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Country: Indonesia **Participants:** 6–12 years old students from two different areas with different levels of drinking water fluoride in Palu City, with no history of head trauma, chronic disease, or were not undergoing treatment. **Sampling time frame:** NR **Sample size:** 100 **Sex: N (%):** Females: 64 (64.0%) **Source of funding / support:** NR **Author declaration of interest:** No COI	**Exposure level(s):** • High fluoride area: 1.6 ppm • Low fluoride area: 0.10 ppm **Outcome(s):** • IQ • Dental fluorosis	○ Total: 37 (61.7%) ○ Questionable (score 1): 1 (0%) ○ Very mild (score 2): 10 (0%) ○ Mild (score 3): 11 (11%) ○ Moderate (score 4): 8 (8%) ○ Severe (score 5): 7 (7%) • Low-fluoride area: ○ Total: 3 (7.5%) ○ Questionable (score 1): 2 (%) ○ Very mild (score 2): 1 (1%) ○ Mild (score 3): 0 (0%) ○ Moderate (score 4): 0 (0%) ○ Severe (score 5): 0 (0%) IQ • High-fluoride area: ○ Low: 17 (28.3%) ○ High: 43 (71.7%) • Low-fluoride area: ○ Low: 0 (0%) ○ High: 40 (100%) IQ and Dental fluorosis • Dental fluorosis: ○ Low: 15 (37.5%) ○ High: 25 (62.5%) • No dental fluorosis: ○ Low: 2 (3.3%) High: 28 (96.6%)	well water and the incidence of fluorosis in students, where the incidence of fluorosis was higher in the high fluorine area than in the low fluorine area." • "The intelligence of children who suffered from fluorosis is lower than the intelligence of children who do not suffer from fluorosis." • "The level of intelligence of students who live in the high-fluorine area is lower than students who live in low fluorine area."	
Yu 2021 [415]				
Reference type: Original study **Study design:** Cross-sectional **Country:** China **Participants:** School children aged 7 to 13 years old **Sampling time frame:** 2015 **Sample size:** 952	• Exposure: Fluoride content in • Drinking water • Urine • Hair and nail **Exposure level(s):**	• Water fluoride (mg/L) ○ High (IQ ≥ 120): 0.70 (0.40–1.00) ○ Non-high (70 ≤ IQ<120): 1.00 (0.50–1.90) • Urinary fluoride (mg/L) ○ High (IQ ≥ 120): 0.33 (0.13–0.81)	• "Our study suggests that fluoride is inversely associated with intelligence." • "The interactions of fluoride with mitochondrial function-related SNP-set,	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Sex: N (%): Girls: 481 (50.5%) **Source of funding / support:** • The State Key Program of National Natural Science Foundation of China (Grant No. 81430076). • The National Program for Support of Top-notch Young Professionals and Health commission of Hubei Province **Author declaration of interest:** No COI	• Water fluoride (mg/L) ○ Tertile 1 (≤0.60) ○ Tertile 2 (0.61–1.40) ○ Tertile 3 (>1.40) • Urinary fluoride (mg/L) ○ Tertile 1 (≤0.22) ○ Tertile 2 (0.23–1.80) ○ Tertile 3 (>1.80) • Hair fluoride (μg/g) ○ Tertile 1 (≤10.40) ○ Tertile 2 (10.41–17.02) ○ Tertile 3 (>17.02) • Nail fluoride (μg/g) ○ Tertile 1 (≤14.64) ○ Tertile 2 (14.65–23.41) ○ Tertile 3 (>23.41 Outcome(s): IQ)	○ Non-high (70 ≤ IQ <120): 0.60 (0.16–2.22) • Hair fluoride (μg/g) ○ High (IQ ≥ 120): 8.26 (5.72–10.48) ○ Non-high (70 ≤ IQ <120): 14.39 (10.25–20.56) • Nail fluoride (μg/g) ○ High (IQ ≥ 120): 11.71 (8.53–14.64) ○ Non-high (70 ≤ IQ <120): 19.76 (14.16–27.32) <u>Fluoride exposure and high intelligence: OR (95% CI)</u> • Water fluoride (mg/L) ○ Tertile 1 (≤0.60) Reference ○ Tertile 2 (0.61–1.40) Crude: 0.95 (0.65, 1.38) Adjusted: 0.94 (0.64, 1.37) ○ Tertile 3 (>1.40) Crude: 0.38 (0.24, 0.59) Adjusted: 0.39 (0.25, 0.61) • Urinary fluoride (mg/L) ○ Tertile 1 (≤0.22) Reference ○ Tertile 2 (0.23–1.80) Crude: 1.26 (0.87, 1.83) Adjusted: 1.26 (0.87, 1.84) ○ Tertile 3 (>1.80) Crude: 0.41 (0.26, 0.65) Adjusted: 0.41 (0.26, 0.66) • Hair fluoride (μg/g) ○ Tertile 1 (≤10.40) Reference ○ Tertile 2 (10.41–17.02) Crude: 0.16 (0.10, 0.29)	genes and pathways may also be involved in high intelligence loss."	

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Adjusted: 0.16 (0.09, 0.29) ○ Tertile 3 (>17.02) Crude: 0.08 (0.04, 0.16) Adjusted: 0.08 (0.04, 0.16) • Nail fluoride (μg/g) ○ Tertile 1 (≤14.64) Reference ○ Tertile 2 (14.65–23.41) Crude: 0.15 (0.08, 0.29) Adjusted: 0.15 (0.08, 0.29) ○ Tertile 3 (>23.41) Crude: 0.09 (0.04, 0.18) Adjusted: 0.09 (0.04, 0.19) <u>Does-response relationships of IQ scores with fluoride exposures</u> • *β and 95% CI for every 0.50 mg/L increment of water fluoride or urinary fluoride* • *β and 95% CI for every 1.00 μg/g increment of hair fluoride or nail fluoride.* • *Adjustment: age, sex, maternal education and paternal education.* • Water fluoride (mg/L) ○ 0.20-3.40 Crude: -1.24 (-1.48, -0.99) Adjusted: -1.16 (-1.41, -0.91) ○ 3.40-3.90 Crude: -5.36 (-8.54, -2.18) Adjusted: -4.21 (-7.54, -0.87) • Urinary fluoride (mg/L) ○ 0.01-1.60 Crude: 0.96 (0.29, 1.63) Adjusted: 1.01 (0.34, 1.68) ○ 1.60-2.50		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Crude: -5.08 (-6.94, -3.22) Adjusted: -5.23 (-7.07, -3.39) ○ 2.50-5.54 Crude: -0.50 (-1.13, 0.14) Adjusted: -0.34 (-0.98, 0.30) • Hair fluoride (µg/g) ○ 3.23-10.50 Crude: -2.34 (-2.69, -1.99) Adjusted: -2.34 (-2.69, -1.99) ○ 10.50-45.04 Crude: -0.41 (-0.49, -0.34) Adjusted: -0.42 (-0.50, -0.34) • Nail fluoride (µg/g) ○ 2.08-14.50 Crude: -1.11 (-1.41, -0.81) Adjusted: -1.10 (-1.41, -0.80) ○ 14.50-99.60 Crude: -0.50 (-0.56, -0.44) Adjusted: -0.49 (-0.55, -0.43) <u>Interaction of SNP-set score with fluoride exposure on high intelligence OR (95% CI).</u> • *The P-value for interaction (p-inter) was adjusted for age, sex, maternal education and paternal education.* • *High SNP: -set score group (-1.59 to 0.00):* • *Low SNP-set score group (-2.90 to -1.59):* • Water fluoride (binary variable based on the limit of 1.00 mg/L) ○ Sample size: 952 ○ High SNP: 0.33 (0.20, 0.55) ○ Low SNP: 0.27 (0.14, 0.54)		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		○ p-inter: 0.030 • Urinary fluoride (binary variable based on the limit of 1.60 mg/L) ○ Sample size: 952 ○ High SNP: 0.37 (0.22, 0.62) ○ Low SNP: 0.32 (0.16, 0.63) ○ p-inter: 0.040 • Hair fluoride (binary variable based on the median level of 14.00 μg/g) ○ Sample size: 719 ○ High SNP: 0.17 (0.08, 0.34) ○ Low SNP: 0.12 (0.04, 0.35) ○ p-inter: 0.010 • Nail fluoride (binary variable based on the median level of 19.60 μg/g) ○ Sample size: 638 ○ High SNP: 0.13 (0.06, 0.31) ○ Low SNP: 0.12 (0.04, 0.37) ○ p-inter: 0.242		
Zhao 2021 [416]				
Reference type: Original study **Study design:** Cross-sectional **Country:** China **Participants:** Children, aged 6–11 years old, from endemic and non-endemic fluorosis areas in Tianjin, China. **Sampling time frame:** 2018 **Sample size:** 567 **Sex: N (%):** Girls: 283 (49.9%) **Source of funding / support:** The National Natural Science Foundation of China (Grant No. 81573107, 81372934).	**Exposure:** Fluoride concentration in • Drinking water • Urine **Exposure level(s):** Fluoride in drinking water: • High fluoride areas: 1.53–2.84 mg/L • Non-endemic fluorosis area (WF: 0.15–0.37 mg/L Fluoride in urine:	<u>Associations between UF and IQ scores</u> • Overall: Log_UF were inversely linear associated with IQ score ($P < 0.05$) in both crude model and adjusted model • β (95% CI): ○ Crude: - 5.159 (- 8.996, - 1.321) ○ Adjusted: - 5.957 (- 9.712, - 2.202) ○ Bootstrapped estimation of the variance: (95% CI: - 10.356, - 1.834; p=0.006)	• "Dopamine relative genes may modify the association between fluoride and intelligence, and a potential interaction among fluoride exposure and DA relative genes on IQ." • "fluoride exposure is inversely related to children's IQ; DA related genes polymorphism (ANKK1 Taq1A, COMT rs4680, DAT1 40 bp VNTR and MAOA	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Author declaration of interest: No COI	• Urinary fluoride concentration was not normally distributed, with a median (quantile 1, quantile 3) of 1.03 (0.72, 1.47) mg/L • After log transformation, the mean (±SD) Log_UF was 0.015 (±0.252) **Outcome(s):** • IQ		uVNTR) have modifying effects of fluoride exposure on IQ; UF, ANKK1 Taq1A, COMT Val 158 Met and MAOA uVNTR have a high-dimensional interaction on IQ."	
Bai 2020 [28]				
Study design: Cross-sectional **Country:** USA **Participants:** US children and adolescents 6–19 years old (NHANES survey) **Sampling time frame:** 2013–2016 **Sample size:** 3,392 **Sex:** Men: 50.6% **Source of funding:** National Natural Science Foundation of China **Author declaration of interest:** No COI	**Exposures:** <u>Fluoride levels in</u> • Drinking water • Serum <u>Exposure level:</u> Water fluoride (mg/L) • Total: 0.36 (0.30, 0.42) • Male children: 0.40 (0.32, 0.47) • Male adolescents: 0.34 (0.28, 0.40) • Female children: 0.37 (0.29, 0.44) • Female adolescents: 0.35 (0.28, 0.41) • p-value: 0.143 Plasma fluoride (umol/L) • Total: 0.35 (0.33, 0.37) • Male children: 0.38 (0.36, 0.41) • Male adolescents: 0.34 (0.32, 0.36)	• Compared with subjects at the first tertile of plasma fluoride, percent changes (95% CI) in testosterone were: ○ Second tertile: –8.08% (–17.36%, 2.25%) ○ Third tertile: –21.65% (–30.44%, –11.75%) ○ P trend <0.001 • Male adolescents at the third tertile of plasma fluoride had decreased levels of testosterone: –21.09% (–36.61% to –1.77%). • Similar inverse associations were also found when investigating the relationships between plasma fluoride and estradiol. • Decreased levels of SHBG associated with water and plasma fluoride ○ Male adolescents (third tertile): –9.39% (–17.25% to –0.78%) ○ Female children (second tertile): –10.78% (–17.55% to –3.45%)	"The data indicated gender- and age-specific inverse associations of fluoride in plasma and water with sex steroid hormones of total testosterone, estradiol and SHBG in U.S. children and adolescents."	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	• Female children: 0.36 (0.34, 0.37) • Female adolescents: 0.33 (0.31, 0.35) • p-value: <0.001 **Outcome(s):** Sex steroid hormones [testosterone, estradiol and sex hormone-binding globulin (SHBG)]	• Percent change in testosterone (95% CI) at tertiles T2 and T3, compared to T1: *Total* ○ T2: -7.95 (-20.47, 6.56) ○ T3: -8.11 (-15.84, 0.33) ○ p trend = 0.069 *Male Children* ○ T2: 10.90 (-8.11, 33.85) ○ T3: -7.56 (-21.80, 9.27) ○ p trend = 0.458 *Male Adolescents* ○ T2: -2.35 (-19.83, 18.94) ○ T3: -7.43 (-24.79, 13.94) ○ p trend = 0.461 *Female Children* ○ T2: -1.07 (-14.11, 13.96) ○ T3: -3.97 (-15.95, 9.72) ○ p trend = 0.549 *Female Adolescents* ○ T2: -2.08 (-11.75, 8.66) ○ T3: -3.58 (-14.75, 9.06) ○ p = trend 0.540 • Percent change in Estradiol (95% CI) at tertiles T2 and T3, compared to T1: *Total* ○ T2: -4.55 (-16.08, 8.56) ○ T3: 1.48 (-6.97, 10.70) ○ p trend = 0.896 *Male Children* ○ T2: 2.08 (-2.97, 7.39) ○ T3: 0.72 (-4.07, 5.75) ○ p trend = 0.705 *Male Adolescents* ○ T2: -4.56 (-19.04, 12.52) ○ T3: -1.25 (-14.54, 14.10) ○ p trend = 0.823		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Female Children ○ T2: -15.59 (-32.04, 4.84) ○ T3: -7.25 (-22.74, 11.35) ○ p trend = 0.337 *Female Adolescents* ○ T2: 3.50 (-21.43, 36.33) ○ T3: 9.49 (-13.47, 38.53) ○ p trend = 0.457 • Percent change in SHBG (95% CI) at tertiles T2 and T3, compared to T1: *Total* ○ T2: 2.71 (-4.84, 10.86) ○ T3: -2.75 (-9.69, 4.74) ○ p = trend 0.557 *Male Children* ○ T2: 5.38 (-2.14, 13.48) ○ T3: -4.14 (-10.65, 2.85) ○ p trend = 0.322 *Male Adolescents* ○ T2: 0.38 (-7.95, 9.47) ○ T3: -9.39 (-17.25, -0.78) ○ p trend = 0.038 *Female Children* ○ T2: -1.74 (-11.50, 9.10) ○ T3: 0.12 (-7.47, 8.34) ○ p trend = 0.984 *Female Adolescents* ○ T2: 2.09 (-13.3, 19.98) ○ T3: -0.37 (-12.06, 12.88) ○ p trend = 0.996		
Cui 2020 [29]				
Study design: Cross-sectional **Country:** China **Participants:** School aged children (7 – 12 years) from Tianjin	**Exposures:** Fluoride levels in • Urine	*Mean (SD) IQ by urinary fluoride levels* • < 1.6 mg/L: 112.16 (±11.50) • 1.6 – 2.5 mg/L: 112.05 (±12.01) • ≥ 2.5 mg/L: 110.00 (±14.92)	Although fluoride was not the main focus[24], the study reported some non-significant frequency	2

[24] RSI conclusion provided as the author's reported conclusion did not include information on effects causes by exposure to fluoride.

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Sampling time frame: 2014 – 2018 **Sample size:** 498 **Sex:** Boys: 49.80% **Source of funding:** • National Nature Science Foundation of China • Tianjin Health Inspection Fund **Author declaration of interest:** No COI	<u>Exposure level:</u> Distribution by urinary fluoride levels (N; %) • < 1.6 mg/L ○ N = 396 (79.52) • 1.6 – 2.5 mg/L ○ N = 66 (13.25) • ≥ 2.5 mg/L ○ N = 36 (7.23) **Outcome(s):** • IQ scores • Thyroid Stimulating Hormone (TSH) • Dopamine (DA)	• p-value: 0.578 *Median (q1-q3) TSH in uIU/mL by urinary fluoride levels* • < 1.6 mg/L: 2.81 (2.21 – 3.81) • 1.6 – 2.5 mg/L: 2.82 (2.01 – 3.82) • ≥ 2.5 mg/L: 3.29 (2.30 – 4.48) • p-value: 0.287 *Median (q1-q3) DA in ng/L by urinary fluoride levels* • < 1.6 mg/L: 5.62 (3.08 – 12.15) • 1.6 – 2.5 mg/L: 5.77 (3.01 – 12.59) • ≥ 2.5 mg/L: 7.24 (2.16 – 15.23) • p-value: 0.925	differences between urinary fluoride and IQ, TSH and DA	
Das 2020 [30]				
Study design: Cross-sectional **Country:** Saudi Arabia **Participants:** Dental college patients (aged 9 to 50 years) **Sampling time frame:** July – December 2019 **Sample size:** 1,150 **Sex:** Men: 53% **Source of funding:** Deanship of Scientific Research **Author declaration of interest:** No COI	**Exposures:** <u>Fluoride levels in</u> • Wells • Filtration plants • Commercial brand water bottles <u>Exposure level:</u> Mean (SD) Fluoride levels in ppm by water source type • Well Water ○ 1.97 (0.20) • Filtered Water ○ 1.05 (0.69) • Bottled Water ○ 1.09 (0.10) **Outcome(s):** Dental Fluorosis	**Results:** Association between dental fluorosis and sources of drinking water • Well Water ○ None: 33% ○ Questionable: 28% ○ Very Mild: 21% ○ Mild:14% ○ Moderate: 2% ○ Severe: 1% • Filtered Water ○ None: 63% ○ Questionable: 30% ○ Very Mild: 5% ○ Mild: 1% ○ Moderate: 0% ○ Severe: 0% • Total ○ None: 50% ○ Questionable: 29% ○ Very Mild: 12% ○ Mild: 7%	"The results revealed that fluoride levels varied between 0.03 and 3.8 ppm. People who drank well water displayed increased fluoride levels (>0.81 ppm). The prevalence of dental fluorosis was established to be 20.43% among the total number of examined patients. The findings of this study show very mild to moderate dental fluorosis prevail among the patients who consume well water in the Asir region."	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		○ Moderate: 1% ○ Severe: 0% • p-value: <0.002		
Fernandes 2020 [31]				
Study design: Cross-sectional **Country:** Brazil **Participants:** Children (6 to 12 years of age) from rural public schools in São João do Rio do Peixe, Poço José de Moura, Marizópolis, and Uiraúna **Sampling time frame:** NR **Sample size:** 610 **Sex %:** Men: 53.9% **Source of funding:** NR **Author declaration of interest:** No COI	**Exposures:** Fluoride level in • Water samples Exposure level: Level of residual fluoride in water (ppm): • Range: 0.06 – 1.98 **Outcome(s):** Dental fluorosis	*Dental fluorosis absent %* • ≤0.7 ppm fluoride: 63.1% • >0.7 ppm F: 55.2% *Dental fluorosis present %* • ≤0.7 ppm fluoride: 36.9% • >0.7 ppm fluoride: 44.8%	"The prevalence of dental fluorosis in group II [>0.7 ppm fluoride] was higher (44.8%), but it was not significantly different from group I [<0.7 ppm fluoride] (36.9%)." (p. 477)	2
Godebo 2020 [32]				
Study design: Cross-sectional **Country:** Ethiopia **Participants:** Adolescents and adult farmers living in the MER rural area **Sampling time frame:** 2018-2019 **Study population:** 341 **Sex:** (men): 55.1% **Funding/support:** National Institute of Environmental Health Sciences **Author declaration of interest:** NR	**Exposures** Fluoride levels in • Drinking water • Urine Exposure level: Mean (SD) water F- concentrations: • Water intake (liter/day): 1.3 ± 0.63 • Fl in groundwater (mg/L): 6.8 ± 4.30 • Fl intake (mg/day): 9.13 ± 7.30 Mean (SD) urinary F- concentrations: • F- in 24-h urine (mg/L): 8.2 ± 7.6 • F- excretion (mg): 5.01 ± 4.5	• 1 mg/L increase in F- in drinking water was related to reduction of 15.8 m/s (95% CI: −21.3 to −10.3) of adult tibial SOS. • 1 mg/L increase in 24-h urinary F- (range: 0.04–39.5 mg/L) was linked to a reduction of 8.4 m/s (95% CI: −12.7, −4.12) of adult tibial SOS. • Adolescents: weaker and non-significant inverse associations between F- exposure and SOS • Age, gender, and BMI were more significant predictors than in adults	• Negative association between fluoride exposure and bone quality at all three bone sites • Fluoride-induced deterioration of bone quality in humans, likely reflecting a combination of factors related to SOS: net bone loss, abnormal mineralization and collagen formation, or altered microarchitecture.	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	Outcome: Skeletal fluorosis			
Kim 2020 [33]				
Study design: Case-control **Country:** USA **Participants:** Phase 1 • Cases: all patients younger than 40 years old, who were diagnosed with osteosarcoma • Controls: patients with other bone tumors or non-neoplastic conditions, identified during the same periods, and from the same orthopedic surgery department as cases. **Sampling time frame:** • Phase 1: 1989–1993 • Phase 2: 1994–2000 **Sample size:** • Phase 1: cases (209), controls (440) • Phase 2: cases (108), controls (296) **Sex:** Phase 1 & 2 combined: • Cases: men: 142 (60.2%) • Controls: men 248 (60.6%) **Source of funding:** • Statistical analysis: CDI Research, Inc. • Phase 1: the National Institute of Environmental Health Sciences (NIH). • Data collection: the New England Research Institute. • Phase 2: the National Cancer Institute (NIH) and the National	**Exposures:** Fluoride levels in Water Exposure level: Lived in a fluoridated area (0.7 ppm) • No ○ Cases: 58 (24.6%) ○ Controls: 81 (19.8%) • Yes ○ Cases: 178 (75.4%) ○ Controls: 328 (80.2%) **Outcome(s):** Osteosarcoma (bone cancer)	• A modestly significant interaction existed between fluoridation living status and bottled water use (P = 0.047). • Risk of osteosarcoma (adjusted): ○ For ever having lived in a fluoridated area for nonbottled water drinkers: [OR= 0.51 (95% CI: 0.31 – 0.84) P = 0.008)]. ○ For bottled water drinkers: [OR=1.86 (95% CI: 0.54 – 6.41; P = 0.326).	"Findings from this study demonstrated that community water fluoridation is not associated with an increased risk for osteosarcoma."	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Institute of Dental and Craniofacial Research (NIH). **Author declaration of interest:** Declaration provided				
Krishna 2020 [34]				
Study design: Case-control **Country:** India **Participants:** Patients (45 – 75 years of age) from RL Jalappa Hospital and Research Center **Sampling time frame:** July 2019 – September 2019 **Sample size:** 90 **Sex:** NR **Source of funding:** NR **Author declaration of interest:** NR	**Exposures:** Fluoride levels in Serum Exposure level: Mean (SD) levels of fluoride in ppm by study groups • Controls: 0.0949 (0.12) • T2DM without CKD: 0.6318 (0.59) • T2DM with CKD: 0.5128 (0.30) • p-value: 0.001 **Outcome(s):** Diabetes Mellitus and Diabetic nephropathy using serum renal parameters	**Results:** Pearson correlation between serum fluoride and parameters (N= 30). * = significant at 0.05. • Fasting Blood Sugar: 0.28 • Post Prandial Blood Sugar: 0.44* • Urea: 0.107 • Serum Creatinine: 0.08 • Albumin: 0.102 • Sodium: 0.005 • Potassium: 0.101	• "Our results showed that Fasting, post prandial blood glucose values and serum Fluoride were significantly higher in T2DM without CKD group as compared to the controls and T2DM with CKD." • "This study also supports the hypothesis of increase serum Fluoride increases DM and DN which is evident from the results."	1
Lee 2020 [35]				
Study design: Ecological **Country:** South Korea **Participants:** All residents in the Cheongju region **Sampling time frame:** 1 January 2004 – 31 December 2013 **Sample size:** • Fluoridated areas: 4,406,021 • Non-fluoridated areas: 2,270,959 **Sex:** • Fluoridated areas: Men: 49.9% • Non-fluoridated areas: Men: 49.6%	**Exposures:** Fluoride levels in • Water Exposure level: • NR **Outcome(s):** • Hip fracture • Osteoporosis • Bone cancer	• The posterior relative risks (RR): ○ Hip fracture: RR: 0.95, 95% CI: 0.87- 1.05 ○ Osteoporosis RR: 0.94, 95% CI: 0.87-1.02 ○ Bone cancer RR: 1.20, 95% CI: 0.89-1.61 (a little high due to smaller sample size compared to the other bone diseases) • The RR of the selected bone diseases increased over time but did not	"These findings suggest that CWF is not associated with adverse health risks related to bone diseases."	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Source of funding: Division of Oral Health Policy, Ministry of Health and Welfare, Republic of Korea **Author declaration of interest:** No COI		increase in the CWF area compared to non-CWF areas.		
Nanayakkara 2020 [36]				
Study design: Cross-sectional **Country:** Sri Lanka **Participants:** Males with chronic kidney disease of uncertain aetiology (CKDu) and healthy controls **Sampling time frame:** NR **Sample size (N):** • Males with CKDu = 311 • Healthy Controls = 276 **Sex:** • Cases: Men: 100% • Controls: NR **Source of funding:** Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology **Author declaration of interest:** No COI	**Exposures:** <u>Fluoride levels in</u> • Serum • Water <u>Exposure level:</u> Mean (SD) levels of fluoride in drinking water • 0.68 mg/L (0.48) Mean (SD) levels of fluoride in serum by stages of CKD • Stage 0 (N = 276) ○ 35.5 µg/L (16.3) • Stage 1 (N = 10) ○ 38.1 (18.1) • Stage 2 (N = 60) ○ 53.9 (34.2) **Outcome(s):** Chronic kidney disease of unknown origin (CKDu)	Mean serum fluoride level (±SD) by CKDu stage • Stage 0 (N= 276): 35.5 µg/L (±16.3) • Stage 1 (N= 10): 38.1 µg/L (±18.1) • Stage 2 (N= 60): 53.9 µg/L (±34.2) * • Stage 3 (N= 160): 82.8 µg/L (±41.9) * • Stage 4 (N= 72): 123.4 µg/L (±59.9) * • Stage 5 (N= 9): 123.9 µg/L (±52.6) * * *p<0.05 compared to controls*	"Serum samples from CKDu patients and healthy controls and water samples from their common drinking water sources were analysed for fluoride content. Over 95% of water samples met the WHO guideline of 1.5 mg/L. CKDu patients showed significantly higher serum fluoride concentrations than the healthy controls. The estimated glomerular filtration level was inversely proportional to the serum fluoride concentration, indicating the accumulation of fluoride in the body with the progression of CKDu, which can further aggravate renal tissue damage." (p. 4)	2
Russ 2020 [37]				
Study design: Cohort **Study design:** Cohort study **Country:** Scotland **Participants:** all people born in 1921 and at school in Scotland in June 1932, and took part in a comprehensive national intelligence test at a mean age of 11 years	**Exposures:** Aluminum and fluoride levels in drinking water <u>Exposure level:</u> Fluoride in drinking water: • Mean: 53.4 µg/L ±16.0 • Range: 23.8–181.1	• Fluoride ○ Mean (SD): 53.4 µg/L (*16.0)* ○ *Range: 23.8–181.1* • No statistical interaction between aluminum and fluoride levels in relation to dementia. • A dose-response pattern was observed between mean fluoride levels	Higher levels of fluoride were related to dementia risk in a population of men and women who consumed relatively low drinking-water levels of fluoride.	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
(Scottish Mental Survey 1932, SMS1932) **Sampling time frame:** 2005-2014 **Sample size (N):** 6,980 **Sex:** Men: 39% **Funding/support:** Alzheimer Scotland, Marjorie MacBeath bequest **Author declaration of interest:** No COI	**Outcome:** Dementia	and dementia in women [HR: 1.34 (95% CI: 1.28–1.41, P <0.001)] and men [HR: 1.30 (95% CI: 1.22–1.39), P <0.001], with dementia risk more than doubled in the highest quartile compared with the lowest.		
Stangvaltaite-Mouhat 2020 [38]				
Study design: Cross-sectional **Country:** Lithuania **Participants:** Adults between 35 and 74 years old **Sampling time frame:** NR **Sample size:** 1,397 **Sex:** Men: 33.1% **Source of funding:** The Borrow Foundation **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Drinking water Exposure level: • ≤ 1 ppm • > 1 ppm **Outcome(s):** Dental fluorosis	**Dental fluorosis prevalence by age group and gender** 35–44 years *Men* • Yes: 5 (4%) • No: 125 (96%) *Women* • Yes: 8 (4%) • No: 215 (96%) 45–54 years *Men* • Yes: 2 (2%) • No: 102 (98%) *Women* • Yes: 3 (1%) • No: 204 (99%) 55–64 years *Men* • Yes: 1 (1%) • No: 111 (99%) *Women* • Yes: 0 (0%) • No: 248 (100%) 65-74 years *Men* • Yes: 2 (2%) • No: 114 (98%)	"Signs of fluorosis were detected in 2% of participants (N=21) and the presence of fluorosis did not associate significantly with higher levels of fluoride in the drinking water (data not shown)."	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Women • Yes: 0 (0%) • No: 253 (100%) **Dental fluorosis prevalence by water fluoride level** ***≤ 1 ppm*** 35–44 years • Men: 121 (93%) • Women: 198 (88%) 45–54 years • Men: 95 (91%) • Women: 181 (87%) 55–64 years • Men: 100 (89%) • Women: 201 (80%) 65-74 years • Men: 96 (83%) • Women: 204 (80%) ***>1ppm*** 35–44 years • Men: 9 (7%) • Women: 26 (12%) 45–54 years • Men: 9 (9%) • Women: 26 (13%) 55–64 years • Men: 12 (11%) • Women: 49 (20%) 65-74 years • Men: 20 (17%) • Women: 50 (20%)		
Sun 2020 [39]				
Study design: Cross-sectional **Country:** China **Participants:** Female farmers (20 – 60 years of age) from 6 villages (3 endemic fluorosis villages with	**Exposures:** Fluoride levels in • Urine Exposure level:	*Adjusted association of fluoride with CALCA exon 1 methylation levels* • r = 0.022 • p = 0.576	"…decreased BMD in women may be associated with exposure to excessive fluoride in an age-specific manner, which may be	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
fluoride levels > 1.0 mg/L; 3 control villages with fluoride levels < 1.0 mg/L) in Tongxu County **Sampling time frame:** NR **Sample size:** 722 **Sex:** Women: 100% **Source of funding:** • National Natural Science Foundation of China • Scientific and Technological Project of Henan Province **Author declaration of interest:** No COI	• NR **Outcome(s):** • Reduction of bone mineral density (BMD) via CALCA gene methylation	*Adjusted association (β; 95% CI) of fluoride (mg/L) with CALCA exon 1 methylation levels by age groups* • 20 – 60 yrs: 0.270 (-0.621, 1.162) • 20 – 39 yrs: 1.656 (-1.464, 4.776) • 40 – 44 yrs: 4.953 (1.162, 8.743) • 45 – 49 yrs: -0.152 (-2.673, 2.369) • 50 – 54 yrs: 0.405 (-0.797, 1.607) • 55 – 60 yrs: -1.643 (-3.657, 0.370) *Correlation between fluoride and T-score* • r = 0.019**,** p = 0.611 *Adjusted association (β; 95% CI) of fluoride (mg/L) with T-score by age groups* • 20 – 60 yrs: 0.010 (-0.032, 0.051) • 20 – 39 yrs: 0.001 (-0.139, 0.139) • 40 – 44 yrs: 0.106 (-0.021, 0.233) • 45 – 49 yrs: 0.095 (-0.022, 0.212) • 50 – 54 yrs: -0.063 (-0.129, -0.002) • 55 – 60 yrs: 0.035 (-0.044, 0.114) *Interaction between fluoride and CALCA exon 1 methylation on BMD was assessed* • *"…found evidence of a significant association, as manifested by increased BMD in women aged 45-49 years induced by the interactive effect of the highest methylation of CALCA exon 1 (tertile 3) and fluoride exposure (β = 5.338, P = 0.016)"*	modified by methylation of CALCA exon 1."	
Till 2020 [40]				
Study design: Cohort study **Country:** Canada **Participants:** >17 years old, and less than 14 weeks gestation **Sampling time frame:** 2008-2011	**Exposures:** Fluoride levels in • Drinking water • Urine samples (maternal)	• An increase of 0.5 mg/L in water fluoride concentration *(almost equal to the difference between fluoridated and non-fluoridated regions)* corresponded to reduction in performance IQ in breastfed children:	Exposure to increasing levels of fluoride in tap water was associated with diminished non-verbal intellectual abilities; the effect was more	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Sample size (N): 398 mother-child pairs **Sex:** Girls *Breastfed, fluoride: 51%* *Breastfed, non-fluoride: 53%* **Funding/support:** NIEHS, Health Canada, Ontario Ministry of Environment, CIHR **Author declaration of interest:** No COI	Exposure level: Water Fluoride concentration (mg/L) • Breastfed≥6 mo. ○ *Fluoridated*: 0.58 (0.08) ○ Non- Fluoridated: 0.13 (0.06) • Formula-fed ○ Fluoridated: 0.59 (0.07) ○ Non- Fluoridated: 0.13 (0.05) • P-value: 0.18 Infant fluoride intake (mg/day) • Breastfed≥6 mo. ○ Fluoridated: 0.12 (0.07) ○ Non- Fluoridated: 0.02 (0.02) • Formula-fed ○ Non- Fluoridated: 0.34 (0.12) ○ Non- Fluoridated: 0.08 (0.04) • P-value: <.001 **Outcomes:** Intellectual function	○ *6.2-point (95% CI: −10.45, −1.94).* • Association remained significant upon controlling for fetal fluoride exposure ○ *(B=−6.30, 95% CI: −10.92, −1.68)*	pronounced among formula-fed children.	
Wang 2020 [41]				
Study design: Cross-sectional **Country:** China **Participants:** 7–13 years old children, selected at random from endemic and non-endemic fluorosis areas in Tianjin, China.	**Exposures:** Fluoride levels in • Drinking water • Urine samples Exposure level:	Fluoride (mg/L): Mean ±SD • Water fluoride: 1.39 ±1.01 • Urinary fluoride: 1.28 ±1.30 Thyroid hormones: • TT3 (ng/mL): *Mean (SD): 1.32 ± 0.19*	Low-moderate fluoride exposure is associated with alterations in childhood thyroid function that may modify the association	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Sampling time frame: 2015 **Sample size (N):** 571 **Sex:** Boys: 292 (51.1%) **Funding/support:** State Key Program of National Natural Science Foundation of China, Fundamental Research Funds for the Central Universities **Author declaration of interest:** No COI	• Mean water fluoride level (mg/L): 1.39 ±1.01 **Outcomes:** • Thyroid hormone dysfunction (TT3, TT4, FT3, FT4 and TSH levels in serum) • Intelligence (IQ)	• FT3 (pg/mL): *Mean (SD): 3.28 ± 0.32* • TT4 (µg/dL): *Mean (SD): 6.86 ± 1.16* • FT4 (ng/dL): *Mean (SD): 1.13 ± 0.12* • TSH (uIU/mL): *Mean (SD): 2.57 ± 1.29* • Every 1 mg/L increment of water fluoride was associated with 0.13 uIU/mL increase in TSH. • Every 1 mg/L increment of urinary fluoride was associated with ○ *0.09 ug/dL decrease in TT4* ○ *0.009 ng/dL decrease in FT4* ○ *0.11 uIU/mL increase in TSH.* • Water fluoride exposure was inversely related to IQ scores ○ *Change in IQ scores per 1 mg/L increment of water fluoride (continuous): β =−1.59 (−2.61, −0.57), p=0.002* ○ *Change in IQ scores per quartile increment of water fluoride compared to the reference (≤0.70 mg/L): (1.00–1.90 mg/L): β= −3.07 (−5.64, −0.49), p: 0.02* • Urinary fluoride exposure was inversely related to IQ scores ○ *Change in IQ scores per 1 mg/L increment of urinary fluoride (continuous): β −1.21 (−1.99, −0.44), p=0.002* ○ *Change in IQ scores per quartile increment of urinary fluoride compared to the reference (≤0.15 mg/L): (> 2.28 mg/L): β= −4.10 (−6.91, −1.29), p: 0.004*	between fluoride and intelligence	

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• Higher TT3, FT3 were related to the increased odds of children having high normal intelligence ○ *TT3: OR=3.41 (1.04, 11.12)* ○ *FT3: OR=3.277 (1.62, 6.62)* • A significant modification effect by TSH on the association between urinary fluoride and IQ scores, without mediation by thyroid hormones		
An 2019 [42]				
Study design: Cross-sectional **Country:** China (Henan Pr) **Participants:** 18-55 male farmers **Sampling time frame:** 2011-2012 **Sample size (N):** 348 **Sex:** Males (100%) **Funding/support:** National Natural Science Foundation of China, Henan Department of Science and Technology, China **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Urine Exposure level: • Mean (SD) urinary fluoride (mg/L) ○ *HEG 2.66 ± 1.03* ○ *LEG 0.95 ± 0.31* *P-value: <0.001* **Outcomes:** Levels of reproductive hormones (SHBG and ABP) in serum	Reproductive hormones (Mean ± SD) • ABP (nmol/L) ○ *HEG 19.86 ± 22.46* ○ *LEG 24.04 ± 26.94* *P-value= 0.144* • SHBG (nmol/L) ○ *HEG 30.07 ± 28.32* ○ *LEG 35.90 ± 28.58* *P-value= 0.012*	Chronic fluoride exposure from drinking water is associated with alterations of serum SHBG and ABP concentrations in local male farmers and that the effect of fluoride exposure on ABP levels vary depending on ESRα gene polymorphisms	1
Crnosija 2019 [43]				
Study design: Ecological study **Country:** USA (NY State) **Participants:** >18 years old inpatients with metastatic bone cancer **Sampling time frame:** 2008–2010 **Sample size (N):** 24,661 **Sex:** NR **Funding/support:** NR **Author declaration of interest:** NR	**Exposures:** Fluoride levels in • Drinking water Exposure level: • Fluoride in drinking water (mg/L): ○ 0.7 (45 counties) ○ 0.8 (2 counties) ○ 0.5 (1 county) ○ 0.4 (1 county)	Percentage of population in county with fluoridation • <25% (reference) ○ *No. counties: 27* ○ *2ry bone cancer: 12.9%* • 25%-75% (16 counties) ○ *2ry bone cancer: 12.9%* ○ *Coefficient: 0.02* ○ *p-value: 0.96* • >75% ○ *No. counties: 19* ○ *2ry bone cancer: 12.9 %*	We found no evidence of an association between community water fluoridation category and secondary bone cancer from 2008 to 2010 at the county level in New York State	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	Outcomes: Secondary bone cancer	○ *Coefficient: 0.02* ○ *p-value: 0.97*		
Fernando 2019 [44]				
Study design: Case-control **Country:** Sri Lanka **Participants:** Cases: 19-76 years old, non-dialysis biopsy-proven patients Controls (matched): Healthy volunteers **Sampling time frame:** NR **Sample size (N):** 193 (116 cases and 77 controls) **Sex:** Cases: Men (81.1%) Controls: Men (70.1%) **Funding/support:** National Research Council (NRC) **Author declaration of interest:** No COI	**Exposures:** Fluoride level in • Serum Exposure level: • Fluoride in ground water: 1.33 - 5.30 mg/L • Fluoride MAC in drinking water: 0.60 mg/L **Outcomes:** Chronic kidney disease of unknown origin (CKDu)	• Serum fluoride: Mean ±SD [range] mg/L ○ *CKDu patients: 1.43 ± 1.2 [0.47 – 9.58]* ○ *Controls: 1.07 ± 0.3 [0.51 – 1.92]* ○ *p = 0.000 (showed a significant difference based on CKDu stage but not with sex or age)* • Urinary fluoride: Mean ±SD [range] mg/L ○ *CKDu patients: 1.53 ± 0.8 [0.45 – 6.92]* ○ *Controls: 1.26 ± 0.63 [0.36 – 3.80]* ○ *p = 0.004* • Patients in the age group 19–29 years showed lower serum fluoride levels than other age groups	Higher fluoride exposure via drinking water is possibly the reason for higher fluoride in serum, while excessive urinary excretion would be due to deterioration of the kidney, suggesting a possible nephrotoxic role of environmental fluoride exposure.	2
Jimenez-Cordova 2019 [45]				
Study design: Cross-sectional **Country:** Mexico (Chihuahua) **Participants:** 5-12 years old Mexican school children **Sampling time frame:** 2015 **Sample size (N):** 374 **Sex:** Boys: 46.8% **Funding/support:** Children's Environmental Health Network, National Council of Science and Technology, Mexico **Author declaration of interest:** No COI	**Exposures:** Fluoride level in • Drinking water Exposure level: • Mean (IQR): *0.3 mg/mL (0.01–1.9)* • MPL: *1.5* **Outcomes:** Kidney dysfunction, using Kidney injury biomarkers [glomerular filtration rate (eGFR), and the urinary concentrations of kidney injury molecule 1 (KIM-1) and cystatin-C (uCys-C)]	**Urinary fluoride showed** • Positive association with ○ *eGFR (β=1.3, p=0.015),* ○ *VCAM-1 (β=111.1, p=0.019)* ○ *ICAM-1 (β=57, p=0.032)* ○ *cIMT (β=0.01, p=0.032)* • Inverse association with ○ *uCys-C (β=−8.5, p=0.043)* ○ *sCys-C (β=−9.6, p=0.021)* • No significant association with ○ *ET-1 (β=0.069, p=0.074)* ○ *KIM-1 (β=29.1, p=0.212)*	• Fluoride exposure is related to early vascular alterations, which may increase the susceptibility of cardiovascular diseases in adult life. • Inconclusive results regarding fluoride exposure and kidney injury	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Jiménez-Córdova 2019a [46]				
Study design: Cross-sectional **Country:** Mexico **Participants:** Adult participants recruited directly from information sessions **Sampling timeframe:** 2013 **Sample size (N):** 236 **Sex:** Men: 29% **Funding/support:** National Council of Science and Technology, Mexico **Author declaration of interest:** No COI	**Exposures** Fluoride level in • Drinking water Exposure level: • Water fluoride: 1.6 mg/L ±1.6 **Outcome** Change in products and indicators of arsenic (As) metabolism in the body	A statistically significant interaction of F and as exposure on the following was observed: • Increase in MAs% (β = 0.16, p = 0.018) • Decrease in DMAs% (β = −0.3, p = 0.034), • Decrease in PMI (β=−0.07, p=0.052) • Decrease in SMI (β=−0.13, p=0.097)	Fluoride exposure decreases Arsenic methylation capacity, and increases its toxicity	1
Khanoranga 2019 [47]				
Study design: Cross-sectional **Country:** Pakistan **Participants:** Male brick kiln workers and controls (17 to 45 years of age) from three districts of Balochistan. Controls were office and university workers residing in locations with no fluoride exposure **Sampling time frame:** August – September 2017 **Sample size:** • Brick kiln workers: 100 • Controls: 20 **Sex:** Men: 100% **Source of funding:** None **Author declaration of interest:** NR	**Exposures:** Fluoride levels in • Groundwater samples • Urinary samples Exposure level: Fluoride levels (mg/L) found in groundwater samples of the three districts (Quetta Pishin, and Mastung) • Range: 0.87 – 1.59 Mean (SD) Fluoride levels (mg/L) found in urinary samples of participants from the three districts and controls • Quetta (n = 25) ○ *Mean*: 0.17 (0.15) ○ Range: 0.013 – 0.54 • Pishin (n = 50) ○ Mean: 0.19 (0.21)	Correlation between groundwater fluoride levels and CFI • r = 0.90 Correlation between urinary fluoride levels and CFI • r = 0.96	"The relationship among the groundwater fluoride concentration, urinary F, and dental fluorosis was assessed through Pearson's correlations. A strong positive relationship was determined by the aforementioned parameters (groundwater F, urinary F, and dental fluorosis)" (p. 419)	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	○ Range: 0.002 – 0.842 • Mastung (n = 25) ○ Mean: 0.30 (0.19) ○ Range: 0.092 – 0.811 • Control (n = 20) ○ Mean: 0.003 (0.002) ○ Range: 0.0003 – 0.007 **Outcome(s):** • Community Fluoride Index (CFI)			
Liu 2019 [48]				
Study design: Cross-sectional **Country:** China **Participants:** Randomly selected 7–13 years old residents from ground water-supplied areas of Baodi District, Tianjin, China, with low to moderate fluoride exposure **Sampling time frame:** May - October 2015 **Sample size (N):** 2430 **Sex:** Boys: 51.1% **Source of funding/ support:** National Natural Science of China, National Natural Science Foundation of China, Fundamental Research Funds for the Central Universities **Author declaration of interest:** No COI	**Exposures:** <u>Fluoride levels in</u> • Ground water <u>Exposure level:</u> • Water fluoride: ○ *0.83 mg/L (95%CI: 0.81, 0.86)* ○ *p-value: 0.414* **Outcomes:** age- and sex-standardized height, weight and BMI z-scores, and childhood overweight/obesity (BMI z-score > 1)	• Linear dose-dependent positive association between water fluoride levels and height z-score, as indicated by the trend across fluoride quartiles (*P-trend*=0.022). • Each log unit (roughly 10-fold) increase in urinary fluoride concentration was associated with a ○ *0.136 unit increase in weight z-score (95% CI: 0.039, 0.233)* ○ *0.186 unit increase in BMI z-score (95% CI: 0.058, 0.314)* ○ *1.304-fold increased odds of overweight/obesity (95% CI: 1.062, 1.602)* ○ *These associations were stronger in girls than in boys (P interaction= 0.016)* ○ *Children of fathers with lower education levels were more vulnerable to fluoride (P interaction=0.056)*	• Low-to-moderate fluoride exposure is associated with overweight and obesity in children. • Gender and paternal education level may modify the relationship	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• Each log unit (roughly 10-fold) increase in water fluoride concentration was associated with a 0.129 unit increase in height z-score (95% CI: 0.005, 0.254), but not with other anthropometric measures.		
Malin 2019 [49]				
Study design: Cross-sectional **Country:** United States **Participants:** US adolescents: 12–19 years old (NHANES survey) **Sampling time frame:** 2013–2016 **Sample size (N):** 4470 **Sex:** Men: 52.7% **Source of funding/ support:** Mount Sinai Children's Center Foundation, NIH/NIEHS **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Drinking water Exposure level: • Tap water fluoride: *0.48 mg/L ± 0.03* • Plasma fluoride: *0.40 µmol/L ± 0.01* **Outcomes:** • Estimated glomerular filtration rate • Serum uric acid • Albumin to creatinine ratio • Blood urea nitrogen • AST/ALT • ALP • Gamma-glutamyl transferase • Serum albumin	• A 1 µmol/L increase in plasma fluoride was associated with: ○ *10.36 mL/min/1.73m2 lower estimated glomerular filtration rate (95% CI: −17.50, −3.22; p=0.05),* ○ *0.29 mg/dL higher serum uric acid concentration (95% CI: 0.09, 0.50; p=0.05),* ○ *1.29 mg/dL lower blood urea nitrogen concentration (95%CI: −1.87, −0.70; p < 0.001).* • A 1 mg/L increase in water fluoride was associated with: ○ *0.93 mg/dL lower blood urea nitrogen concentration (95% CI: −1.44, −0.42; p=0.007).* ○ *eGFR: -1.03 mL/min/m² (95% CI: -2.93, 0.87); p > 0.99; water fluoride was log2 transformed in this model.* ○ *SUA: 0.05 mg/dL (95% CI: -0.07, 0.18); p > 0.99* ○ *ACR: -0.01 mg/g (95% CI: -0.07, 0.06); p = > 0.99; water fluoride and outcome variables were log2 transformed.*	Fluoride exposure may contribute to complex changes in kidney-related (lower estimated glomerular filtration rate, higher serum uric acid concentration, and lower blood urea nitrogen concentration) and liver-related parameters among US adolescents	1
Malin 2019a [50]				
Study design: Cross-sectional **Country:** US **Participants:** 16-19 years old adolescents with fluoride biomonitoring data and self-	**Exposures:** Fluoride level in • Drinking water Exposure level:	• An IQR increase in water fluoride was associated with 1.97 times higher odds of:	Fluoride exposure may contribute to changes in sleep cycle regulation and sleep behaviors among	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
reported sleep outcome measures (NHANES 2015–2016) **Sampling time frame:** 2015–2016 **Sample size (N):** 419 **Sex:** Men: 49 % **Source of funding/ support:** NIH/NIEHS **Author declaration of interest:** No COI	• Tap water fluoride mean (SE): *0.39 mg/L (0.05)* • Plasma fluoride mean (SE): *0.35 µmol/L (0.02)* • Median (IQR) water and plasma fluoride concentrations were 0.27 (0.52) mg/L and 0.29 (0.19) µmol/L respectively **Outcomes:** Self-reported sleep outcome measures	○ *Reporting symptoms suggestive of sleep apnea (95% CI: 1.27, 3.05; p = 0.02)* ○ *a 24 min later bedtime (B = 0.40, 95% CI: 0.10, 0.70; p = 0.05)* ○ *a 26 min later morning wake time (B = 0.43, 95% CI: 0.13, 0.73; p =0.04)* ○ *Among males, a 38% reduction in the odds of reporting snoring (95% CI: 0.45, 0.87, p =0.03).*	older adolescents in the US.	
Pei 2019 [51]				
Study design: Cross-sectional **Country:** China **Participants:** Residents aged 16 or older who lived in one of five villages that are endemic in skeletal fluorosis, (Zhao Dong County, Heilongjiang Province) **Sampling time frame:** NR **Sample size (N):** 302 **Sex:** Men: 30% **Source of funding/ support:** National Natural Science Foundation of China, Translational Medicine Special Foundation of China-Russia Medical Research Center, Harbin Medical University, China, Science Foundation for Distinguished Young Scholars of Heilongjiang Province, China	**Exposures:** Fluoride levels in • Drinking water Exposure level: • Water fluoride groups: ○ *1.2 mg/L* ○ *>1.2 mg/L - ≤2 mg/L* ○ *>2 mg/L - ≤4 mg/L* ○ *>4 mg/L* **Outcomes:** Genetic biomarkers of skeletal fluorosis	• 31 miRNAs were significantly and differentially expressed between cases and controls. Of these, 21 miRNAs were up-regulated and 10 miRNAs were down-regulated • 3 additional miRNAs (miR-200c-3p, miR-1231 and miR-3185) were significantly up-regulated in the cases	• Multiple signaling pathways were found to be regulated by the differentially expressed miRNAs • Dysregulation of molecular signaling pathways are involved in the process of fluoride-induced damage of osteoblasts and osteoclasts. However, the regulatory mechanism of fluoride on molecular pathways is still not very clear	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Author declaration of interest: No COI				
Riddell 2019 [52]				
Study design: Cross-sectional **Country:** Canada **Participants:** Youth 6-17 years old from the Canadian Health Measures Survey (Cycles 2 and 3). **Sampling timeframe:** • 2009–2011 • 2012–2013 **Sample size (N):** • Cycle 2: N=2,520 • Cycle 3: N=2,667 **Sex:** Men: 50.8%–52.7% **Funding/support:** Faculty of Health, York University **Author declaration of interest:** No COI	**Exposures** <u>Fluoride levels in</u> • Community source • Drinking water • Urine <u>Exposure level:</u> • Mean (SD) concentration of urinary fluoride adjusted for specific gravity (mg/L) ○ Urinary fluoride – sample 1: 0.61 (0.39) ○ CWF status - sample 2: 0.64 (0.45) ○ Tap water fluoride – sample 3: 0.62 (0.48) • Mean (SD) concentration of water fluoride (mg/L) ○ Urinary fluoride – sample 1: 0.23 (0.24) ○ CWF status – sample 2: 0.26 (0.26) ○ Tap water fluoride – sample 3: 0.23 (0.24) • Mean (SD) ○ Urinary fluoride: 11.3 (3.4)	• **Water fluoride** *Mean ±SD: 0.23 mg/L ±0.24 (cycle 3 only)* • **Urinary fluoride** *Mean ±SD: 0.61 mg/L ±0.39 (cycles 2 & 3)* • An increase of 1.0 mg/L in water fluoride concentration was associated with 6.1 times higher odds of an ADHD after adjusting for potential confounders • **UF_{SG} did not significantly predict ADHD:** *aOR=0.96 (95% CI: 0.63, 1.46); p=0.84* • **UF_{SG} did not significantly predict SDQ hyperactive/ inattentive subscale scores** *aOR = 0.31 (-0.04, 0.66); p = 0.08* **ADHD diagnosis & tap water fluoride** ○ *aOR = 6.10 (1.60, 22.8); p < 0.05* ○ *Exposure-response relationship: yes* **SDQ hyperactive/inattentive subscale score & tap water fluoride** ○ *aOR = 0.31 (0.04, 0.58); p < 0.05* ○ *Exposure-response relationship: yes* **ADHD diagnosis & UF_{SG}** ○ *aOR = 0.96 (0.63, 1.46); p < 0.05* ○ *Exposure-response relationship: yes* **SDQ Hyperactive/Inattentive Subscale Score & UF_{SG}** ○ *aOR = 0.31 (-0.04, 0.66); p = 0.05*	Higher tap water fluoride levels were significantly associated with a higher risk of ADHD and increased symptoms of hyperactivity and inattention, especially among adolescents.	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	○ CWF status: 11.3 (3.3) ○ Tap water fluoride: 11.2 (3.5) **Outcome** Attention-related outcomes	○ *Exposure-response relationship: yes*		
Shaik 2019 [53]				
Study design: Cross-sectional **Country:** India **Participants:** Healthy children 9-13 years old with normal nutritional status, and consuming Iodized salt, with lifelong residence in one of 19 villages in Mysore Taluk, India, with water fluoride levels 0.01-1.8 ppm). **Sampling time frame:** NR **Sample size (N):** 293 **Sex:** Boys: 46% **Source of funding/ support:** NR **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Drinking water Exposure level: Water fluoride mean: • Group I (0.01-0.6 ppm): 0.22 • Group II (0.7-1.2 ppm): 0.89 • Group III (1.3-2.0 ppm): 1.44 **Outcomes:** Thyroid function biomarkers (TSH, T3, T4 in serum)	• TSH: 40% of children of group I had deranged levels followed by group III (20%) and Group II (16%) • T4: 24% of children of both groups I and III had deranged levels followed by group II (20%) • Inter group correlation of drinking water fluoride levels to number of deranged serum T3, T4, and TSH of the children showed non-significant association	Long term intake of fluoridated drinking water (0.02 -1.4 ppm) does not seem to have any effect on the thyroid function in the children with normal nutritional status and optimal iodine intake	2
Soto-barreras 2019 [54]				
Study design: Cross-sectional **Country:** Mexico **Participants:** Children (9 to 10 years of age) in grade 4 attending public elementary schools in Chihuahua **Sampling time frame:** May – December 2017 **Sample size:**161 **Sex:** Men: 54.7%	**Exposures:** Fluoride levels in • Drinking water samples • Urine samples Exposure level: • See results **Outcome(s):** • Intellectual ability • Dental fluorosis	• *Mean (SD) water fluoride levels (mg/L) by dental fluorosis categories* ○ TF 0 (N= 32): 0.75 ± 0.95 ○ TF 1 – 2 (N= 45): 0.67 ± 0.15 ○ TF 3 – 4 (N= 60): 1.22 ± 1.09 ○ TF > 5 (N= 24): 1.66±0.93 p–value: 0.008 • *Mean (SD) urinary fluoride levels (mg/L) by dental fluorosis categories* ○ TF 0 (N= 32): 0.48 ± 0.23 ○ TF 1 – 2 (N= 45): 0.51 ± 0.38	• "No evidence was found for fluoride-associated cognitive deficits. As the level of fluoride consumption remains a public health concern and its implications for health are still uncertain, further research is needed to clarify whether or not fluoride may possibly	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Source of funding: PRODEP program of the Mexican Minister of Education (SEP) **Author declaration of interest:** No COI		○ TF 3 – 4 (N= 60): 0.62 ± 0.32 ○ TF > 5 (N= 24): 0.67±0.41 ○ p-value: 0.088 • *Mean (SD) exposure dose to fluoride (EDI) (mg/kg bw/day) by dental fluorosis categories* ○ TF 0 (N= 32): 0.016 ± 0.02 ○ TF 1 – 2 (N= 45): 0.017 ± 0.02 ○ TF 3 – 4 (N= 60): 0.035 ± 0.03 ○ TF > 5 (N= 24): 0.047±0.03 ○ p-value: 0.001 • *Mean (SD) water fluoride levels (mg/L) by intellectual grade categories* ○ Grade I (N= 6): 1.48 ± 1.13 ○ Grade II (N= 44): 1.05 ± 1.06 ○ Grade III (N= 79): 1.04 ± 1.06 ○ Grade IV (N= 28): 0.97 ± 1.10 ○ Grade V (N= 4): 0.79 ± 1.17 ○ p–value: 0.645 • *Mean (SD) urinary fluoride levels (mg/L) by intellectual grade categories* ○ Grade I (N= 6): 0.45 ± 0.34 ○ Grade II (N= 44): 0.54 ± 0.29 ○ Grade III (N= 79): 0.61 ± 0.38 ○ Grade IV (N= 28): 0.56 ± 0.33 ○ Grade V (N= 4): 0.35 ± 0.19 ○ p–value: 0.559 • Mean (SD) exposure dose by intellectual grade categories Grade I (N= 6): 0.03 ± 0.03 Grade II (N= 44): 0.026 ± 0.03 Grade III (N= 79): 0.027 ± 0.03 Grade IV (N= 28): 0.029 ± 0.03 Grade V (N= 4): 0.016 ± 0.02 p–value: 0.389	have adverse effects on brain development." (p. 481) • "The fluoride content in the drinking water and the exposure dose were significantly higher in the moderate-to-severe fluorosis cases. The urinary fluoride level increased as the level of the severity of the dental fluorosis increased but no statistically significant difference was present." (p. 477 – 478)	

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Zhang 2019 [55]				
Study design: Cross-sectional **Country:** US **Participants:** Massachusetts (MA) resident women with a live birth (2009- 2016) who responded to the PRAMS survey (Pregnancy Risk Assessment Monitoring System) **Sampling time frame:** 2009-2016 **Sample size (N):** 9,234 **Sex:** Women: 100% **Exclusions:** • Women with multiple births • Missing data for dental cleaning during pregnancy, CWF, and/or gestational age • Missing data on relevant maternal characteristics **Source of funding/ support:** CDC **Author declaration of interest:** NR	**Exposures:** • Dental cleaning during pregnancy (DC) alone • Community water fluoridation (CWF) alone • DC and CWF combined Exposure level: • Water fluoride levels: NR **Outcomes:** Prevalence of preterm births (birth < 37 weeks gestation)	• Prevalence of preterm birth among women with a singleton live birth was 8.5% in Massachusetts. • Overall, 58.7% of women had dental cleaning during pregnancy, and 63.6% lived in CWF. • Compared to women without DC and CWF and adjusting for potential confounders: ○ *Dental cleaning alone and preterm birth: significant (aRR = 0.74 [95% CI 0.55–0.98])* ○ *DC–CWF and preterm birth: significant (aRR = 0.74 [95% CI 0.57–0.95]) were significant* ○ *CWF alone and preterm birth: non-significant (aRR = 0.81 [95% CI 0.63–1.05])*	Women who had dental cleaning during pregnancy and lived in a community with water fluoridation had lower prevalence of preterm births	1
Zhou 2019 [56]				
Study design: Cross-sectional **Country:** China **Participants:** >10-years residents, of the Han nationality, in any of 12 villages in north east China, aged ≥40 years old, with no congenital eye disease or ocular trauma **Sampling time frame:** NR **Sample size (N):** 1,813 **Sex:** Men: 30% **Source of funding/ support:** Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Drinking water Exposure level: • Drinking-water fluoride: >1.2 mg/L **Outcomes:** Prevalence of one of seven eye diseases	• Fluoride in the drinking water was closely associated with: ○ *Cataract: OR: 0.543 (95% CI 0.310–0.845).* ○ *Pterygium: OR: 1.991 (95% CI 1.931–3.622).* ○ *Arteriosclerotic retinopathy: OR: 2.011 (95% CI 1.121–3.637).* ○ *Primary angle closure glaucoma: OR:1.179 (95% CI: 0.788–1.489).* ○ *Diabetic retinopathy: OR: 1.845 (95% CI: 0.931–3.120).* ○ *Age-related macular degeneration: OR: 1.048 (95% CI: 0.735–2.221).* ○ *Strabismus: OR: 1.598 (95% CI: 0.936–2.689).*	• High intake of fluoride may act directly and/or indirectly on the eyeball. • Significant positive association of water fluoride levels with pterygium and arteriosclerotic retinopathy, and significant inverse association with cataract. • Non-significant associations with primary angle closure glaucoma, diabetic retinopathy, age-related macular	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• Compared to the control group: ○ *Significant decrease in the exposed group for cataract (14.9% in exposed group, 24.7% in control group)* ○ *Significant increases in the exposed group for pterygium (7.7% in exposed group, 3.2% in control group)* ○ *Significant increases in the exposed group for arteriosclerotic retinopathy (17.6% in exposed group, 6.4% in control group).*	degeneration, and strabismus.	
Zhou 2019a [57]				
Study design: Cross-sectional **Country:** China **Participants:** Children (7 to 13 years to age), from rural areas with low-to-moderate fluoride exposure in Tianjin **Sampling time frame:** 2015 **Sample size:** 616 **Sex N (%):** • Non-DF group: Men: 45.42% • DF group: Men 53.72% **Source of funding:** • The State Key Program of National Natural Science of China • The National Natural Science Foundation of China • The Fundamental Research Funds for the Central Universities **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Drinking water samples • Urine samples Exposure level: Exposure level in mg/L (P25 – P75): • Non-DF group ○ Water: 0.70 (0.40 – 0.80) ○ Urine: 0.17 (0.09 – 0.31) • DF group ○ Water: 1.60 (1.20 – 2.60) ○ Urine: 2.11 (0.45 – 2.69) **Outcome(s):** • Mitochondrial DNA (mtDNA) levels • Dental fluorosis (DF)	***Mitochondrial DNA (mtDNA)*** • *Change (95% CI) in mtDNA levels among those with water fluoride levels in T2 and T3 compared to T1 (mg/L)* T1 (≤ 0.70) Reference T2 (0.71 – 1.50) B = -0.24 (-0.32, -0.15), P = 0.035 T3 (> 1.50) B = -0.32 (-0.39, -0.24), P <0.001 Trend test: P <0.001 • *Change (95% CI) in mtDNA levels per 1 mg/L increase in water fluoride level* B = -0.10 (-0.14, -0.06), P <0.001 • *Change (95% CI) in mtDNA levels among those with urinary fluoride levels in T2 and T3 compared to T1 (mg/L)* T1 (≤ 0.21) Reference T2 (0.22 – 2.08) B = -0.03 (-0.12, 0.06), P = 0.516 T3 (> 2.08) B = -0.27 (-0.35, -0.20), P <0.001	"In conclusion, we have showed that low-to-moderate concentrations of water fluoride and urinary fluoride were positively associated with DF prevalence, while inversely associated with circulating mtDNA levels. Additionally, our study indicates that the gender potentially modifies the associations of DF prevalence with relative mtDNA levels and low-to-moderate fluoride exposure, and that the reduced mtDNA levels may partly mediate the elevated prevalence of moderate DF in children under such exposure."	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Trend Test: P <0.001 • *Change (95% CI) in mtDNA levels per 1 mg/L increase in urinary fluoride level* B = -0.12 (-0.14, -0.09), P <0.001 ***Total DF*** • *Odds (95% CI) of total DF among those with water fluoride levels in T2 and T3 compared to T1 (mg/L)* T1 (≤ 0.70) Reference T2 (0.71 – 1.50) OR = 2.58 (2.02, 3.30), P <0.001 T3 (> 1.50) OR = 3.64 (2.91, 4.55), P <0.001 Trend Test: P <0.001 • *Odds (95% CI) of total DF per 1 mg/L increase in water fluoride level* OR = 1.47 (1.40, 1.55), P <0.001 • *Odds (95% CI) of total DF among those with urinary fluoride levels in T2 and T3 compared to T1 (mg/L)* T1 (≤ 0.21), Reference T2 (0.22 – 2.08) OR = 1.49 (1.26, 1.77), P <0.001 T3 (> 2.08) OR = 3.16 (2.53, 3.95), P <0.001 Trend Test: P <0.001 • *Odds (95% CI) of total DF per 1 mg/L increase in urinary fluoride level* OR = 1.39 (1.32, 1.46), P <0.001 ***Very Mild DF*** • *Odds (95% CI) of very mild DF among those with water fluoride levels in T2 and T3 compared to T1 (mg/L)* T1 (≤ 0.70) Reference		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		T2 (0.71 – 1.50) OR = 2.33 (1.55, 3.51), P <0.001 T3 (> 1.50) OR = 4.93 (3.48, 6.98), P <0.001 Trend Test: P <0.001 • *Odds (95% CI) of very mild DF per 1 mg/L increase in water fluoride level* OR = 1.85 (1.63, 2.11), P <0.001 • *Odds (95% CI) of very mild DF among those with urinary fluoride levels in T2 and T3 compared to T1 (mg/L)* T1 (≤ 0.21) Reference T2 (0.22 – 2.08) OR = 1.31 (0.92, 1.86), P = 0.135 T3 (> 2.08) OR = 4.02 (2.81, 5.74), P <0.001 Trend Test: P <0.001 • *Odds (95% CI) of very mild DF per 1 mg/L increase in urinary fluoride level* OR = 1.57 (1.41, 1.76), P <0.001 ***Mild DF*** • *Odds (95% CI) of mild DF among those with water fluoride levels in T2 and T3 compared to T1 (mg/L)* T1 (≤ 0.70) Reference T2 (0.71 – 1.50) OR = 4.17 (2.80, 6.20), P <0.001 T3 (> 1.50) OR = 6.88 (4.78, 9.92), P <0.001 Trend Test: P <0.001 • *Odds (95% CI) of mild DF per 1 mg/L increase in water fluoride level* OR = 1.68 (1.57, 1.79), P <0.001 • *Odds (95% CI) of mild DF among those with urinary fluoride levels in T2 and T3 compared to T1 (mg/L)*		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		T1 (≤ 0.21) Reference T2 (0.22 – 2.08) OR = 1.79 (1.44, 2.23), P <0.001 T3 (> 2.08) OR = 5.99 (4.15, 8.66), P <0.001 Trend Test: P <0.001 • *Odds (95% CI) of mild DF per 1 mg/L increase in urinary fluoride level* OR = 1.56 (1.45, 1.67), P <0.001 ***Moderate DF*** • *Odds (95% CI) of moderate DF per 1 mg/L increase in water fluoride level* OR = 3.85 (3.01, 4.92), P <0.001 • *Odds (95% CI) of moderate DF per 1 mg/L increase in urinary fluoride level* OR = 2.85 (2.39, 3.39), P <0.001		
Bashash 2018 [58]				
Study design: Cohort **Country:** Mexico **Participants:** Mother-child pairs (ELEMENT study) **Sampling time frame:** • 1997–1999 • 2001–2003 **Sample size (N):** 213 Mother-child pairs **Sex:** Girls: 54% **Funding/ support:** US NIH, NIEHS/EPA, National Institute of Public Health, Ministry of Health of Mexico, American British Cowdray Hospital **Author declaration of interest:** NR	**Exposures:** Fluoride levels in • Maternal urinary samples (prenatal fluoride exposure biomarker) Exposure level: Mean (95% CI) level of fluoride in maternal urine adjusted for creatinine • 0.85 mg/L (0.81, 0.90) **Outcomes:** Attention-deficit/ hyperactivity disorder (ADHD) related symptoms in children between 6 to 12 years of age	• Mean (95% CI) level of fluoride in maternal urine adjusted for creatinine: 0.85 mg/L (0.81, 0.90) • Change (95% CI) in outcome per 0.5 mg/L unit increase in maternal urinary fluoride levels adjusted for creatinine • **CRS-R scores** *Cognitive Problems + Inattention* *β= 2.54 (0.44, 4.63), p= 0.0178* *Restless-Impulsive* *β= 1.92 (-0.07, 3.91), p= 0.0586* *Hyperactivity* *β= 1.05 (-0.91, 3.00), p= 0.2953* *ADHD Index* *β= 2.47 (0.43, 4.50), p= 0.0175* *DSM-IV Inattention* *β= 2.84 (0.84, 4.84), p= 0.0054* *DSM-IV Hyperactivity-Impulsivity* *b= 1.69 (-0.33, 3.70), p= 0.1016* *DSM-IV ADHD Total*	Observed a positive association between higher prenatal fluoride exposure and more behavioral symptoms of inattention, but not hyperactivity or impulse control, in a large Mexican cohort of children aged 6 to 12 years. The current findings provide further evidence suggesting neurotoxicity of early-life exposure to fluoride.	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		β= 2.38 (0.42, 4.34), p= 0.0176 • **CPT-II scores** <u>*Omission Errors*</u> *β= 0.22 (-2.30, 2.74), p= 0.8643* <u>*Commission Errors*</u> *β= -0.43 (-2.38, 1.51), p= 0.6641* <u>*Hit Reaction Time*</u> *β= 1.07 (-1.19, 3.32), p= 0.3546*		
Cui 2018 [59]				
Study design: Cross-sectional **Country:** China **Participants:** Children (7-12) from four schools in Tianjin Municipality in fluorosis-endemic compared to fluorosis-non-endemic areas **Sampling time frame:** 2014 – 2015 **Sample size (N):** 323 **Sex:** Boys: 54.8% **Source of funding/ support:** National Nature Science Foundation of China, Scientific and Technological Project of Tianjin Medicine (2014), Scientific and Technological Project of Tianjin Centers for Disease Control and Prevention **Author declaration of interest:** No COI	**Exposure:** Fluoride levels in urine samples <u>Exposure level:</u> Median (interquartile range) levels of fluoride in urine by DRD2 single nucleotide polymorphism (SNP) • CC (N = 103) 1.3 (0.9 – 1.6) • CT (N = 179) 1.2 (0.8 – 1.8) • TT (N = 44) 1.3 (1.0 – 2.0) **Outcomes:** Intelligence quotient (IQ)	• **Water fluoride:** <u>Nonendemic fluorosis area:</u> 0.20–1.00 mg/L. <u>Endemic fluorosis area:</u> 1.52–2.49 mg/L. • **Urinary fluoride:** Median (IQR) by DRD2 single nucleotide polymorphism (SNP): ○ *CC: 1.3 (0.9 – 1.6)* ○ *CT: 1.2 (0.8 – 1.8)* ○ *TT: 1.3 (1.0 – 2.0)* • **Change (95% CI) in IQ score per log-unit increase in urinary fluoride among all participants and by subgroups** <u>Overall (N= 323)</u> *b = -2.47 (-4.93, - 0.01), p = 0.049* *[Bootstrapped estimate: 95%CI = -4.97, 0.03; p = 0.053]* <u>DRD2 SNP of CC or CT (N= 279)</u> *b = - 1.59 (- 4.24, 1.05), p = 0.236* *[Bootstrapped estimate: 95%CI= -4.14, 0.95; p = 0.220]* <u>DRD2 SNP of TT (N= 44)</u> *b = -12.31 (-18.69, -5.94), p=< 0.001* *[Bootstrapped estimate: 95%CI= -19.66, -4.96; p = 0.001]*	• There is heterogeneity in the relation between urine fluoride and IQ across children carrying different DRD2 Taq 1A genotypes • Overall, the DRD2 Taq 1A polymorphism itself was not related to IQ scores in children with high level of urine fluoride. • In the CC/CT subgroup, urinary fluoride levels were not related to IQ scores in children. • Among participants with the TT genotype, there was a strong and robust negative linear relationship between log-urine fluoride and IQ scores in children after adjusting for child age and have a cold more than 5 times a year.	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		• The safety threshold of urine fluoride levels in the subgroup TT: 1.73 mg/L (1.51-1.97)		
Jimenez-Cordova 2018 [60]				
Study design: Cross-sectional **Country:** Mexico **Participants:** 18 to 77 years old of age) who were exposed to fluoride via drinking water **Sampling time frame:** 2013 **Sample size (N):** 239 **Sex:** Men: 28.8% **Funding/support:** Mexican National Council of Science and Technology **Author declaration of interest:** No COI	**Exposures:** <u>Fluoride levels in</u> • Drinking water samples • Urine samples <u>Exposure level:</u> • Geometric mean (Interquartile range; IQR) level of water fluoride (mg/L); N = 232 ○ 1.5 (0.19 – 1.8) • Geometric mean (IQR) level of urinary fluoride (µg/mL); N = 236 ○ 2.0 (1.1 – 3.5) • Geometric mean (IQR) level of urinary tAS (ng/mL); N = 236 ○ 18.55 (10.6 – 34.1) • Geometric mean (IQR) level of urinary inorganic As (ng/mL); N = 236 ○ 1.8 (0.91 – 4.4) **Outcomes:** <u>Kidney injury</u> • Urine levels of albumin (ALB), cystatin-C (Cys-C), kidney injury molecule 1 (KIM-1), clusterin (CLU), osteopontin (OPN), and trefoil factor 3 (TIFF-3)) <u>Kidney function</u>	• Geometric mean (Interquartile range; IQR) level of water fluoride (mg/L); N=232; 1.5 (0.19 – 1.8) • Geometric mean (IQR) level of urinary fluoride (µg/mL); N=236; 2.0 (1.1 – 3.5) • Geometric mean (IQR) level of urinary tAS (ng/mL); N=236; 18.55 (10.6 – 34.1) • Change in outcome (p-value) per unit increase of fluoride in water (mg/L) and urine (µg/mL) <u>ALB (µg/mL)</u> *Water: β= 1.20 (p= <0.001)* *Urine: β= 0.56 (p= <0.001)* <u>Cys-C (mg/mL)</u> *Water: β= 0.03 (p= 0.005)* *Urine: β= 0.022 (p= 0.001)* <u>OPN (mg/mL)</u> *Water: β= 0.10 (p= 0.028)* *Urine: β= 0.038 (p= 0.041)* <u>CLU (µg/mL)</u> *Water: β= 0.09 (p= 0.118)* *Urine: β= 0.07 (p= 0.100)* <u>KIM-1 (ng/mL)</u> *Water: b= 0.045 (p= 0.162)* *Urine: b= 0.048 (p= 0.008)* <u>TFF-3 (ng/mL)</u> *Water: β= 2.88 (p= 0.010)* *Urine: β= 1.14 (p= 0.115)* <u>eGFR (mL/min/1.73 m^2)</u> *Water: β= 0.19 (p= 0.675)* *Urine: β= 0.49 (p= 0.030)*	Urinary excretion of 4 early kidney injury biomarkers (ALB, Cys-C, KIM-1 and OPN) is related to environmental F exposure in an adult population, without an As interaction effect. Our results suggest a possible tubular dysfunction from F exposure that might increase susceptibility to the future development of CKD.	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	• Glomerular filtration rate (eGFR)			
Kumar, V 2018 [61]				
Study design: Cross-sectional **Country:** India **Participants:** 8 to 15 years old children **Sampling time frame:** NR **Sample size (N):** 400 Group A (N= 200, endemic): Group B (N= 200, non-endemic) **Sex:** NR **Funding/support:** None **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Serum samples • Urine samples Exposure level: • Mean (range) level of water fluoride (ppm) by study groups ○ A1: 1.1 (1.5 – 5) ○ A2: 3.3 (1.8 – 5.8) ○ B: 0.99 (0.94 – 1.08) • Range of urinary fluoride (ppm) level by study groups ○ A1: 0.27 – 8.6 ○ A2: 0.6 – 7.64 ○ B: 0.22 – 1.07 • Range of serum fluoride (ppm) level by study groups ○ A1: 0.05 – 0.71 ○ A2: 0.05 – 0.71 ○ B: 0.03 – 0.10 **Outcomes:** Thyroid functions Serum levels of free triiodothyronine (T3), free thyroxine (T4), and thyroid stimulating hormone (TSH)	• Mean free T3 (pg/ml) by study group ○ *A: 3.125; β: 2.698, p = 0.26* • Mean free T4 (ng/dL) by study group ○ *A: 1.282; β: 1.193, p = 0.41* • Mean TSH (µlU/m) ○ *A: 3.849; β: 2.588, p = 0.02* • Mean water fluoride (ppm) ○ *A: 2.877; β: 1.020, p = 0.01* • Mean urinary fluoride (ppm) ○ *A: 2.982; β: 0.761, p = 0.02* • Mean serum fluoride (ppm) ○ *A: 0.195; β: 0.059, p = 0.03* • Percent (%) of thyroid hormone level derangement ○ *A: 67.5; β: 54*	• Mean TSH, water fluoride levels, urine fluoride levels and serum fluoride levels of subjects of group 1 were found to be significantly higher than that of subjects of group 2 (p-value < 0.05). • Fluorosis and thyroid functional activity are positively correlated with each other. • Excessive fluoride levels also lead to alteration in thyroid hormones activity	2
Kumar, S 2018 [62]				
Study design: Cross-sectional **Country:** India	**Exposures:** Fluoride levels in	Correlation between water fluoride levels (ppm) and DF severity	"The severity of dental fluorosis is positively	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Participants: Adolescents (12 to 15 years of age) from 16 schools in Jhabua and Dhar districts **Sampling time frame:** January 2015 to July 2015 **Sample size:** 800 **Sex:** Boys: (49.75%) **Source of funding:** None that would influence the results **Author declaration of interest:** No COI	• Water samples <u>Exposure level:</u> Mean (SD) water fluoride levels • Jhabua: 1.29 (±0.52) • Dhar: 1.23 (±0.39) • Total: 1.27 (±0.46) **Outcome(s):** Severity of Dental Fluorosis (DF)	• r = 0.967; p = 0.000 <u>Odds (95% CI) of DF at >1.2ppm compared to ≤ 1.2ppm</u> • OR = 1.764 (1.309, 2.377); p < 0.0001	correlated with the fluoride content in the water. The water fluoride content is the strongest predictor for dental fluorosis." (p. 6)	
Malin 2018 [63]				
Study design: Cross-sectional **Country**: Canada **Sampling period:** 2012–2013 **Participants:** 3-79 years old from 16 cities, Canada **Sample size (N):** 6,914,124 **Sex:** Men: 51.54% **Source of funding:** SSHRC, CIHR, CFI, Statistics Canada **Author declaration of interest:** No COI	**Exposures** <u>Fluoride levels in</u> • Drinking water • Urine <u>Iodine level in</u> • Urine <u>Exposure level:</u> Water fluoride • 0.22 mg/L ± 0.24 Urinary fluoride • 0.94 mg/L ± 1.05 **Outcome(s)** Thyroid functions	**Water fluoride:** 0.22 mg/L ± 0.24 **Urinary fluoride:** 0.94 mg/L ± 1.05 **Change in serum TSH (mIU/L) per unit increase in UFsg (mg/L)** <u>No iodine deficiency</u> *ß = -0.02 (-0.19, 0.15), p = 0.43* <u>Iodine deficiency</u> *ß = 0.36 (-0.03, 0.75), p = 0.03*	Adults living in Canada who have moderate-to-severe iodine deficiencies and higher levels of urinary fluoride may be at an increased risk for underactive thyroid gland activity.	1
Mohd Nor 2018 [25]				
Study design: Cross-sectional **Country:** Malaysia **Participants:** Lifelong residents aged 9- and 12-year-olds **Sampling time frame:**	**Exposures:** Fluoride levels in public drinking water supply <u>Exposure level:</u> • Original: 0.7 ppm • Reduced: 0.5 ppm **Outcome(s):**	• "The prevalence of fluorosis (Dean's score ≥ 2) among children in the fluoridated area (35.7%, 95% CI: 31.9%-39.6%) was significantly higher (P < 0.001) than children in the nonfluoridated area (5.5%, 95% CI: 3.6%-7.4%)."	"Findings indicate that the change in fluoride level from 0.7 to 0.5 ppm has reduced fluorosis and maintains a caries-preventive effect. Although there is a reduction in fluorosis prevalence, the	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
2015 (calculated using the following information reported by the authors) • 9-year-old children (born between 1 January and 31 December 2006 • 12-year-old children (born between 1 January and 31 December 2003) **Sample size:** 1143 children aged 9-12 years old **Sex:** Boys: 43% **Source of funding:** Ministry of Higher Education, Malaysia **Author declaration of interest:** No COI	Dental fluorosis	• "Of those in the fluoridated area, the prevalence of fluorosis decreased from 38.4% (95% CI: 33.1% 44.3%) for 12-year-olds to 31.9% (95% CI: 27.6%-38.2%) for 9-year-olds, although this difference was not statistically significant (P = 0.139)." **Fluorosis prevalence no. (%)** (0) Normal • Fluoridated: 342 (56.3%) • Nonfluoridated: 494 (90.1%) (1) Questionable • Fluoridated: 41 (6.8%) • Nonfluoridated: 23 (4.2%) (2) Very mild • Fluoridated:95 (15.7%) • Nonfluoridated: 23 (4.2%) (3) Mild • Fluoridated: 65 (10.7%) • Nonfluoridated: 5 (0.9%) (4) Moderate • Fluoridated:53 (8.7%) • Nonfluoridated: 2 (0.4%) (5) Severe • Fluoridated:0 • Nonfluoridated: 0 Not able to score • Fluoridated:11 (1.8%) • Nonfluoridated: 1 (0.2%) Total • Fluoridated:607 (100%) • Nonfluoridated: 548 (100%) Fluorosis (Deans > 0) Fluoridated: 254 (42.6%), *P<0.001* Nonfluoridated: 53 (9.7%) Fluorosis (Deans ≥ 2) Fluoridated:213 (35.7%), *P<0.001*	difference was not statistically significant."	

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Nonfluoridated: 30 (5.5%) **Bivariate analysis of fluorosis prevalence with different fluoride exposures** <u>Fluorosis Deans ≥2</u> *0 ppm lifetime* • N (%): 30 (12.30%) • OR (95% CI), p-value: Ref. *0.5 ppm lifetime* • N (%): 100 (41.2%) • OR (95% CI), p-value: 8.45 (5.45-13.10), 0.001 *0.7 ppm for first 2 years and then 0.5 ppm* • N (%): 113 (46.5%) • OR (95% CI), p-value: 10.88 (7.03-16.84), 0.001 <u>Any fluorosis: Deans > 0</u> *0 ppm lifetime* • N (%): 53 (9.7%) • OR (95% CI), p-value: Ref. *0.5 ppm lifetime* • N (%): 123 (40.5%) • OR (95% CI), p-value: 6.33 (4.40-9.12), 0.001 *0.7 ppm for first 2 years and then 0.5 ppm* • N (%): 161 (55.1%) • OR (95% CI), p-value: 7.58 (5.26-10.93), 0.001 **Fluorosis prevalence after fluoride concentration in the water supply was reduced** <u>Fluorosis (Deans > 0)</u> *% Prevalence 12-year-old (PreReduction)* • Fluoridated: 44.6% • Nonfluoridated (control): 10.3%		

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		% Prevalence 9-year-old (PostReduction) • Fluoridated: 39.3% • Nonfluoridated (control): 8.9% *% Difference (post-pre)* • Fluoridated: −5.3 • Nonfluoridated (control): −1.4 *% Difference (pre)* • Fluoridated: 34.3 *% Difference (post)* • Fluoridated: 30.4 <u>Fluorosis (Deans ≥ 2)</u> *% Prevalence 12-year-old (PreReduction)* • Fluoridated: 38.4% • Nonfluoridated (control): 4.7% *% Prevalence 9-year-old (PostReduction)* • Fluoridated: 31.9% • Nonfluoridated (control): 6.5% *% Difference (post-pre)* • Fluoridated: −6.5 • Nonfluoridated (control): 1.8 *% Difference (pre)* • Fluoridated: 33.7 *% Difference (post)* • Fluoridated: 25.4		
Mustafa 2018 [64]				
Study design: Ecological **Country:** Sudan **Participants: P**rimary school students (6 to 14 years of age) residents of rural areas in Khartoum state **Sampling time frame:** NR **Sample size (N):** 775 **Sex:** Boys: 40.6%	**Exposure:** <u>Fluoride levels in</u> Groundwater samples <u>Exposure level:</u> Range for levels of fluoride in groundwater by season • Dry season	• Ground water fluoride: ○ Dry season: 0.14–2.07 mg/L ○ Rainy season: 0.01–1.34 mg/L • Correlation between average level of fluoride in drinking water (mg/L) and average school performance score (%) <u>*Overall score*</u>*: r = -0.51; p = 0.007* • Correlation between average level of fluoride in drinking water (mg/L) and	• Significant correlations undoubtedly exist between the drinking water F level and the schooling performances in all the subjects except for one, technology, which might be due to the nature of the subject	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Source of funding/ support: Ministry of Education-Khartoum State, Ministry of Higher Education and Scientific Research, Sudan **Author declaration of interest:** NR	○ 0.14 – 2.07 mg/L • Rainy season ○ 0.01 – 1.34 mg/L **Outcomes:** Schooling performance (average score and high score [> 70%] prevalence)	the prevalence of high school performance score (%) *Overall score: r = -0.48; p = 0.012*	• There may be an inverse relationship between the F level in drinking water and the schooling performance.	
Oweis 2018 [65]				
Study design: Cohort **Country:** USA **Participants:** Adolescents (17 years of age) whose families were recruited into the Iowa Fluoride Study (IFS) from hospitals following birth **Sampling time frame:** IFS: 1992 - 1995 Iowa Bone Development Study (IBDS) – IFS Subset: 1998 - 2000 **Sample size (N):** 380 **Sex:** Boys: 46.3% **Source of funding/ support:** NIH grants, Wright-Bush Shreves Endowed Professor Fund, University of Iowa **Author declaration of interest:** NR	**Exposure:** Period-specific daily intake of fluoride • Birth to 8.5 years • 8.5 to 14 years • 14 to 17 years • Birth to 17 years Cumulative average daily intake of fluoride • Birth to 17 years Exposure level: Range for level of fluoride intake • Women: 0.7 - 0.8 mg /day • Men: 0.7 - 0.9 mg /day **Outcomes:** Radial and tibial bone characteristics • Cortical content • Cortical density • Trabecular content • Trabecular density • Compression strength • Torsion strength	**Fluoride intake (water and other sources)** Women: 0.7 - 0.8 mg /day Men: 0.7 - 0.9 mg /day. **Change (SE) per 1 mg unit increase in daily fluoride intake in (0-17 years)** **RADIAL BONE** • **Trabecular content (mg)** *Girls: β = 0.59 (3.30), p = 0.86* *Boys: β =-5.63 (4.28), p = 0.19* • **Trabecular density (mg/cm^3)** *Girls: β = 0.99 (12.14), p = 0.94* *Boys: β = -7.88 (11.51), p = 0.50* • **Cortical content (mg)** *Girls: β = -3.19 (3.33), p = 0.34* *Boys: β = 0.37 (4.10), p = 0.93* • **Cortical density (mg/cm^3)** *Girls: β = -2.28 (5.46), p = 0.68* *Boys: β = -0.21 (6.16), p = 0.98* • **Compression strength** *Girls: β = -2.00 (3.10), p = 0.52* *Boys: β = 0.72 (4.43), p = 0.88* • **Torsion strength** *Girls: β =-21.00 (14.95), p = 0.17* *Boys: β = 8.05 (19.62), p = 0.69* **TIBIAL BONE** • **Trabecular content (mg)** *Girls: b = 0.24 (10.07), p = 0.98*	"In summary, the findings show that the effects of life-long fluoride intake from combined sources for adolescents in the United States were not strongly associated with pQCT bone measures at age 17... the study findings provide support to the assertion that fluoride intakes, within these ranges, are not associated with adverse consequences on bone outcome measures by age 17."	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		Boys: b = -5.82 (9.37), p = 0.54 • **Trabecular density (mg/cm^3)** *Girls: b = -8.66 (11.63),* p = 0.46 *Boys: b = 7.31 (10.37), p = 0.49* • **Cortical content (mg)** *Girls: b = 14.24 (11.95), p = 0.24* *Boys: b = 16.19 (13.63), p = 0.24* • **Cortical density (mg/cm^3)** *Girls: b = -0.86 (6.07), p = 0.89* *Boys: b = -0.06 (5.52), p = 0.99* • **Compression strength (mg^2/mm^4)** *Girls: b = -1.62 (6.82), p = 0.82* *Boys: b = 9.37 (8.34), p = 0.27* • **Torsion strength (mm^3)** *Girls: b = 64.15 (74.10), p = 0.39* *Boys: b = 90.24 (95.28), p = 0.35*		
Quadri 2018 [66]				
Study design: Case-control (Only cross-sectional analysis results relevant to the review are included) **Country:** India **Participants:** 4-12 years old children minimal change nephrotic syndrome (NS-MCD) **Sampling time frame:** 2012–2015 **Sample size (N):** 156 **Sex:** NR **Funding/support:** None **Author declaration of interest:** No COI	**Exposure:** Fluoride levels in • Urine samples • Serum samples Exposure levels: Urinary fluoride, mean ±SD • Gp 0: 0.56 ppm ±0.15 • Gp 1: 0.61 ppm ±0.17 • Gp 2: 4.01 ppm ±1.83 Serum fluoride, mean ±SD • Gp 0: 0.07 ppm ±11 • Gp 1: 0.07 ppm ±0.01 • Gp 2: 0.1 ppm ±0.013 **Outcomes:** Nephrotoxicity: • Renal tubule ultrastructural changes	**Urinary fluoride, mean ±SD** ○ *Gp 1: 0.61 ppm ±0.17* ○ *Gp 2: 4.01 ppm ±1.83* ○ *Gp 0: 0.56 ppm ±0.15* **Serum fluoride, mean ±SD** ○ *Gp 1: 0.07 ppm ±0.01* ○ *Gp 2: 0.1 ppm ±0.013* ○ *Gp 0: 0.07 ppm ±11* • Significantly higher level of fluoride in urine in G-2 compared to Gp 1 and Gp 0 (p = 0.001) • Significantly higher level of fluoride in serum was reported among Gp -2 compared to G-1 and G-0 (p = 0.001) **Renal tubule apoptosis** Level of renal tubule apoptosis *Gp 1 = 7%* *Gp 2 = 22%*	• Increased levels of apoptosis were observed in high fluoride group (Gp 2) compared to normal fluoride group (Gp 1), which leads to cell death and renal injury. • Various degrees of fluoride-associated damages to the architecture of tubular epithelia, such as cell swelling and lysis, cytoplasmic vacuolation, nuclear condensation, apoptosis, and necrosis, were observed.	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	• Renal tubule apoptosis	*p = 0.001*		
Rathore 2018 [67]				
Study design: Cross-sectional **Country:** India **Participants:** 8-14 years old children **Sampling time frame:** NR **Sample size (N):** 100 **Sex:** NR **Funding/support:** NR **Author declaration of interest:** NR	**Exposures:** <u>Fluoride levels in</u> • Drinking water samples • Urine samples • Blood samples <u>Exposure level:</u> • Urinary fluoride, mean ±SD ○ *Gp 1: 1.25 mg/L ±0.42* ○ *Gp 2: 1.23 mg/L ±0.32* ○ *Gp 3: 3.03 mg/L ±0.58* ○ *Gp 4: 4.49 mg/L ±1.21* • Serum fluoride, mean ±SD ○ *Gp 1: 0.046 mg/L ±0.02* ○ *Gp 2: 0.046 mg/L ±0.02* ○ *Gp 3: 0.11 mg/L ±0.09* ○ *Gp 4: 0.20 mg/L ±0.13* **Outcomes:** <u>Thyroid hormone derangement</u> Serum levels of free T4 (FT4), free T3 (FT3) and TSH	• **Urinary fluoride, mean ±SD** ○ *Gp 1: 1.25 mg/L ±0.42* ○ *Gp 2: 1.23 mg/L ±0.32* ○ *Gp 3: 3.03 mg/L ±0.58* ○ *Gp 4: 4.49 mg/L ±1.21* • **Serum fluoride, mean ±SD** ○ *Gp 1: 0.046 mg/L ±0.022* ○ *Gp 2: 0.046 mg/L ±0.019* ○ *Gp 3: 0.11 mg/L ±0.09* ○ *Gp 4: 0.20 mg/L ±0.13* • **Mean (SD) of free T3 (pg/mL); [range]** ○ *Gp 1: 2.66 (0.46); [2.11 – 3.89]* ○ *Gp 2: 2.73 (0.36); [2.13 – 3.56]* ○ *Gp 3: 2.84 (0.46); [2.02 – 4.26]* ○ *Gp 4: 3.06 (0.78); [1.91 – 4.42]* • **Mean (SD) of free T4 (ng/dL); [range]** ○ *Gp 1: 0.98 (0.21); [0.79 – 1.79]* ○ *Gp 2: 1.02 (0.26); [0.78 – 1.89]* ○ *Gp 3: 1.11 (0.28); [0.76 – 1.98]* ○ *Gp 4: 1.22 (0.33); [0.75 – 1.89]* • **Mean (SD) of TSH (uIU/mL); [range]** ○ *Gp 1: 1.33 (0.78); [0.4 – 2.99]* ○ *Gp 2: 1.64 (0.88); [0.29 – 3.76]* ○ *Gp 3: 1.86 (0.77); [0.76 – 3.74]* ○ *Gp 4: 1.91 (1.10); [0.75 – 4.99]*	• "When serum FT3, FT4 and TSH of different category of our study were compared we found significant difference between these." • "FT3 levels was highest in category IV with minor difference in other groups; concentration of FT4 levels was maximum in category III, whereas TSH levels were significantly higher in category IV." • "The results of this study question the validity of the fluoridation of drinking water, milk, fruit juices, and salt by public health authorities … It is recommended to reduce the fluoride content of drinking water in the high fluoride area by making either de fluoridation of water or alternative water source."	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Shruthi 2018 [68]				
Study design: Cross-sectional **Country:** India **Participants:** Adolescent and adult residents of three randomly selected villages in Bangarpet taluk, India. **Sampling time frame:** Study duration of 1 year **Sample size (N):** High fluoride group: N= 486 Normal fluoride group: N= 417 **Sex:** High fluoride group: Men: 55.1% Normal fluoride group: Men: 44.9% **Source of funding/ support:** None **Author declaration of interest:** No COI	**Exposure:** Fluoride levels in • Drinking water samples Exposure level: • High fluoride group ○ > 1.5 mg/L fluoride in water • Normal fluoride group ○ < 1.0 mg/L fluoride in water **Outcomes:** • Non-skeletal manifestations	**Exposure levels:** Fluoride level in water: *High fluoride group: > 1.5 mg/L* *Normal fluoride group: < 1.0 mg/L* **Results:** • **Number (%) of participants with non-skeletal manifestations of fluorosis by study groups** Dyspepsia = 32 (100.0) ○ *High fluoride group: 24 (75.0)* ○ *Normal fluoride group: 8 (25.0)* Muscle weakness: 13 (100.0) ○ *High fluoride group: 9 (69.23)* ○ *Normal fluoride group: 4 (30.77)* Fatigue: 32 (100.0) ○ *High fluoride group: 19 (59.38)* ○ *Normal fluoride group: 13 (40.62)* • None of the study participants had complaints of polyuria, polydipsia, repeated abortions, and repeated stillbirths. • The study subjects with clinical manifestations of non-skeletal fluorosis were higher compared to those without clinical manifestations of non-skeletal fluorosis at nearly same doses of fluoride exposure in both high and normal fluoride groups.	• Higher proportion of study subjects with clinical manifestations of non-skeletal fluorosis compared to those without clinical manifestations of non-skeletal fluorosis at nearly same doses of fluoride exposure in both high and normal fluoride groups indicates that these manifestations may be due to fluoride exposure through water or other sources like food. • Participants with dyspepsia in the high fluoride group are three-times higher than those in the normal fluoride group.	2
Yu 2018 [69]				
Study design: Cross-sectional **Country:** China **Participants:** 7-13 years old children **Sampling time frame:** 2015 **Sample size (N):** 2,886 **Sex:** Normal-fluoride gp.: Boys:51.9%	**Exposures:** Fluoride levels in • Urine samples • Drinking water samples Exposure level:	• **Mean (SD) levels of fluoride in water (mg/L) (p <0.001)** ○ *Normal-fluoride: 0.50 (0.27)* ○ *High-fluoride: 2.00 (0.75)* • **Mean (SD) levels of fluoride in urine (mg/L) (p <0.001)** ○ *Normal-fluoride: 0.41 (0.49)* ○ *High-fluoride: 1.37 (1.08)*	• "In our study, urinary fluoride levels presented a positive relationship with water fluoride concentration, indicating that fluoride from drinking water makes important	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
High-fluoride gp.: Boys: 53.4% **Funding/support:** National Natural Science of China, Fundamental Research Funds for the Central Universities **Author declaration of interest:** No COI	• Mean (SD) levels of fluoride in water (mg/L) (p <0.001) ○ Normal-fluoride exposure: 0.50 (0.27) ○ High-fluoride exposure: 2.00 (0.75) • Mean (SD) levels of fluoride in urine (mg/L) (p <0.001) ○ Normal-fluoride exposure: 0.41 (0.49) ○ High-fluoride exposure: 1.37 (1.08) **Outcomes:** Intelligence quotient (IQ)	• **IQ scores per 0.5 mg/L increment of fluoride in water by concentration** ○ *0.20 – 3.40 mg/L:* *β = -0.04 (- 0.33, 0.24)* ○ *3.40 – 3.90 mg/L:* *β = - 4.29 (- 8.09, - 0.48)* • **IQ scores per 0.5 mg/L increment of fluoride in urine by concentration** ○ *0.01 – 1.60 mg/L* *β = 0.36 (- 0.29, 1.01)* ○ *1.60 – 2.50 mg/L* *β = -2.67 (-4.67, -0.68)* ○ *2.50 – 5.54 mg/L* *β = -0.84 (-2.18, 0.50)* • **IQ level among children exposed to high compared to normal water fluoride** ○ *Excellent: OR= 0.47 (0.32, 0.71)* ○ *Superior: OR= 0.89 (0.69, 1.15)* ○ *High normal: OR= 0.96 (0.80, 1.15)* ○ *Dull normal: OR= 0.85 (0.62, 1.17)* ○ *Marginal: OR= 1.25 (0.69, 2.26)* • **IQ level among children exposed to high compared to normal urine fluoride** ○ *Excellent: OR= 0.49 (0.26, 0.93)* ○ *Superior: OR= 0.84 (0.58, 1.20)* ○ *High normal: OR= 0.87 (0.68, 1.12)* ○ *Dull normal: OR= 0.63 (0.39, 1.01)* ○ *Marginal: OR= 1.44 (0.72, 2.91)* • **IQ level per 0.5 mg/L increment of fluoride in water** ○ *Excellent (Fluoride: 0.20–1.40 mg/L): OR= 0.60 (0.47, 0.77)* ○ *Excellent (Fluoride:1.40–3.90 mg/L): OR= 1.09 (0.88, 1.36)* ○ *Superior: OR= 0.99 (0.93, 1.06)* ○ *High normal: OR= 0.98 (0.94, 1.03)*	contribution to urinary fluoride." • "Chronic exposure to excessive fluoride, even at a moderate level, was inversely associated with children's … intelligence scores, especially excellent intelligence performance, with threshold and saturation effects observed in the dose-response relationships."	

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		○ *Dull normal: OR= 0.96 (0.88, 1.05)* ○ *Marginal: OR= 1.04 (0.89, 1.23)* • **IQ level per 0.5 mg/L increment of fluoride in urine** ○ *Excellent: OR= 0.87 (0.76, 1.01)* ○ *Superior: OR= 0.96 (0.89, 1.04)* ○ *High normal: OR= 0.99 (0.94, 1.04)* ○ *Dull normal: OR= 0.90 (0.81, 1.00)* ○ *Marginal: OR= 1.07 (0.91, 1.25)*		
Arulkumar 2017 [70]				
Study design: Case-control **Country:** India **Participants:** Cases with dental and skeletal fluorosis and matching controls **Sampling time frame:** NR **Sample size (N):** 508 **Sex:** Men (58%) **Funding/support:** Periyar University, Indian Council of Medical Research **Author declaration of interest:** NR	**Exposures:** Fluoride level in serum <u>Exposure level:</u> • Drinking water fluoride concentration: > 1.5 mg/l • Mean (SD) level of fluoride (mg/L) in serum by study groups ○ Group I (controls): 0.07 (0.08) ○ Group II (mild fluorosis): 0.13 (0.02) ○ Group III (moderate fluorosis): 0.19 (0.03) ○ Group IV (severe fluorosis): 0.28 (0.03) **Outcomes:** • Degree of lipid peroxidation • Lipid profiles • Enzyme activity	**Drinking water fluoride concentration:** *> 1.5 mg/l* **Mean (SD) level of fluoride (mg/L) in serum by study groups** ○ *Gp I (controls): 0.07 (0.08)* ○ *Gp II (mild fluorosis): 0.13 (0.02)* ○ *Gp III (moderate fluorosis): 0.19 (0.03)* ○ *Gp IV (severe fluorosis): 0.28 (0.03)* **Correlation between serum fluoride and outcomes in patients with fluorosis** ○ *Plasma TBARS: r = 0.095; p = 0.019* ○ *Erythrocyte TBARS: r = 0.783; p = 0.000* ○ *Cholesterol: r = 0.121; p = 0.003* ○ *TGL: r = -0.043; p = NS* ○ *HDL: r = -0.075; p = 0.006* ○ *LDL: r = 0.157; p = 0.000* ○ *VLDL: r = -0.038; p = NS* **Correlation between serum fluoride and outcomes in patients with fluorosis** ○ *PON1: r = -0.738; p = 0.000* ○ *ARE: r = -0.447; p = 0.000* ○ *Lactonase: r = -0.645; p = 0.000* **Serum levels of IQ-related enzymes by study group**	• Positive correlation with erythrocyte TBARS (p <0.01), plasma TBARS (p <0.05), cholesterol (p <0.01) and LDL (p <0.01). Significant inverse association of serum fluoride levels with PON1, ARE, and lactonase. No significant association of serum fluoride levels with TGL and VLDL. No observed correlation with serum HDL; however, serum fluoride modulates the activities of PON1, ARE and lactonase. Results support the chances of cardiovascular-related complications in fluorosis patients. • Increased LDH5 isoenzyme (liver synthesized) activity is an indication of possible liver damage in fluorosis patients. Prolonged	2

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
		○ *AChE* • *Controls: 6.29 ± 0.68* • *Mild: 4.64 ± 0.54* • *Moderate: 4.11 ± 0.4* • *Severe: 3.78 ± 0.35* ○ *ATPase/Na+ K+ ATPase* • *Controls: 2.41 ± 0.34* • *Mild: 2.56 ± 0.31* • *Moderate: 2.64 ± 0.29* • *Severe: 2.87 ± 0.4*	fluoride ingestion (observed in moderate and severe groups) caused continuous multifaceted calamities beyond the regenerative capacity of the liver tissues. • The decreased activity of the membrane bound enzymes, AChE and ATPase indicates the prevalence of memory loss with lower IQ scores as well as defect in signaling and energy metabolism in fluorosis patients.	
Bashash 2017 [71]				
Study design: Cohort **Country:** Mexico **Participants:** Mother-child pairs (ELEMENT study) **Sampling time frame:** • 1997–1999 • 2001-2003 **Sample size (N):** 299 mother-child pairs **Sex:** Girls: GCI analysis: 56% IQ analysis: 55% **Funding/support:** NIH, NIEHS/EPA, National Institute of Public Health (MOH, Mexico), American British Cowdray Hospital **Author declaration of interest:** No COI	**Exposures:** <u>Fluoride levels in</u> • Maternal urinary samples during gestation • Child urinary samples at 6 to 12 years of age <u>Exposure level:</u> • Water fluoride levels in Mexico City: ○ 0.15 - 1:38 mg/L (Juárez-López et al. 2007; Martínez-Mier et al. 2005). • Maternal urinary fluoride (Mean ±SD) ○ 0.88 mg/L ±0.34	**Water fluoride levels in Mexico City:** ○ *0.15 - 1:38 mg/L (Juárez-Lópezetal.2007; Martínez-Mier et al.2005).* **Maternal urinary fluoride (Mean ±SD)** ○ *0.88 mg/L ±0.34* **Child urinary fluoride (Mean ±SD)** ○ *0.84 mg/L ±0.40* • **Change in outcome per 0.5 mg/L increase in maternal urinary fluoride levels** ○ *GCI: β = -3.15 (-5.42, -0.87); p = 0.01* ○ *IQ: β = -2.50 (-4.12, -0.59); p = 0.01* • **Change in outcome per 0.5 mg/L increase in child urinary fluoride levels**	• Higher prenatal exposure to fluoride (as indicated by average creatinine-adjusted maternal urinary fluoride concentrations during pregnancy) was associated with lower GCI scores in children at approximately 4y old, and with lower Full-Scale IQ scores at 6–12 y old. • In models that focused on the cross-sectional relationship between children's exposure to fluoride (reflected by their specific gravity–adjusted urinary fluoride levels) and IQ score and that	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	• Child urinary fluoride (Mean ±SD) ○ 0.84 mg/L ±0.40 **Outcomes:** Neurocognitive function in children at 4 years of age, and 6 to 12 years of age	○ *IQ – Without adjustment of maternal urinary fluoride levels:* *β = - 0.89 (-2.63, 0.85)* ○ *IQ – With adjustment of maternal urinary fluoride levels* *β = - 0.77 (-2.53, 0.99)*	contained the main covariates of interest, there was not a clear, statistically significant association between contemporaneous children's urinary fluoride (CUFsg) and IQ either unadjusted or adjusting for MUFcr.	
Chauhan 2017 [15]				
Study design: Abstract (design not reported) **Country:** India **Participants:** Adult fluorosis patients **Sample size (N):** 100 **Sex:** Men (100%) **Funding/support:** NR **Author declaration of interest:** NR	**Exposure:** • Fluoride Exposure level: • NR **Outcomes:** • Semen morphological parameters • Hypothalamic-testicular axis hormones (LH, FSH, prolactin, testosterone) • Oxidative stress markers	• LH, FSH, testosterone and prolactin values were significantly ($p<0.05$) altered in fluoride exposed population. • Increased lipid peroxidation and Protein carbonyl content and decreased antioxidant status i.e., SOD, CAT, GPx and GSH was observed. • Sperm count, motility and viability was delineated in exposed population.	This study suggests that hypothalamic testicular axis hormones and oxidative stress parameters can be useful as early markers for determination of disease fluorosis in population those residing in high fluoride regions.	N/A
Stephenson 2017 [16]				
Study design: Abstract (design not reported) **Country:** US **Participants:** NR **Sampling time frame:** 2010, 2012, 2014 **Sample size (N):** 201 **Sex:** NR **Funding/support:** USTAR **Author declaration of interest:** NR	**Exposure:** • Fluoridated water Exposure level: • NR **Outcomes:** • Suicide rates	Relationship between fluoridated water and suicide rates: ○ *2010: r= -0.386; p= 0.05* ○ *2012: r= -0.324; p= 0.020* ○ *2014: r= -0.342; p= 0.014*	"These results suggest that … fluoridation may be correlated with a decrease in the rate of suicide by reducing the levels of microorganisms found in drinking water."	N/A
Verma 2017 [72]				

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Study design: Cross-sectional **Country:** India **Participants:** High school adolescents (12–17 years) from randomly selected government and private schools in urban and rural areas of Kolar taluka (6 villages). All students who were residents of the area since birth were included in the study. **Sampling time frame:** February - August 2013 **Sample size:** 1,026 **Sex:** Boys: 49.6% **Source of funding:** None **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in ground water Exposure level: Mean water fluoride: • Holur: 0.85 mg/L. • Other 5 villages: ≥1.2 mg/L • All 6 villages: 1.4 ±0.38 **Outcome(s):** Dental fluorosis	Karl Pearson correlation coefficient (all 6 villages) • Mean fluoride level in water: 1.4 mg/L ± 0.38 • Community fluorosis index: 2.3 ± 0.37 Multivariable regression analysis (GEE) by drinking water source: • Fluorosis present: ○ Bore well water: 551 (63.7%) ○ Pipe/tape water: 79 (64.8%) • Total: ○ Bore well water: 865 ○ Pipe/tape water:122 • β estimate (95%CI): ○ Bore well water: 0.92(−0.32,2.16), p-value: 0.145 ○ Pipe/tape water: 0	• "Prevalence of dental fluorosis was considerably high, affecting nearly two-thirds of the students, and mainly in government schools and long-term residents of the area."	1
Cardenas-Gonzalez 2016 [73]				
Study design: Cross-sectional **Country:** Mexico **Participants:** 5-12 years old students (grades 1-6) **Sampling time frame:** 2014 **Sample size (N):** 83 **Sex:** Boys: 56.63% **Funding/support:** National Council on Science and Technology, NIH/NIEHS, Harvard-NIEHS Centre for Environmental Health, HSPH-NIEHS **Author declaration of interest:** No COI	**Exposures:** Fluoride levels in • Urine samples • Drinking water samples Exposure level: • Mean (range) tap water fluoride (ppm) ○ 2.47 (2.08 - 2.94) • Mean (range) urinary fluoride (ppm) ○ 2.18 (0.34 - 8.60) **Outcome(s):** Kidney injury biomarkers • Kidney injury molecule 1 (KIM-1) • Neutrophil gelatinase-associated lipocalin (NGAL)	**Tap water fluoride, mean (range)** ○ *2.47 ppm (2.08 - 2.94)* **Urinary fluoride, mean (range)** ○ *2.18 ppm (0.34 - 8.60)* • Correlation between urinary levels of fluoride (ppm) and kidney injury biomarkers: ○ *KIM-1 (pg/mL): r = 0.09; p = 0.38* ○ *NGAL (ng/mL): r = -0.2; p = 0.07* ○ *miR-21 (copies/µl): r = 0.05; p = 0.67* ○ *miR-200c (copies/µl): r = 0.27; p = 0.01* ○ *miR-423 (copies/µl): r = 0.14; p 0.22* ○ *SCr (mg/dL): r = 0.07; p = 0.53* ○ *eGFR (mL/min): r = - 0.19; p = 0.07* ○ *ACR (mg/gCr): r = 0.08; p = 0.45*	• "The correlation of … fluoride levels between urine and water samples was significant … suggesting that water is the main source of fluoride exposure." • "Urinary miR-200c was correlated with … fluoride ... There was no correlation between any of the other biomarkers and toxicants exposure levels." • "Regression models examining the association between urine … fluoride … and the kidney injury biomarkers did not show any statistically significant	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
	• Serum creatinine (SCr) • MircroRNAs (miRNAs): miR-21, miR200c, and miR-423 • Estimated glomerular filtration rate (eGFR) • Albumin-creatinine ratio (ACR)	• No statistically significant differences reported between fluoride levels in urine and outcome biomarkers	differences (data not shown)."	
de Moura 2016 [74]				
Study design: Cross-sectional **Country:** Brazil **Participants:** 11 to 14-year-old school children with fully erupted permanent teeth, signed informed consent, and completed socio-demographic questionnaire. **Sampling time frame:** 2011 **Sample size:** 571 **Sex:** NR **Source of funding:** NR **Author declaration of interest:** NR	**Exposures:** Fluoride levels in • Water Exposure level: • 0.6-0.8 ppm (as reported by the same author in in earlier study (Moura et al. 2010), for the same city of residence of the study participants **Outcome(s):** • Dental fluorosis	• The prevalence of fluorosis was 77.9% (N= 445). • 12.1% (N= 69) of all participants had fluorosis of TF3, and 0.4% of TF4 and TF5 (n=2). • Of the participants with higher severity of fluorosis: ○ *98.6% (N= 70) belonged to the lowest social class (≥ B2),* ○ *91.5% were born and always lived in Teresina,* ○ *94.4% consumed fluoridated water supply* ○ *76% used infant toothpaste* ○ *64% reported swallowing this toothpaste*	"The prevalence of fluorosis was high, though the severity was low in individuals exposed to fluoridation since birth."	2
Heck 2016 [75]				
Study design: Cross-sectional **Country:** US **Participants:** Non-institutionalized children (14 to 15 years old) and adults (17 to 90 years old); NHANES III **Sampling time frame:** NR **Sample size (N):** > 500,000 **Sex:** NR **Funding/support:** NR **Author declaration of interest:** NR	**Exposures:** • Fluoridated water Exposure level: • NR **Outcomes:** • Trouble working • Retardation • General health	**Change in outcome from the effect of residential optimal water fluoridation among children** ○ *Trouble working: b = 0.039 (0.039)* ○ *Retardation: b = 0.001 (0.002)* ○ *General Health: b = -0.159 (0.165)* **Change in outcome from the effect of optimal water fluoridation among adults** ○ *Trouble working: b = 0.041 (0.043)* ○ *General health: b = -0.028 (0.143)*	No evidence of an effect of water fluoridation on general health, trouble working for children or adults, retardation in children.	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Kousik 2016 [76]				
Study design: Ecological **Country:** India **Participants:** Children (6 to 18 years of age) from Simlapal Block in Bankura District **Sampling time frame:** NR **Sample size (N):** 149 **Sex:** Boys: 44.3% **Source of funding/ support:** NR **Author declaration of interest:** NR	**Exposure:** Fluoride levels in • Urine samples • Ground water samples Exposure levels: • Mean (SD) levels of fluoride in water samples ○ 2.11 mg/L (1.64) • Levels of fluoride in urine samples ○ Min = 0.45 mg/L ○ Max = 17.00 mg/L **Outcomes:** • Body mass index (BMI) • Intelligence quotient (IQ)	**Exposure levels:** **Mean (SD) levels of fluoride** ○ *Water samples: 2.11 g/L (1.64)* ○ *Urine samples* *MiN= 0.45 mg/L, Max = 17.00 mg/L* **Results:** • Correlation between urinary fluoride and exposure dose: r = 0.513; p = <0.01 • Correlation between urinary fluoride and BMI: r = 0.022; p not <0.01 • Correlation between urinary fluoride and IQ: r = -0.751; p = <0.01 • Correlation between exposure dose and BMI: r = -0.083; p not < 0.01 • Exposure dose & IQ: *r = -0.343; p = < 0.01* • Exposure dose & BMI ○ 6-8 years old *Boys: BMI = 13.9 - 2.7 ED, r = 0.073, p = 0.832* *Girls: BMI = 13.3 + 29.3 ED, r = 0.092, p = 0.716* ○ 8-10 years old *Boys: BMI = 15.3 – 12.7 ED, r = 0.124, p = 0.451* *Girls: BMI = 14.1 – 5.69 ED, r = 0.144, p = 0.362* ○ >10 years old *Boys: BMI = 17.3 – 20.1 ED, r = 0.217, p = 0.371* *Girls: BMI = 14.3 + 3.63 ED, r = 0.133, p = 0.575*	• "The results also reveal that exposure dose has a positive correlation with … urinary fluoride (r=0.513, P < 0.01), a negative correlation with IQ (r = −0.343, P<0.01), and a non-significant correlation with BMI (r = 0.083)." • "[C]hildren residing in areas with higher than normal water fluoride level demonstrated more impaired development of intelligence"	2
Sabokseir 2016 [77]				
Study design: Cross-sectional **Country:** Iran	**Exposures:** Fluoride levels in	*Percentage of genuine fluorosis by exposure categories*	"Fluorosis indices, if used alone, could result in	1

Study	Exposure Outcome	Results	Authors' reported conclusions	Quality of evidence
Participants: Children (9 years of age) randomly selected from locations with high, optimal, and low fluoride drinking water levels in Fars **Sampling time frame:** NR **Sample size:** 376 **Sex:** Boys: 53% **Source of funding:** Vice-Chancellery for Research of Shiraz University of Medical Science **Author declaration of interest:** No COI	• Water <u>Exposure level:</u> Fluoride levels by town and category of exposure: • Gerash (high fluoride) ○ 2.12 – 2.85 ppm • Sepidan (low fluoride) ○ 0.24 – 0.29 ppm • Shiraz (optimal fluoride) ○ 0.62 – 1.22 ppm **Outcome(s):** Dental fluorosis	• High Water Fluoride: 47.7% • Optimal Water Fluoride: 20.6% • Low Water Fluoride: 3.3% • p-value: <0.001 • *Odds (95% CI) of genuine fluorosis with optimal compared to high fluoride levels:* • 0.292 (0.168 – 0.506) *Odds (95% CI) of genuine fluorosis with low compared to high fluoride levels:* • 0.037 (0.011 – 0.127)	misdiagnosis of dental fluorosis and misguide health policymakers in their decision about public health measure related to use of fluoride. Information about adverse health-related conditions linked to DDEs at specific positions on teeth could help to differentiate between genuine fluorosis and fluorosis-resembling defects." (p. 8)	
Xiang 2016 [78]				
Study design: Cross-sectional **Country:** China **Participants:** Children (8 – 14 years of age) from Wamiao and Xinhuai **Sampling time frame:** • 2002: before defluoridation • 2013: 10 years after defluoridation **Sample size (N):** <u>2002:</u> • Wamiao = 236 • Xinhuai = 290 <u>2013:</u> • Wamiao = 68 • Xinhuai = 65 **Sex:** • Wamiao in 2002: Men: 55.1% • Xinhuai in 2002: Men: 54.8% **Source of funding:** National Natural Science Foundation of China **Author declaration of interest:** No COI	**Exposures:** <u>Fluoride levels in</u> • Taps, • Deep wells • River sources <u>Exposure level:</u> Mean fluoride level in tap water (SD) in 2013 • Wamiao: 0.91 mg/L (0.02) • Xinhuai: 0.89 mg/L (0.03) **Outcome(s):** • Dental fluorosis • Defect dental fluorosis	• **"**The prevalence of dental fluorosis and defect dental fluorosis in 2002 had a significant positive dose–response correlation with the drinking water fluoride with the coefficient correlations, regression equations, and p values being r=0.999, y=99.552/(1+40.049×e–3.464x), and p=0.017; and r=0.987, y=17.520x – 6.950, and p=0.001, respectively." (p. 23) • "The prevalence of dental fluorosis and defect dental fluorosis were significantly decreased with the decreased drinking water fluoride in Wamiao in 2013 after defluoridation compared with the results in 2002." (p. 23)	"This study suggests that defluoridation of drinking water is effective for controlling endemic fluorosis in China and that the role of fluoridation of public water supplies for the of control dental caries needs to be further studied." (p. 23)	2

Assessment of quality of newly identified original human studies

The quality of included studies was assessed using the OHAT risk of bias tool [5] as summarized in Table 4. Fifty-one percent of studies (n=45) were of high quality, compared to forty-seven percent that were of acceptable quality (n=42). Detailed assessment for individual studies is included in Section 3 of the Supplementary Material.

Table 4: Quality assessment for included human studies using OHAT risk of bias tool

Study	Selection bias			Confounding bias	Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	Other sources of bias	Overall quality of evidence (Score)
	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were the comparison groups appropriate?	Did the study design or analysis account for important confounding and modifying variables?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Other potential threats to internal validity	
Mercado 2023 [396]	N/A	N/A	++	–	N/A	N/A	++	++	++	++	++	2
Tang 2023 [397]	N/A	N/A	+	++	N/A	N/A	++	++	++	++	++	2
Ahmad 2022 [398]	NA	NA	–	–	N/A	N/A	–	–	–	++	++	3
Feng 2022 [417]	N/A	N/A	++	++	N/A	N/A	++	++	–	++	++	2
García-Escobar 2022 [399]	NA	NA	+	–	NA	NA	++	++	++	++	++	2
Goodman 2022 [418]	N/A	N/A	+	++	N/A	N/A	++	++	++	++	++	1
Gupta 2022 [400]	N/A	N/A	++	–	N/A	N/A	++	++	– –	++	++	2
Ibarluzea 2022 [419]	NA	NA	++	++	NA	NA	++	++	++ ++	++	++	1
Kaur 2022 [401]	N/A	NA	NA	++	–	NA	NA	++	++	+	++	1

Study	Selection bias			Confounding bias	Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	Other sources of bias	Overall quality of evidence (Score)
	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were the comparison groups appropriate?	Did the study design or analysis account for important confounding and modifying variables?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Other potential threats to internal validity	
Marques 2022 [402]	NA	NA	++	++	NA	NA	++	++	++	++	++	1
McLaren 2022 [403]	NA	NA	++	++	NA	NA	++	+	++	++	++	1
Rani 2022 [404]	NA	NA	+	–	NA	NA	–	++	++	++	++	2
Saeed 2022 [405]	N/A	N/A	+	++	N/A	N/A	–	++	+ ++	++	++	2
Tawfik 2022 [406]	N/A	N/A	++	–	N/A	N/A	++	+	++	++	++	2
Thilakarathne 2022 [407]	NA	NA	+	–	NA	NA	++	++	++	++	++	2
Al-Omoush 2021 [17]	N/A	N/A	+	–	N/A	N/A	–	++	++	++	++	2
Ayele 2021 [18]	N/A	N/A	++	+	N/A	N/A	++	++	++ –	++	++	2
Cao 2021 [408]	N/A	N/A	++	–	N/A	N/A	–	++	++	++	++	2
Dong 2021 [19]	N/A	N/A	++	++	N/A	N/A	–	++	++	++	++	1
Du 2021 [20]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Farmus 2021 [409]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Fernandes 2021 [410]	NA	NA	++	–	NA	NA	++	++	++	++	++	2
Helte 2021 [21]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
James 2021 [22]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1

Study	Selection bias			Confounding bias	Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	Other sources of bias	Overall quality of evidence (Score)
	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were the comparison groups appropriate?	Did the study design or analysis account for important confounding and modifying variables?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Other potential threats to internal validity	
Meghe 2021 [23]	N/A	N/A	+	–	N/A	N/A	+	++	–	++	++	2
Meng 2021 [24]	N/A	N/A	++	–	N/A	N/A	–	++	++	++	++	2
Mohd Nor 2021 [25]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Rojanaworarit 2021 [411]	NA	NA	++	++	NA	NA	++	++	++	++	++	1
Sharma 2021 [26]	N/A	N/A	+	–	N/A	N/A	–	++	–	++	++	2
Silva 2021 [412]	N/A	N/A	+	++	N/A	N/A	++	++	++	++	++	1
Tkachenko 2021 [27]	N/A	N/A	+	–	N/A	N/A	++	+	++	++	++	2
Wang 2021 [413]	N/A	N/A	++	++	N/A	N/A	++	++	++ ++	++	++	1
Yani 2021 [414]	N/A	N/A	+	–	N/A	N/A	++	–	+ +	++	++	2
Yu 2021 [415]	N/A	N/A	++	++	N/A	N/A	++	++	+	++	++	1
Zhao 2021 [416]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Bai 2020 [28]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Cui 2020 [29]	N/A	N/A	++	–	N/A	N/A	++	++	+ ++ ++	++	++	2[25]

[25] *Cui 2020: Assessment of question 9 (Outcome assessment) was (+) for IQ and (++) for thyroid dysfunction and dopamine outcomes. However, the overall study assessment did not change due to this difference.*

Study	Selection bias			Confounding bias	Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	Other sources of bias	Overall quality of evidence (Score)
	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were the comparison groups appropriate?	Did the study design or analysis account for important confounding and modifying variables?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Other potential threats to internal validity	
Das 2020 [30]	N/A	N/A	++	–	N/A	N/A	–	++	++	++	++	2
Fernandes 2020 [31]	N/A	N/A	+	–	N/A	N/A	++	++	++	++	++	2
Godebo 2020 [32]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Kim 2020 [33]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Krishna 2020 [34]	N/A	N/A	++	+	N/A	N/A	++	++	++	++	++	1
Lee 2020 [35]	N/A	N/A	++	+	N/A	N/A	++	++	++	++	++	1
Nanayakkara 2020 [36]	N/A	N/A	+	–	N/A	N/A	–	++	++	++	++	2
Russ 2020 [37]	N/A	N/A	++	+	N/A	N/A	++	++	++	++	++	1
Stangvaltaite-Mouhat 2020 [38]	N/A	N/A	+	–	N/A	N/A	–	++	++	++	++	2
Sun 2020 [39]	N/A	N/A	+	++	N/A	N/A	++	++	++	++	++	1
Till 2020 [40]	N/A	N/A	++	++	N/A	N/A	++	+	++	++	+	1
Wang 2020 [41]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
An 2019 [42]	N/A	N/A	++	+	N/A	N/A	++	++	++	++	++	1
Crnosija 2019 [43]	N/A	N/A	++	– –	N/A	N/A	++	– –	++	++	++	2

Study	Selection bias			Confounding bias	Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	Other sources of bias	Overall quality of evidence (Score)
	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were the comparison groups appropriate?	Did the study design or analysis account for important confounding and modifying variables?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Other potential threats to internal validity	
Fernando 2019 [44]	N/A	N/A	–	– –	N/A	N/A	++	++	+	++	– –	2
Jimenez-Cordova 2019 [45]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Jimenez-Cordova 2019a [46]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Khanoranga 2019 [47]	N/A	N/A	++	–	N/A	N/A	–	++	++	++	++	2
Liu 2019 [48]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Malin 2019 [49]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Malin 2019a [50]	N/A	N/A	++	++	N/A	N/A	++	+	++	++	++	1
Pei 2019 [51]	N/A	N/A	+	– –	N/A	N/A	++	++	++	++	++	2
Riddell 2019 [52]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Shaik 2019 [53]	N/A	N/A	+	– –	N/A	N/A	++	++	++	++	++	2
Soto-barreras 2019 [54]	N/A	N/A	++	–	N/A	N/A	++	++	++	++	++	2
Zhang 2019 [55]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Zhou 2019 [56]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Zhou 2019a [57]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1

Study	Selection bias			Confounding bias	Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	Other sources of bias	Overall quality of evidence (Score)
	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were the comparison groups appropriate?	Did the study design or analysis account for important confounding and modifying variables?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Other potential threats to internal validity	
Bashash 2018 [58]	N/A	N/A	++	++	N/A	N/A	++	++	+ ++	++	++	1[26]
Cui 2018 [59]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Jimenez-Cordova 2018 [60]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Kumar, V 2018 [61]	N/A	N/A	+	–	N/A	N/A	++	++	++	++	++	2
Kumar, S 2018 [62]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Malin 2018 [63]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1
Mohd Nor 2018 [25]	N/A	N/A	++	–	N/A	N/A	++	++	++	++	++	2
Mustafa 2018 [64]	N/A	N/A	+	–	N/A	N/A	++	++	++	++	+	2
Oweis 2018 [65]	N/A	N/A	++	++	N/A	N/A	++	–	++	++	++	2
Quadri 2018 [66]	N/A	N/A	++	–	N/A	N/A	–	++	+	+	+	2
Rathore 2018 [67]	N/A	N/A	+	–	N/A	N/A	++	++	++	++	+	2
Shruthi 2018 [68]	N/A	N/A	++	–	N/A	N/A	++	++	–	++	++	2
Yu 2018 [69]	N/A	N/A	++	++	N/A	N/A	++	++	++	++	++	1

[26] *Bashash 2018: Assessment of question 9 (outcome assessment) was different for both outcomes. However, the overall study assessment did not change due to this difference.*

Study	Selection bias			Confounding bias	Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	Other sources of bias	Overall quality of evidence (Score)
	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were the comparison groups appropriate?	Did the study design or analysis account for important confounding and modifying variables?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Other potential threats to internal validity	
Arulkumar 2017 [70]	N/A	N/A	+	–	N/A	N/A	++	++	++	++	++	2
Bashash 2017 [71]	N/A	N/A	++	+	N/A	N/A	–	+	++	++	++	1
Chauhan 2017 [15]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Stephenson 2017 [16]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Verma 2017 [72]	N/A	N/A	++	++	N/A	N/A	–	++	++	++	++	1
Cardenas-Gonzalez 2016 [73]	N/A	N/A	++	++	N/A	N/A	++	++	++	+	++	1
de Moura 2016 [74]	N/A	N/A	++	–	N/A	N/A	++	–	++	++	++	2
Heck 2016 [75]	N/A	N/A	+	+	N/A	N/A	–	++	++	++	+	1
Kousik 2016 [76]	N/A	N/A	+	–	N/A	N/A	++	++	++	++	++	2
Sabokseir 2016 [77]	N/A	N/A	+	+	N/A	N/A	++	++	++	++	++	1
Xiang 2016 [78]	N/A	N/A	++	–	N/A	N/A	++	++	++	++	++	2

Assessment criteria

Key criterion *Other (non-key) criterion*

Level of bias

DL: *definitely low risk of bias* ++ ***PL:*** *probably low risk of bias* + ***PH:*** *probably high risk of bias* – ***DH:*** *definitely high risk of bias* – –

Newly identified reviews of human studies

While a total of five published original reviews of the fluoride scientific literature passed the three-question screening criteria for further consideration, none were ultimately of sufficient methodological rigor or comprehensive to be considered as updates of the CADTH or NTP reviews. A brief summary of the assessment and the authors' reported conclusions are shown in Table 5.

Table 5: Summary of eligible reviews of human evidence[27]

Reference (Study Design)	Comprehensiveness	Methodological Rigor	Summary of Author-reported conclusions
Grandjean 2019 [79] *(Literature Review)*	**Time Period Covered by Search** • Restricted to most recent 10 years **Sources Searched** • Electronic databases (PubMed) • Grey literature (Yes) • Reference list of articles (Yes) **Number of References Included** • N= 14 cross-sectional studies • N= 5 prospective studies • N= 2 retrospective studies	**Was screening conducted by two independent reviewers?** • NR **Was data abstraction conducted by two independent investigators?** • NR **Was quality assessment conducted by two independent investigators?** • NR	The recent epidemiological results support the notion that elevated fluoride intake during early development can result in IQ deficits that may be considerable. Recognition of neurotoxic risks is necessary when determining the safety of fluoride-contaminated drinking water and fluoride uses for preventive dentistry purposes.
Saeed 2019 [80] *(Literature Review)*	**Time Period Covered by Search** • 1989 to 2019 **Sources Searched** • Electronic databases (PubMed, Environmental Health Perspectives, MEDLINE, Google	**Was screening conducted by two independent reviewers?** • NR **Was data abstraction conducted by two independent investigators?**	The significant downsides of fluoride outweigh its benefits for dental problems across many endemic areas of the world. The findings in past literature studies are alarming and the seriousness of this debate urges expediting policymaking and awareness campaigns for public safety.

[27] Information and data in this table was taken directly from the original publications

Reference (Study Design)	Comprehensiveness	Methodological Rigor	Summary of Author-reported conclusions
	Scholar, Fluoride Action Network, Elsevier, and Springer) • Grey literature (NR) • Reference list of articles (NR) **Number of References Included** N= 57	• NR **Was quality assessment conducted by two independent investigators?** • NA	The current review highlights some gaps in past literature, needing to be bridged in future research on fluoride toxicity among the human population. The review also prompts the need for more research work on school-going children to curb the rise of dental and skeletal fluorosis and mental disabilities related to early childhood exposure to fluoride in developing countries.
Chaitanya 2018 [81] *(Systematic Review)*	**Time Period Covered by Search** • January 1981 to November 2015 **Sources Searched** • Electronic databases (PubMed, Medline, Embase, Cochrane Library, EBSCO) • Grey literature (NR) • Reference list of articles (NR) **Number of references included** • N= 10	**Was screening conducted by two independent reviewers?** • No **Was data abstraction conducted by two independent investigators?** • No **Was quality assessment conducted by two independent investigators?** • NA	The present systematic review suggests a positive correlation between excess fluoride and hypothyroidism. This calls the need for further well-controlled studies in this otherwise emerging alarming issue. It also calls for considerable community network through health informatics for problem sensitization.
Duan 2018 [82] *(Meta-analysis)*	**Time Period Covered by Search** • Electronic databases searched throughout November 2016 **Sources Searched** • Electronic databases (PubMed, Embase, and Cochrane Library) • Grey literature (NR) • Reference list of articles	**Was screening conducted by two independent reviewers?** • Yes **Was data abstraction conducted by two independent investigators?** • Yes **Was quality assessment conducted by two independent investigators?** • Yes	Greater exposure to high levels of fluoride in water was significantly associated with reduced levels of intelligence in children. Therefore, water quality and exposure to fluoride in water should be controlled in areas with high fluoride levels in water.

Reference (Study Design)	Comprehensiveness	Methodological Rigor	Summary of Author-reported conclusions
	(Yes) **Number of References Included** N= 26		
PHO 2018 [83] *(Literature Review)*	**Time Period Covered by Search** • January 1, 2009 – May 10, 2017 **Sources Searched** • Electronic databases (Ovid MEDLINE, Embase, CINAHL, and Dentistry) • Grey literature (Yes) • Reference list of articles (NR) **Number of References Included** • N= 29 articles (Systematic reviews N= 2; cross-sectional studies N= 20; prospective cohort studies N= 5; case-control studies N= 2) • N= 6 documents from grey literature search	**Was screening conducted by two independent reviewers?** • NR **Was data abstraction conducted by two independent reviewers?** • NR **Was quality assessment conducted by two independent investigators?** • NR	• Outcomes include dental fluorosis, enamel opacities, hypo-mineralization, and bone health, cancers including bone cancers, reproductive, neurobehavioral effects, mutagenicity, hypothyroidism, and urolithiasis. • Overall, the existing literature suggests that at an optimal concentration of water fluoridation, the only adverse health consequence observed is a mild form of dental fluorosis. • The 2010 Health Canada fluoride document states that there is no evidence to support a link between exposure to fluoride in drinking water at or below 1.5 mg/L and any adverse health effects such as any types of cancer, developmental defects, neurobehavioral effects, or genotoxicity. The studies conducted and the organizational reports published after the 2010 Health Canada fluoride document and until May 10, 2017 corroborate these findings.

NR = Not Reported; NA = Not Applicable

Animal evidence

The search strategy resulted in retrieval of 2,119 non-duplicate records from bibliographic databases. Upon excluding 1,628 irrelevant studies during title and abstract screening, there were 446 studies left for full-text examination. One hundred and ninety-nine original animal studies were finally retained for data abstraction and detailed analysis. A detailed PRISMA flow diagram [14] showing the selection process for human studies is shown in Figure 3. A summary of studies excluded at levels 1 and 2, grouped by reason of exclusion is shown in Table 6, and detailed in full in Section 4 of the Supplementary Material.

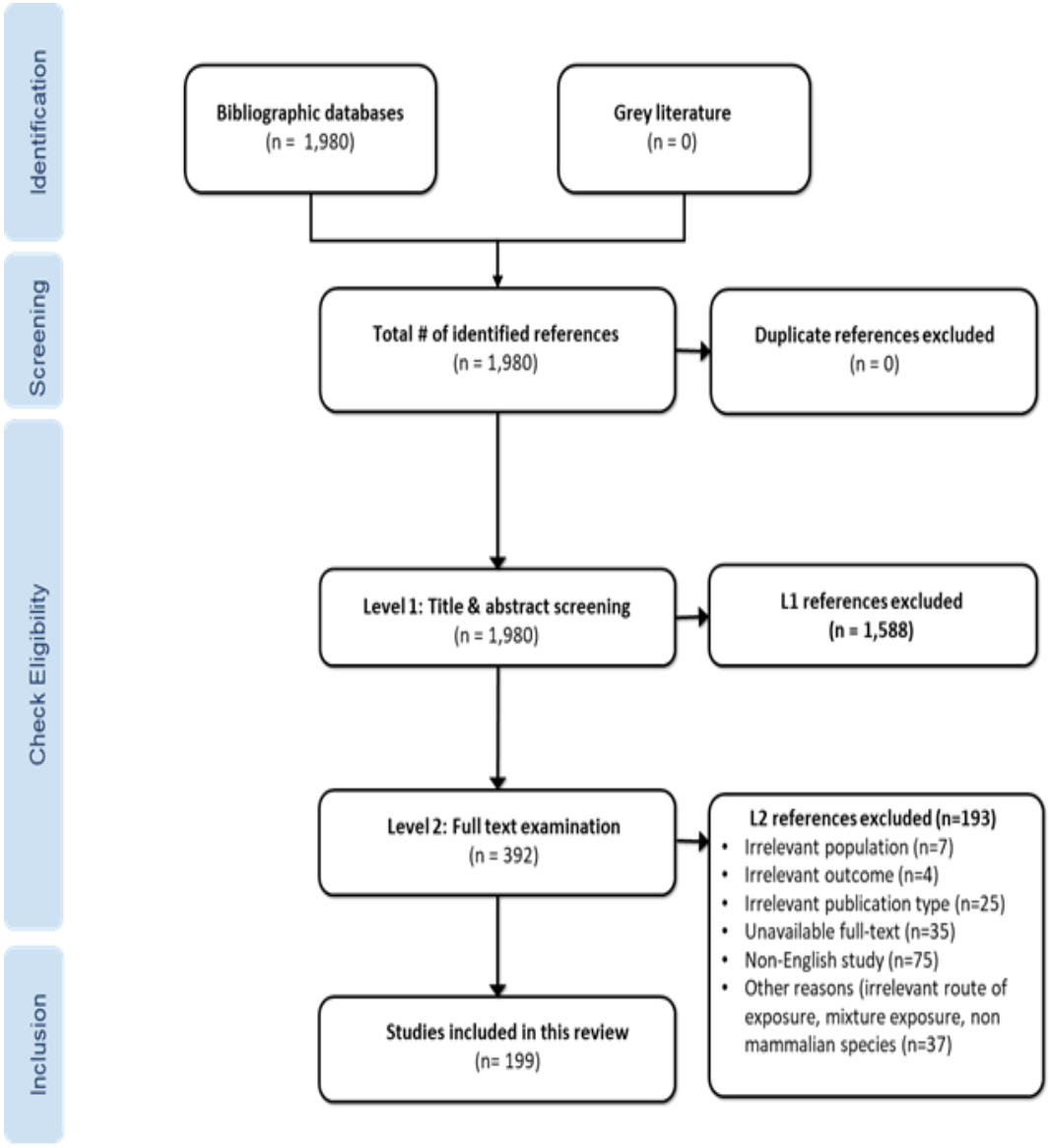

Figure 3: PRISMA flow diagram for animal studies

Table 6: Animal studies excluded at levels 1 and 2 by exclusion reason/group

Level	Exclusion group	Reason for exclusion	# of studies
L1	Ineligible	One or more exclusion criteria	1,628
L2	Language	Non-English publication	76
	Duplicate	Duplicates	7
	Unavailable full-text	No pdf available	37
	Irrelevant outcome	Outcome out of scope	8
	Irrelevant population	Human subjects	5
		In vitro models (e.g., mammalian cells/ tissues, bacterial cells, plant cells)	3
	Irrelevant publication type	Non-systematic reviews	11
		Commentary/communication/editorial/letter/ conference abstract/poster/presentation	16
	Other exclusion reasons	Including route of exposure other than drinking water, mixture exposure, non-mammalian species	43

Data abstraction and prioritization of animal Studies

After screening, based on title, abstract and full-text reading, of 2,119 articles, 199 non-human mammalian studies were found eligible for further consideration. Table 7 shows the list of animal studies by outcome. This data covers most of the primary outcomes with the largest body of evidence on neurotoxicity and developmental/ reproductive toxicity. To handle the large volume of animal data, following similar approaches by US NTP and EU REACH, a tiered approach was implemented where only a subset (tier-1) of eligible studies would be considered for complete data abstraction, and the data from remaining studies (tier 2 and below) were generally not extracted but used as supporting information.

This criteria were designed to give preference to studies with exposure doses relevant to humans (≤20 ppm), studies that provide understanding of the dose-response relationship (more than single dose), and those that were not included in another authoritative review, as well as guideline studies; lower tier studies include those where the primary focus was protection against or interaction with fluoride toxicity or mechanism of action (e.g. oxidative stress), or were single exposure dose studies.

Table 7: List of animal studies retained for qualitative analysis, by endpoints

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
	n= 0	n= 34	n= 59	n= 46	n= 13	n= 18	n= 7	n= 1	n= 18	n= 11	n= 15	n= 27	n= 5	n= 47
Adedara 2017 [84]					✓							✓		
Ahmad 2012 [85]				✓										
Akimov 2020 [86]														Oxidative stress
Al-Sabaawy 2020 [87]				✓										
Ali 2019 [88]				✓										
Altindag 2021 [89]				✓										
Altintas 2010 [90]														Oxidative stress
Baba 2016 [91]												✓		
Balaha 2021 [92]													✓	
Balaji 2015 [93]			✓											
Bartos 2018 [94]				✓										
Basha 2013 [95]												✓		
Basha 2011 [96]			✓		✓									
Basha 2011 [97]														Oxidative stress
Basha 2012 [98]														Oxidative stress

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Bataineh 2006 [99]														
Bharti 2011 [100]														Oxidative stress
Bharti 2011 [101]														Oxidative stress
Birkner 2006 [102]												✓		Oxidative stress
Blaszczyk 2012 [103]		✓												
Blaszczyk 2009 [104]														Oxidative stress
Bondu 2017 [105]		✓												
Bondu 2019 [106]		✓												
Bouaziz 2007 [107]														Oxidative stress
Bulduk 2020 [108]							✓							
Cao 2016 [109]				✓										
Cao 2019 [110]			✓											
Cao 2021 [111]			✓											
Cárdenas-González 2013 [112]												✓		
Cenesiz 2008 [113]										✓				
Chaithra 2019 [114]				✓										
Chaithra 2019 [115]				✓										

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Chattopadhyay 2011 [116]									✓			✓		
Chaudhary 2010 [117]														metabolism
Chen 2013 [118]		✓												
Cheng 2008 [119]		✓												
Choudhary 2020 [120]		✓		✓										
Chiba 2010 [121]						✓								
Chiba 2015 [122]						✓								
Chiba 2019 [123]						✓								Metabolism
Chioca 2008 [124]			✓											
Chouhan 2013 [125]														Oxidative stress
Chu 2020 [126]		✓												
Das 2006a [127]				✓										
Das 2006b [128]										✓				
de Cássia Alves Nunes 2016 [129]		✓				✓								
Dec 2018 [130]									✓					Oxidative stress
Dec 2019 [131]			✓											
Dey 2021 [132]		✓												
Dhurvey 2016 [133]				✓										

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Dong 2015 [134]			✓											
Dong 2017 [135]			✓											
Faruk 2021 [136]					✓									
Feng 2012 [137]														Oxidative stress
Ferreira 2021 [138]														Oxidative stress, gene expression
Foda 2021 [139]					✓									
Gao 2009 [140]			✓											
Garcia-Montalvo 2009 [141]						✓								
Ge 2018 [142]			✓											
Geng 2014 [143]				✓										Oxidative stress
Grucka-Mamczar 2009 [144]														Oxidative stress
Gupta 2016 [145]		✓												
Gupta 2015 [146]		✓												
Gutierrez-Salinas 2010 [147]										✓				
Han 2014 [148]			✓											
Hosokawa 2010 [149]												✓		

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Hosokawa 2016 [150]		✓												
Hosokawa 2015 [151]										✓				
Hu 2012 [152]						✓								
Inkielewicz-Stepniak 2012 [153]														Oxidative stress
Interlandi 2018 [154]				✓										
Izquierdo-Vega 2008 [155]				✓										
Jaiswal 2020 [156]			✓											Oxidative stress
Jana 2018 [157]				✓		✓				✓				
Jetti 2016 [158]			✓											
Jiang 2014 [159]				✓										
Jiang 2014 [160]			✓											
Kanagaraj 2015 [161]									✓					
Kanbur 2009 [162]														Oxidative stress
Kant 2010 [163]														Blood biochemistry
Karadeniz 2008 [164]														Blood biochemistry
Kaya 2012 [165]		✓												
Khan 2019 [166]									✓					

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Khandare 2011 [167]														
Khandare 2007 [168]						✓								
Kido 2017 [169]												✓		
Kido 2017 [170]												✓		
Kivrak 2012 [171]			✓											
Kobayashi 2014 [172]		✓												
Kobayashi 2011 [173]														Urinary analysis
Kobayashi 2009 [174]												✓		
Krishnamoorthy 2015 [175]										✓				
Kuang 2017 [176]										✓				
Leite Ade 2007 [177]											✓			
Li 2017 [178]		✓												
Li 2019 [179]			✓											
Li 2021a [180]		✓	✓	✓					✓			✓	✓	
Li 2021b [181]		✓								✓				
Liang 2020 [182]				✓										
Liang 2020 [183]				✓										
Lima Leite 2014 [184]						✓								

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Liu 2014 [185]			✓											
Liu 2012 [186]					✓									
Liu 2016 [187]					✓									
Liu 2008 [188]				✓										
Liu 2019 [189]										✓	✓			
Liu 2015 [190]				✓										
Liu 2010 [191]			✓											
Liu 2020 [192]														Protein expression
Liu 2021 [193]				✓										
Lobo 2015 [194]						✓								
Lombarte 2016 [195]						✓								
Lopes 2020 [196]			✓											
Lou 2013 [197]			✓											
Lu 2014 [198]				✓										
Łukomska 2020 [199]			✓											mRNA and protein expression
Lupo 2011 [200]						✓								
Ma 2020 [201]		✓												

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Madhusudhan 2009 [202]				✓										
Mahaboob Basha 2013 [203]			✓											
Mahaboob Basha 2013 [204]												✓		
Malvezzi 2019 [205]						✓								
Mandic 2020 [206]			✓						✓		✓			
Martin-Pardillos 2014 [207]		✓												
McPherson 2018 [208]			✓		✓									
Miao 2013 [209]									✓					
Min 2021 [210]				✓										Protein, RNA expression
Mohamed 2016 [211]														Oxidative stress
Mrvelj 2020 [212]					✓									
Mujahid 2015 [213]							✓							
Nabavi 2013 [214]												✓		
Nadei 2019 [215]			✓											
Nageshwar 2018 [216]			✓											
Niu 2009 [217]			✓											
Nkpaa 2018 [218]			✓											
Oka 2020 [219]														Autophagy

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Ola-Davies 2018 [220]									✓			✓		hypertension
Omóbòwálé 2018 [221]							✓							
Oncu 2006 [222]								✓						lipid
Oncu 2007 [223]				✓							✓			
Oner 2020 [224]									✓					
Owumi 2019 [225]									✓			✓		
Oyagbemi 2018 [226]							✓							hypertension
Oyagbemi 2018 [227]							✓							
Oyagbemi 2021 [228]				✓			✓				✓			Oxidative stress
Pei 2017 [229]		✓												
Pereira 2011 [230]			✓											
Pereira 2017 [231]		✓				✓								
Perera 2018 [232]									✓			✓		
Podder 2011 [233]											✓			
Podder 2008 [234]											✓			
Puranik 2015 [235]					✓									
Qing-Feng 2019 [236]			✓											
Radovanovic 2021 [237]			✓						✓	✓	✓			
Raju 2019 [238]			✓											

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Raju 2020 [239]									✓			✓		
Ran 2021 [240]			✓											Proteomics, dental fluorosis
Ranjan 2009 [241]									✓			✓		Oxidative stress
Ray 2020 [242]				✓										Oxidative stress
Reddy 2014 [243]														Oxidative stress
Sakallioglu 2014 [244]		✓												
Sanchez-Gutierrez 2019 [245]				✓										
Sarkar 2014 [246]					✓									
Shalini 2015 [247]			✓											
Shankar 2013 [248]		✓												
Shankar 2021 [249]		✓												Protein expression, serum biochemistry
Sharma 2018 [250]			✓											
Sharma 2019 [251]				✓										
Sharma 2021 [252]														Bodyweight
Sharma 2021 [253]		✓	✓											Antioxidants,

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
														blood biochemistry
Shashi 2017 [254]				✓							✓			
Saumya 2017 [255]				✓										
Song 2014 [256]											✓	✓		
Song 2013 [257]									✓			✓		
Song 2011 [258]		✓												
Stawiarska-Pieta 2012 [259]									✓					
Stawiarska-Pieta 2009 [260]														Oxidative stress
Sudhakar 2018 [261]			✓											
Sun 2010 [262]			✓											
Sun 2014 [263]											✓			
Sun 2009 [264]				✓										
Sun 2020 [265]			✓										✓	Antioxidants, gene expression, stool bacteria
Teng 2018 [266]			✓											
Tian 2019 [267]				✓										

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Tian 2019 [268]												✓		
Trivedi 2012 [269]			✓											
Turkekul 2020 [270]		✓												
Usuda 2016 [271]									✓			✓		
Validandi 2017 [272]						✓								
Vasant 2010 [273]						✓								
Vasant 2012 [274]						✓								
Vasant 2011 [275]						✓								
Wan 2006 [276]				✓										
Wang 2018 [277]				✓										
Wang 2009 [278]					✓									
Wang 2019 [279]													✓	
Wang 2017 [280]				✓										
Wang 2021 [281]			✓											Gene expression
Wasana 2015 [282]												✓		
Wei 2018 [283]			✓											
Wei 2016a [284]				✓							✓			
Wei 2016b [285]														biomarkers of fluorosis

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Whitford 2009 [286]			✓											
Wu 2008 [287]				✓										
Wu 2019 [288]				✓							✓			
Xin 2020 [289]			✓											Serum biochemistry, mRNA expression
Xin 2021 [290]			✓										✓	Serum biochemistry, mRNA expression, gut flora changes
Xu 2007 [291]												✓		
Xu 2010 [292]					✓									
Yan 2007 [293]		✓												
Yan 2011 [294]		✓												
Yan 2016 [295]			✓											
Yang 2015 [296]		✓												
Yang 2013 [297]												✓		
Yao 2019 [298]		✓												
Yildirim 2018 [299]							✓		✓			✓		

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
Yildiz 2006 [300]		✓												
Yu 2013 [301]		✓												
Yu 2019 [302]			✓											
Yue 2020 [303]														Metabolism
Zhang 2020 [304]			✓											
Zhang 2013 [305]			✓											
Zhang 2012 [306]										✓				
Zhang 2016 [307]				✓										
Zhang 2013 [308]			✓											
Zhang 2011 [309]			✓											
Zhang 2017 [310]				✓							✓			
Zhang 2015 [311]			✓											
Rui 2017 [312]												✓		
Zhang 2013 [313]				✓										
Zhang 2008 [314]			✓											
Zhao 2017 [315]				✓										
Zhao 2018 [316]				✓										
Zhao 2019 [317]			✓											
Zhao 2021 [318]		✓												Blood biochemistry,

Study	Cancer	Bone / Skeletal	Neuro/ Cognitive	Developmental/ Reproductive	Endocrine including thyroid	Diabetes or Glucose or Lipid Metabolism	Cardiovascular	Respiratory	Hepatic	Immunotoxicity	Genotoxicity	Renal/ Kidney	Intestinal/ GIT	Others
														urine fluoride levels, mRNA expression
Zheng 2016 [319]			✓											
Zhou 2013 [320]				✓										
Zhu 2014 [321]											✓			
Zhu 2011 [322]			✓											
Zigui 2017 [323]			✓											

Characteristics of included animal studies

A total of 35 tier-1 and 55 tier-2 studies were included in examining (and updating) the evidence of fluoride induced adverse health effects in experimental animals. Information on all primary endpoints was extracted from each study, and only tier-1 studies were assessed for study quality. Across all endpoints examined, excluding neurological outcomes, the largest amount of data was on reproductive outcomes. A comprehensive summary of important study characteristics of all tier 1 and tier 2 studies included in the current review is provided in Table 8 and Table 9.

Table 8: Study characteristics and results of included tier-1 animal studies

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
Reproductive toxicity				
Cao 2016 [109]				
Oral (drinking water), subchronic mice study • 8- weeks-old Kunming mice, males only • 10 animals per group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 2, 4, 8 mg/kg bw/day** (0, 11, 22, 44 mg F/ L) • Vehicle - drinking water • 11 weeks of exposure Outcomes assessed • **Reproductive toxicity** • Specific outcomes: - Organ weights (femur, epididymis, testis) - Histological examination of testis - Testosterone concentration in blood and testis - Sperm count - Expression levels of spermatogenesis related	D-R relationship: **increase in all reproductive endpoints assessed with increase in NaF concentration** • Results: - Overall growth: animals in all NaF-treated groups showed poor development, rough coats and even rough teeth with dark brown stains - Bone F levels: significantly increased in all treatment groups - Sperm quality: significant decrease in sperm count and significant increase in the deformity ratio of sperm and sperm head of higher treatment groups (50 and 100 mg/L). - Testis histology: tissues of all treated mice showed a few vacuoles in seminiferous tubules, irregular arrangement and decreased layers of spermatogenic cells with most obvious damage in 100 mg/L NaF that includes abnormal arrangement and morphological malformations of spermatogenic cells, and decreased number of sperm in	"NaF did have toxic effects on male reproductive system, which reduced the testosterone content and sperm number, and increased the abnormality ratio of sperm and sperm head, supported by the damages of the testicular structure, as a consequence of depressed HSF2 level, which resulted in the downregulation of Ssty2 and Sly mRNA and protein."	1

[28] When not reported by authors, exposure concentrations (mg/L or ppm fluoride) are converted into doses (indicated with an asterisk '*') using the following default conversion factors, as recommended by Health Canada (1994) 324. Canada, H., *Human health risk assessment for priority substances. Ottawa, ON: Ministry of Health*. 1994, Health Canada.: 1 ppm or mg/L chemical in water equals to 0.14 mg/kg bw/day in rats or 0.20 mg/kg bw/day in mice.

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
	genes (qPCR) and proteins (ELISA)	the lumen; overall histological examination indicated aggravated testicular tissue damage in all treatment groups. - Testosterone levels in serum and testis: both levels were significantly decreased in higher treatment groups (50 and 100 mg/L). - Gene and Protein expression: the mRNA expressions of spermiogenesis specific genes (Ssty2, Sly, HSF2) corresponding protein levels were changed markedly in higher treatment groups (50 and 100 mg/L).		
Chaithra 2019 [114]				
Oral (gavage), subchronic rat study • Adult Wistar rats, males only • 5 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • 0, 0.45, 2.26, 4.5 mg/kg bw/day • Vehicle - de-ionized water • 52 days of exposure Outcomes assessed • **Reproductive toxicity** • Specific outcomes: - Sperm count, motility, and abnormality - Activity of testicular 3β-hydroxysteroid dehydrogenase (3β-HSDH) - Testosterone concentration in serum - Histology of testis (germ cell count in spermatogenesis) - Activity of oxidative stress markers (superoxide dismutase SOD, catalase CAT and malondialdehyde MDA)	D-R relationship: **increase in several reproductive parameters with increase in dose** • Results: - Body or tissues weights: significant decrease in testis, epididymis and seminal vesicle tissue weight (relative) of 5 and 10 mg/kg bw groups; and decrease in % body weight gain in all treated animals - Sperm count: significant reduction in sperm count and increase in abnormal spermatozoa of all treated animals - Testosterone levels: serum testosterone levels were significantly reduced with increase in NaF dose; 3β-HSDH levels were significantly reduced in 5 and 10 mg/kg bw/day groups. - Histology: distorted & shrunken seminiferous tubules and loss of spermatogonial cells with increased severity (complete loss) in highest dosed animals. A significant dose-dependent decrease in counts of various germ cell types of spermatogenesis. - Antioxidant enzymes: significant dose-dependent reduction in testicular SOD and CAT enzymatic levels; significant increase in testicular MDA levels of 5 and 10 mg/kg bw/day groups	"Exposure to F-contaminated groundwater affects spermatozoa, steroidogenesis, and spermatogenesis by inducing oxidative stress. The alterations induced by NaF on the male reproductive system are dose-dependent and increased concentration of NaF causes severe damage to the system. Further, the study reveals that F-induced alterations in reproductive system are reversible."	2
Chaithra 2019 [115]				
Oral (drinking water and gavage) reproductive/ developmental toxicity rat study	Exposure • Sodium fluoride (NaF) • **0, 0.7*, 10 mg/kg bw/day** • Vehicle – deionized water • 52 days of exposure	D-R relationship: **significant increase in several reproductive parameters at both doses tested** • Results:	"F exposure affected the reproductive performances of male rats. The present study further revealed the	2

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
• Adult Wistar rats, males only • 25 animals/ group, 3 groups	Outcomes assessed • **Reproductive toxicity** • Specific outcomes: - Sperm Parameters - Serum Concentration of Testosterone - Histology of Testis - Fertility indices - Number of pups delivered	- Sperm motility: significant decrease in 10 mg/kg bw and 5 mg/L group - Sperm abnormality: significant increase in NaF 10 mg/kg bw and 5 mg/L group - Serum testosterone concentration: significant decrease in 10 mg/kg bw and 5 mg/L group - Histology of the Testis: distorted and shrunken seminiferous tubules with loss of different stages of spermatogonial cells and Leydig cells, especially in the 5 mg/L group - Fertility indexes: significant decrease in 10 mg/kg bw group - Number of pups delivered: significant decrease in 10 mg/kg bw and 5 mg/L group	fact that F-induced decline in testosterone levels, reduced sperm motility, and loss of spermatogonial cells affected the reproductive performances of male rats."	
Liang 2020a [182]				
Oral (drinking water) subchronic mice study • 8-weeks old C57BL-6 mice, males only • 10 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 2.2, 4.5, 9 mg/kg bw/day*** (0, 25, 50, 100 mg/L NaF) • Vehicle – deionized water • 90 days of exposure Outcomes assessed • **Reproductive toxicity** • Specific outcomes: - Mitochondrial structural impairment and mitophagy in mice testes - Expressions of mitophagy key proteins PHB2 and PINK1 in mice testes	D-R relationship: **higher F doses induced mitochondrial impairment and mitophagy in testicular cells** • Results: - The altered mitochondrial structures in various degrees were observed either in germ cells or Sertoli cells in NaF treated groups. - In spermatogenic cells, the mitochondrial cristae and the membranes of mitochondrion disintegrated in all NaF-treated groups.	"Fluoride can induce mitochondrial impairment and mitophagy in testicular cells, especially in Leydig cells, and PINK1/Parkin mediated mitophagy participants in this process, which will contribute to the mechanisms of F-induced male reproductive toxicity."	2

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
		- The expressions of PHB2, both in mRNA and protein levels, were increased significantly in testes, especially in the Leydig cells from fluoride-treated groups. - The mRNA expressions of PINK1 increased significantly in the 2.4 mM NaF group. PINK1 protein levels in the 1.2 and 2.4 mM NaF groups with a dose-dependent manner.		
Liang 2020b [183]				
Oral (drinking water) subchronic mice study • ICR mice, males only • 10 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 2.2, 4.5, 9 mg/kg bw/day*** (0, 25, 50, 100 mg/L NaF) • Vehicle – deionized water • 8 weeks of exposure Outcomes assessed • **Reproductive toxicity** • Specific outcomes: - testicular morphology - ultra-structure of the sperm - genes expressions of spermatozoa and testis	D-R relationship: **higher F doses caused changes in testicular morphology and ultra-structure of the sperm** • Results: - Testicular morphology: In 25 mg/L group, the intervals among seminiferous tubules were widened; in the 50 and 100 mg/L groups, the pattern of the seminiferous epithelial cells were disordered, the spermatogenic cells at different development stages were reduced and the boundary was blurred, and many spermatogenic cells fell off into the lumen. Vacuolar-like lesions appeared in 100 mg/L NaF group indicating spermatogenesis and sperm structure were affected by fluoride exposure - Ultra-structure of the sperm: No abnormal changes were seen in 25 mg/L group. Fiber sheath was thin and irregular, and defective structure or even multiple fractures were observed in the 50 and 100 mg/L NaF-treated group. mRNA and protein expression levels of Akap3 and Akap4 were significantly decreased in the 100 mg/L group.	"In summary, our study revealed that fluoride exposure altered the structures of the fibrous sheathes and axonemal in sperm flagellum via down-regulating the mRNA and protein expression levels of AKAP3, AKAP4, CFAP43, CFAP44, and HYDIN, which provides a new insight of fluorine alters the structure of sperm flagella"	2

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
		- Ultra-structure of spermatozoa flagellum axoneme: in the 50 and 100 mg/L groups, the center pairs were irregular and absent, the "9" in the axial filament were not clearly arranged between the outer twinned microtubules, and some microtubules were irregular in shape. The mRNA expressions of Cfap 43, Cfap44 and Hydin were significantly decreased in the testis of mice from 100 mg/L treatment group. - CFAP44 and HYDIN protein levels of testis were significantly decreased in the 50 and 100 mg/L		
Min 2021 [210]				
Oral (drinking water) chronic study: • Male mice • 13 animals per group, 4 groups	Exposure: ● Sodium Fluoride (NaF) ● **0, 2, 4, 8 mg F/kg bw/day** ● Drinking water ● 90 days of exposure Outcomes assessed: Reproductive toxicity • Specific outcomes: • Organ coefficient of testis • Sperm count and deformity rate • Histopathological analysis • Testosterone content in serum • Identification of gene expression	**Dose response(D-R) relationship: increase in sperm deformity rate, decrease in sperm survival** • Results: ○ 50 mg/L NaF exposure at 90 days significantly reduced the organ coefficient of testis in mice compared to the control. No significant change in 25 mg/L or 100 mg/L NaF groups ○ Sperm count: Significant decrease in sperm counts in 50 mg/L NaF group ○ Sperm deformity: Significant increases in sperm deformity rate in 25, 50, and 100 mg/L NaF groups ○ Sperm viability: Significant decrease in sperm viability in 50 and 100 mg/L NaF groups ○ Serum testosterone: Significant decrease in serum testosterone in 50 and 100 mg/L NaF groups ○ Histopathological changes: the quantity of spermatogenic cells and spermatozoa presented strikingly decreased trend and the gap between spermatogenic tubules increased significantly, especially in 50 and 100 mg/L NaF group ○ Differentially expressed piRNAs: In the 50 mg/L NaF group, there were 1047 up-regulated and 1080 down-regulated piRNAs compared to control	• The reduction of testicular organ coefficients, semen quality, serum testosterone levels, and changes in the testicular microstructure of mice given 50 or 100 mg/L of NaF in water for 90 days showed that these exposures can cause significant testicular damage. Fluoride can induce testicular damage through altered piRNA expression in the testes	1

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
		○ Expression analysis: the target genes expression of Ap4e1, Gga2, Gla and Ap1s3 were increased gradually in 50 and or 100 mg/L NaF group		
Sun 2010 [262]				
Oral (drinking water) subchronic mice study • Adult Kunming mice, males only • 60 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 2.84, 6.28, 14.18 mg/kg bw/day** (0, 30, 70, 150 mg/L NaF) • Vehicle – distilled water • 49 days of exposure Outcomes assessed • **Reproductive toxicity** • Specific outcomes: - Sperm quality evaluation and assessment of hyperactivation - Ca2+ concentration ([Ca2+]) in spermatozoa - Gene/ protein expression changes in sperm	D-R relationship: **Inhibition of sperm hyperactivation in a dose-dependent manner** • Results: - Sperm quality: sperm motility significantly decreased by 15.24 and 18.43%, respectively, in 70 and 150 mg/L groups. Sperm count and survival significantly reduced in 150 mg/L group. - Sperm hyperactivation: 70 and 150 mg/L F concentrations significantly inhibited sperm hyperactivation by 21.70 and 29.73%, respectively, showing a dose-dependent manner. - Sperm Ca2+ levels: significant decrease in sperm Ca2+ concentrations by 16.92% and 30.1% in 70 mg/L and 150 mg/L groups, respectively. - A significant reduction in sperm CAMK2, but not in CALM, protein expression was observed in 70 and 150 mg/L groups	In summary, this study demonstrated that sperm hyperactivation was significantly reduced in mice administrated with 70 and 150 mg/l NaF in drinking water for 49 days, along with the decreased Ca2+ concentration, CAMK2 protein expression, and CatSper1 mRNA level in sperm. It may be one of the mechanisms by which excessive F induced male infertility.	1
Sun 2014 [263]				
Oral (drinking water) subchronic mice study • Adult Kunming mice, males only • 20 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 2.84, 6.28, 14.18 mg/kg bw/day** (0, 30, 70, 150 mg/L NaF) • Vehicle – distilled water • 49 days of exposure Outcomes assessed • **Reproductive toxicity** • Specific outcomes:	D-R relationship: **sperm abnormalities were significantly enhanced with increasing NaF concentration** • Results: - Sperm abnormalities: a significant increase in sperm head abnormality was observed in 150 mg/l group; and significant tail abnormality was found in 70 and 150 mg/L group.	In summary, this study presents evidence that NaF adversely affected mice sperm chromatin structure in a dose dependent manner. Reduced P1 and P2 mRNA expression and altered histones and total thiol groups levels	

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
	- Sperm quality - Sperm morphology - Sperm DNA integrity - Sperm gene and thiol group changes	- Sperm DNA integrity: % DNA denaturation was significantly increased in 70 and 150 mg/L group.	could contribute to the sperm damage resulted from F exposure	
Wang 2018 [277]				
Oral (drinking water) subchronic mice study • 30-day old Kunming mice, males only • 10 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 4.52, 9, 13.5 mg/kg bw/day*** (0, 50, 100, 150 mg/L NaF) • Vehicle – water • 90 days of exposure Outcomes assessed • **Reproductive toxicity** • Specific outcomes: - Sperm quality evaluation - Total RNA extraction and quantitative real-time polymerase chain reaction - Immunohistochemistry for CREM and ACT	D-R relationship: **the sperm count and viability and the percentage of malformed sperm were increased in a dose-dependent manner** • Results: - a significant decrease in testis weight was observed in the 100 and 150 mg/L groups; no significant differences in the average epididymis weights - the sperm count and sperm viability were decreased in all F-treated mice and a statistically significant increase in the percentage of malformed sperm was noted in 100 and 150 mg/L groups - protein expression of CREM and ACT: CREM protein expression levels were significantly decreased in a dose-dependent manner. The protein expression levels of ACT were decreased significantly in all treatment groups	"In conclusion, our results demonstrate that after 90 days of exposure in mice, F impairs sperm quality, which was associated with the downregulation of the testicular transcription factors CREM and ACT. Thus, this could represent one of the molecular mechanisms underlying the effect of F on the male reproductive system."	1
Wei 2016a [284]				
Oral (drinking water) chronic mice study • Adult Kunming mice, males only • 20 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 2.2, 4.5, 9 mg/kg bw/day*** (0, 25, 50, 100 mg/L NaF) • Vehicle – distilled water • 180 days of exposure Outcomes assessed • **Reproductive toxicity** • Specific outcomes: - Sperm quality - Testicular histopathology	D-R relationship: **sperm quality was altered in a dose-dependent manner (between 50 – 100 mg/L)** • Results: - Sperm quality: sperm count, and abnormality were significantly altered with increasing concentrations of NaF at 50 mg/L and above - Testis histopathology: at the 25 mg/L dose, spermatogenic cells changed disorganization and	Taken together, our results demonstrated that, after 180 days exposure to mice, fluoride could induce testicular toxicity, which was associated with up-regulation of testicular inflammatory mediators including IL-17, TNF-a and NO.	1

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
	- Gene and protein expression analysis (testicular interleukin-17(IL-17), interleukin-17 receptor C (IL-17RC), tumor necrosis factor-a (TNF-a) and interleukin-6 (IL-6)) - Concentration of nitric oxide (NO) in testis	denudation; at the 50 mg/L dose, there were a lot of vacuoles in seminiferous tubules; at the 100 mg/L dose, testicular histological alterations included loss and shedding of sperm cells within the lumen - Gene expression: NaF treatment (100 mg/L) altered mRNA levels of f IL-17, IL-17RC, TNFa and IL-6 but not f IL21, TGF-b and IL-1b. Similarly, IL17 and TNFa protein contents were significantly increased in the testicular fluid of 100 mg/L dose compared to controls - NO levels: a significant increase in iNOS mRNA in 50 and 100 mg/L NaF groups and a significant increase in NO content of 100 mg/L NaF group was observed		
Wu 2019 [288]				
Oral (drinking water) chronic mice study • 8-week-old BLB/c mice, males only • 20 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 2.2, 4.5, 9 mg/kg bw/day*** (0, 25, 50, 100 mg/L NaF) • Vehicle – distilled water • 150 days of exposure Outcomes assessed • **Reproductive toxicity** • Specific outcomes: - Sperm Quality - Testicular Histopathology - Influence on Inflammation Cytokines - Status of Immunocyte and Cytokines in Testis	D-R relationship**: higher F doses altered testicular histology and sperm quality** • Results: - Sperm quality: Sperm motility and viability were significantly reduced in 100 mg/L NaF groups, relative controls - Testicular histopathology: normal histological structure was observed in testis of controls and 25 mg/L group mice. However, a different degree of infiltration status in different immune cells, sloughing of cells and vacuolation of spermatogenic epithelium, a reduction of sperms in seminiferous tubule and significant reduction in	Based on this study results, authors confirm that NaF induces adverse effects on testis including testicular inflammation. The presence of specific antisperm autoantibodies in antitesticular autoantibodies and the notable recruitment of immunocyte, these key factors of autoimmune orchitis, are observed in NaF groups. These results indicate that testicular inflammation induced by excessive	1

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
		spermatogenic score was noted in testis of 50 and 100 mg/L group mice. - Inflammation cytokines: relative controls, expression of IL-6, IL-17A, TNF-α and IFN-γ were significantly increased in 100 mg/L group mice.	F exposure is associated with autoimmune orchitis. And IL-17A is a key cytokine to play an important role in this inflammation.	
		Renal or Kidney Toxicity		
Chattopadhyay 2011 [116]				
Oral (drinking water) subchronic mice study • 8 weeks old, Swiss albino mice, males only • 8 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 1.35, 13.5 mg/kg bw/day*** (0, 15 and 150 mg/L NaF) • Vehicle - water • 30 or 90 days of exposure Outcomes assessed • **Hepatotoxicity and Renal or Kidney Toxicity** • Specific outcomes: - Organo-somatic index (OSI) - Liver function tests - Glutathione (GSH) - Glutathione-s-transferase (GST) - Thiobarbituric acid reactive substances (TBARS)	D-R relationship: **severe alteration of renal histological structures, liver enzyme levels in both F treatment groups** • Results: - No death or clinical symptoms; no significant difference in water consumption rate or body weight (weight gain) - No significant difference in the OSI of liver and kidney - Liver function: GPT level increased significantly in all the treatment groups whereas GOT level increased significantly in Gr III (65%) and VI (73%) - GSH-GST response and TBARS production: a significant decrease (32%) in GST of the liver of group II mice; MDA production increased significantly Gr-IV mice. TBARS level increased significantly in the kidneys of Gr-II mice - Histopathology of liver: Gr II mice showed extensive vacuolar degeneration in the cytoplasm and loss of integrity in the epithelium lining of central vein; Gr IV mice showed hepatocellular hypertrophy, cytoplasmic vacuolization and extensive hepatic sinusoidal dilation	"The present study clearly indicates that F induces hepatotoxicity and nephrotoxicity in mice evidenced by oxidative stress, histopathological changes in the liver and kidney with concomitant effects on normal hepatic function."	1

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
		- Histopathology of kidney: severe alteration of renal histological structures, atrophy of glomeruli, blood-filled spaces and varying degree of degeneration of tubular epithelium were noticed in Gr-II and Gr-IV mice - Heat shock protein 70 profile: elevated expression of Hsp 70 in kidneys of group II and group IV than group III; liver Hsp 70 reduced gradually dose and time dependent manner		
Cárdenas-González 2013 [112]				
Oral (drinking water) subchronic rat study • Weanling Wistar rats, males only • 12 animals/ group, 3 groups	Exposure • Sodium fluoride (NaF) • **0, 2, 7 mg/kg bw/day*** (0, 15, 50 ppm F) • Vehicle - water • 40 days of exposure Outcomes assessed • **Renal or Kidney Toxicity** • Specific outcomes: - Urinary and serum creatinine (Cre) - Urinary glomerular filtration rate (eGFR) - Urinary kidney injury biomarkers: Kim-1, Clu, OPN, B2M and CysC - mRNA expression levels of Kim, Clu and OPN in the renal cortex - Histological analysis	D-R relationship: **urinary creatinine and several kidney injury biomarkers were altered at highest F doses** • Results: - A small non-significant dose-dependent increase in the serum creatinine levels in 15 and 50ppm groups. - A small non-significant dose-dependent decrease in the eGFR in 15 and 50 ppm groups. - Urinary kidney injury biomarkers: significant increase in Kim-1, Clu, OPN, B2M and CysC in 50ppm group. - mRNA expression: significant increase in levels of Kim, Clu and OPN in the renal cortex in 50ppm group. - Histological analysis: fluoride exposure induced tubular injury characterized by tubular flattening, loss of proximal tubule brush border, cell detachment and loss of the tubular epithelium continuity. Tubular flattening was observed in both 15 and 50 ppm groups; additionally, 50 ppm group had tubular cell	In summary, our results revealed that the sub-chronic fluoride exposure at environmentally relevant concentrations induces PT injury. This was clearly demonstrated by the increase of early and sensitive kidney injury biomarkers such as Kim-1, Clu, OPN, Hsp72, B2M and CysC at stages where renal function was not altered.	1

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
		detachment. There was a non-significant dose-dependent increase in percentage of injured tubules		
Kobayashi 2009 [174]				
Oral (drinking water) subchronic rat study • Weanling Wistar rats, males only • 6 animals/ group, 3 groups	Exposure • Sodium fluoride (NaF) • **0, 0.3, 3 mg/kg bw/day*** (0, 5, 50 ppm NaF) • Vehicle – deionized water • 60 days of exposure Outcomes assessed • **Renal or Kidney toxicity** • Specific outcomes: - Renal histopathology - Proteomics	D-R relationship: **50 ppm F dose induced marked histological changes in kidneys** • Results: - Renal histopathology: No marked abnormal changes were seen kidneys of controls or 5 ppm group rats (except mild vascular congestion in 5 ppm rats). Kidneys of 50 ppm group rats had markedly increased blood vessels, larger glomerular & medullar capillaries engorged with erythrocytes. - Renal proteomic changes: protein levels related to detoxification, metabolism and endoplasmic reticulum were significantly changed in treated rats, especially in 50 ppm group. Between control vs 50 ppm F, and control vs 5 ppm F groups, 12 and 6 differentially expressed proteins were detected, respectively. Six proteins, mainly related with metabolism, detoxification and housekeeping, were successfully identified. At the high F group, pyruvate carboxylase, a protein involved in the formation of oxaloacetate was found to be downregulated, while enoyl coenzyme A hydratase, involved in fatty acids oxidation, was found to be upregulated."	In summary, the histological analysis revealed no damage in kidneys induced by F, except for a vascular congestion in the high-dose group. The differentially (down-regulated) expressed kidney proteins in F dose groups belong to 3 main functional categories i.e., detoxification-related proteins, metabolism-related proteins and miscellaneous, including endoplasmic reticulum proteins.	1
Wasana 2015 [282]				

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
Oral (drinking water) chronic mice study • 7-8 months old, ICR mice, females only • 6 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 0.012, 0.35, 2.3 mg/kg bw/day** (0, 0.05, 1.5, 10 mg/L F) • Vehicle - drinking water • 295 days of exposure Outcomes assessed • **Renal/ kidney toxicity** • Specific outcomes: - Gross examination - Kidney histopathology - F content in kidneys	D-R relationship: **no significant changes in any outcomes assessed** • Results: - No treatment related deaths or abnormal behavior or visible signs (appetite, depression, lethargy etc.). - No adverse effect on kidney functions due to treatment as indicated by blood urea nitrogen (BUN) and creatinine blood levels. - F treatment didn't induce any histopathological changes in kidney tissues related to CKD including degeneration, necrosis of glomeruli and tubules, atrophy of glomeruli and glomerular capsules, and tubular dilation with leakage	Based on the absence of abnormal histopathological changes and no significant change in blood BUN and creatinine levels, authors conclude that chronic treatment of mice with F in drinking water, within the concentration range of 0.07–15 mg/L, had no adverse effects on kidneys.	1
Hosokawa 2010 [149]				
Oral (drinking water) subchronic mice study • ICGN mice (ICR derived animal model for congenital nephrotic syndrome) with blood urea nitrogen (BUN) ≥36.0 mg/dL in were used as animals with impaired kidney function, and healthy ICR mice used as controls with normal kidney function • 11-14 weeks old mice, males only • 3-9 animals/ group, 5 groups	Exposure • Sodium fluoride (NaF) • **0, 5, 10, 20, 30 mg/kg bw/day*** (0, 25, 50, 100 and 150 ppm F) • Vehicle – water • 4 weeks of exposure Outcomes assessed • **Renal/ Kidney toxicity** • Specific outcomes: - Change in body and tissue weights - Change in kidney function measured by blood urea nitrogen (BUN) and creatinine (CRE) levels	D-R relationship: **highest dose tested caused increase in BUN levels of ICGN mice, not in ICR mice** • Results: - 100% of 150 ppm and 40% of 100 ppm ICGN mice were died within 24 days of treatment. No deaths in 150 ppm ICR mice were recorded. - Relative liver weight was significantly decreased in 150 ppm ICGN mice. - Significant increase in BUN levels were measured in 150 ppm ICGN mice only (increases were rapid just prior to death). No increase in lower dose levels or in any ICR mice was noted. - Significant increase in CRE levels of 150 ppm ICGN mice was reported.	In conclusion, all the kidney impaired ICGN mice exposed to 150 ppm F died in less than a month, and the kidney function in this group deteriorated significantly, since the mean values of BUN and CRE in the serum were dramatically increased. No death or alteration in BUN or CRE levels were noted in ICR mice (with normal kidney function) under same treatment conditions. People with renal insufficiently should therefore be careful to avoid excessive exposure to F.	2
Perera 2018 [232]				

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
Oral (gavage) subchronic rat study • Adult Wistar rats, males only • 9 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 0.03, 0.3, 1.26 mg/kg bw/day*** (0, 0.5, 5, 20 ppm NaF) • Vehicle – distilled water • 15 or 30 or 60 days of exposure Outcomes assessed • **Hepatotoxicity** • Specific outcomes: - Relative organ weight - Hepatic inflammation - Serum creatinine - Serum AST, ALP, and ALT	D-R relationship: **a dose-response relationship was observed for serum AST and ALP** • Results: - Relative organ weight: no significant difference in the relative kidney and liver weights - Hepatic inflammation: mild portal inflammation with lytic necrosis in 0.5 ppm group, multiple areas of focal necrosis and various degrees of portal inflammation appeared in 5 and 20 ppm groups - Serum creatinine: no difference in 15 and 30 days. Significant increase in 20ppm group after 60 days - Serum AST, ALP, and ALT: serum AST activity was higher in 20 ppm group, no significant differences in serum ALT in 15 and 30 days while significantly higher in 5 ppm and 20 ppm after 60 days	"Fluoride exposure impaired hepatocytes and hepatic function, which was strongly supported by the necrosis and portal inflammation histopathologically and increased serum AST, ALT, and ALP activities. Further, it has been demonstrated that there is a possibility of inducing renal damage by high fluoride levels for longer period of administration due to elevated creatinine levels."	1

Endocrine and thyroid related effects

Liu 2016 [187]				
Oral (drinking water) chronic rat study • One-month old Wistar rats, males and females • 20 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 0.3, 0.6, 1.26 mg/kg bw/day*** (0, 5, 10, 20 mg/L NaF) • Vehicle – water • 2 or 8 months of exposure Outcomes assessed • **Endocrine and thyroid related effects** • Specific outcomes: - Thyroid weight and organ coefficient - Thyroid tissue morphology	D-R relationship: • Results: - Thyroid weight and organ coefficient: no obvious changes occurred. - Thyroid tissue morphology: the treatment groups displayed smaller and irregular follicular cavity, or even cell mass without a cavity. Serum T3, T4, FT3, FT4, and TSH: 2 months - No change in serum T3, FT3, and TSH; however, serum T4 and FT4 levels were increased in 10	"Fluoride can damage thyroid structure and function, including thyroid weight and organ coefficient changes, morphological abnormalities in thyroid tissue, alteration of thyroid hormone levels, and an increased apoptosis rate of thyroid cells. ER stress-induced apoptosis is involved in	1

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
	- Serum T3, T4, FT3, FT4, and TSH - Apoptosis rate of thyroid cells - GRP78, IRE1, sXBP-1 and CHOP mRNA expression in rat thyroid tissue	and 20 mg/L groups. T3/T4 ratios showed a dose-dependent reduction 8 months - No change in serum T3, FT3, T4, and FT4; however, TSH levels were reduced in 10 and 20 mg/L groups. T3/T4 ratios decreased only at in 20 mg/L group. - Apoptosis rate of thyroid cells: no significant changes at 2 months. Higher apoptosis rates at 8 months in all groups. - GRP78, IRE1, sXBP-1 and CHOP mRNA expression in rat thyroid tissue: no significant changes at 2 months. Higher GRP78, IRE1, sXBP-1, and CHOP mRNA at 8 months. - GRP78, IRE1, and CHOP protein expression in rat thyroid tissue: increased in treatment groups.	the damage of rat thyroid cells caused by excess fluoride."	
McPherson 2018 [208]				
Oral (drinking water) chronic rat study • GD4 Long-Evans hooded rats, males only • six animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 1.4, 2.8 mg/kg bw/day*** (0, 10, or 20 ppm F) • Vehicle - drinking water • Varying contents F in diet (a standard diet with 20.5 ppm F or a low F diet with 3.24 ppm F) • Exposure from GD6 through PND56 Outcomes assessed • **Endocrine and thyroid related effects** • Specific outcomes: - Serum T3, T4, and TSH	D-R relationship: None • Results: - Serum T3, T4, and TSH: no significant differences were observed across groups for serum T3 or T4 or TSH levels; compared to rats maintained on a standard chow diet, TSH levels were significantly lower in rats maintained on low-F− chow	"Serum triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH) levels were not altered as a function of 10 or 20 ppm F− in the drinking water"	1

Immunotoxicity

Gutiérrez-Salinas 2010 [147]

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
Oral (drinking water) subchronic rat study • Adult Wistar rats, males only • 25 animals/ group, 3 groups	Exposure • Sodium fluoride (NaF) • **0, 0.124, 6.1 mg/kg bw/day** (0, 1, 50 ppm F) • Vehicle - drinking water • Varying contents (low or high) of protein and calcium in diet • 8 weeks of exposure Outcomes assessed • **Immunotoxicity** • Specific outcomes: - Metabolic activity of leukocytes - Expression of Proteins p-53, bcl-2, and Caspase-3	D-R relationship: **the dose intervals are too large to find a dose-dependent trend – only highest dose showed significant changes** • Results: - Metabolic activity of leukocytes: no significant changes in their metabolic activity in 1 ppm group; 50-ppm dose produced a significant decrease ($p < 0.05$) - Expression of Proteins p-53, bcl-2, and Caspase-3: a statistically significant increase in p53 and caspase-3 protein levels of 50 ppm group only. No statistically significant change in bcl-2 expression levels	"Exposure of rats to NaF modifies the expression of p53, bcl-2, and caspase-3 and causes general metabolic changes to leukocytes, which are indicators of changes to normal pattern of apoptosis"	2
Bone/skeletal related toxicity				
Hosokawa 2016 [150]				
Oral (drinking water) subchronic mice study • ICR-derived glomerulonephritis (ICGN) mice, males and females • 5 males and 4 or 7 females/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 5, 10, 20 mg/kg bw/day*** (0, 25, 50, and 100 ppm F) • Vehicle – water • 4 weeks of exposure Outcomes assessed • **Bone/ Skeletal related toxicity** • Specific outcomes: - Microdensitometry examination of the femurs	D-R relationship: **highest test dose induced changes in bone mineral content and bone mineral density of the left femur** • Results: - Microdensitometry of femurs: no significant increase in any bone indexes; bone mineral content and bone mineral density of the left femur from the male ICR 150 ppm group were significantly higher.	"In the present study with mice, 150 ppm of F in drinking water induced bone and dental effects." However, authors note that "work on rodents does not relate to humans because higher levels of fluoride are required to get bone and dental affects similar to those in humans; the ability of rodents to excrete or metabolize F more efficiently than humans are able to explains the discrepancy in the F concentrations that induce osteofluorosis	3

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
			between humans and these experimental animals" and it's worthwhile to examine effects of F on osteofluorosis for a period of more than 2 months.	
Kobayashi 2014 [172]				
Oral (drinking water) subchronic mice study • Weanling mice of 129P3/J and A/J strains, males only • 16 per strain/ group, 3 groups	Exposures • Sodium fluoride (NaF) • **0, 2, 10 mg/kg bw/day*** (0, 10, 50 ppm F**)** • Vehicle - drinking water • 8 weeks of exposure Outcomes assessed • **Bone/ Skeletal related toxicity** • Specific outcomes: - Bone morphology (micro CT analysis) - Bone formation (mineral apposition rate MAR) - Bone modeling (Plasma alkaline phosphatase activity) - Proteomics	D-R relationship: **Dose-specific and strain-specific changes only in proteomics data was noted.** Strain specific, but not dose-specific, changes in bone formation. • Results: - Bone morphology: no significant treatment-related differences in bone mineral density (BMD) or other bone parameters of any bone type (femurs, tibiae and lumbar vertebrae) among all treated groups. - Bone formation: Slight dose-dependent increase in new bone deposition (MAR) was observed only in 129P3/J mice. - Bone modeling: As indicated by plasma ALP activity, no statistical differences were observed among the F treatments for either strain. - Collagen expression: based on western blotting data, no statistically significant differences in collagen type 1 protein levels of femur were found in any treated mice. - Proteomics: Significant changes in several bone proteins (related to osteogenesis and osteoclastogenesis) were found among the F treatment groups within and between each strain indicating an influence of genetic background in bone cell responses to F exposure.	F in drinking water for 8 weeks didn't induce any significant changes in BMD or bone modeling of either strain mice.	1
Song 2011 [258]				
Oral (drinking water) subchronic rat study • Wistar rats, males only • 12 animals/ group, 4 groups **Human spot study:**	Exposure • Sodium fluoride (NaF) • **0, 1.4, 21, 56 mg/kg bw/day*** (0, 10, 150, 400 mg/L F) • Vehicle – water • 15 or 30 or 90 days of exposure • Human study	D-R relationship: **Serum ALP, BALP and BGP levels were affected at highest dose groups** • Results: - Serum alkaline phosphatase activity: serum ALP was significantly increased in 10 and 150 ppm groups on	"In conclusion, changes in serum ALP and BALP activity BGP content are important reference indicators of fluoride exposure. We therefore suggest that serum fluoride, serum	2

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
Eighty-six adult male workers at an aluminum factory in Hubei province, China, without liver, kidney, or bone related diseases were selected	- Age (years), serum F (mg/L), urinary F (mg/L) and air F (mg/m3) of participants in Human study: Fluoride-exposed (n= 58) 38.35±14.24, 0.46±0.22, 2.72±0.16, 2.08±1.01; for non-exposure controls (n=28) 39.70±13.90, 0.16±0.07, 0.63±0.16, 0.10±0.06, respectively. - Spot blood samples Outcomes assessed • **Bone/ Skeletal related toxicity** • Specific outcomes: - Serum alkaline phosphatase (ALP) - Serum bone alkaline phosphatase (BALP) - Serum osteocalcin (BGP)	days 15 and 30, but significantly reduced in the 400-ppm group on day 15 - Serum bone alkaline phosphatase activity: only in the 150 ppm group on day 30 did the vitality of serum BALP showed a significant difference - Serum osteocalcin: the BGP content was lower in 400 ppm group on days 30 and 90; but it was higher in the 150 ppm group on day 90 - In the spot study, the activity of serum ALP and BGP content were higher in the medium working-age group (10 years < working-age ≤ 20 years) than in the short working-age group (≤ 10 years). However, compared with the medium working-age group, the content of BGP was lower in the long working-age group (>20 years).	ALP activity, and BGP content may be important reference indications of fluoride exposure."	

Cardiovascular toxicity

Martin-Pardillos 2014 [207]				
Oral (drinking water) chronic rat study • 2-months old Wistar rats, CKD disease models (5/6-nephrectomized (Nx) or sham-operated controls), males only	Exposure • Sodium fluoride (NaF) • **0, 0.123 and 1.31 mg/kg bw/day** (0, 1.5, 15 mg/L F) • Vehicle - drinking water • 4.5 months of exposure Outcomes assessed • **Cardiovascular toxicity** • Specific outcomes: - Calcium and phosphate deposits in the heart and complete aorta	D-R relationship: **increased MVC and active calcification of the arteries was found in animals exposed to WHO's recommended F concentration** • Results: - CKD: F treatment influenced CKD of the Nx animals (1.2% Pi in diet); 1.5 mg/L and 15 mg/L group animals had higher urea and creatinine levels than controls and sham-operated rats (1.2% Pi diet). (S-1.2Pi). - Calcification of aortas: Nx animals (1.2% Pi diet) of both 1.5 and 15 mg/L group had calcium accumulation in abdominal and thoracic aorta. These calcified spots or lesions were compatible with stage 2 and stage 3 of	Authors conclude that F significantly increased medial vascular calcification (MVC) in animals with CKD and hyperphosphatemia by exacerbating the renal damage; and suggest "adding [F] to municipal drinking water, should be reconsidered and	2

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
		vascular calcification in 1.5 and 15 mg/L groups, respectively.	should be replaced by a fluoridation policy based on the health status of individuals."	
Metabolism (diabetes/glucose or lipid metabolism) related-outcomes				
Lupo 2011 [200]				
Oral (drinking water) subchronic rat study • 7-weeks old, Sprague-Dawley rats (with surgically induced renal insufficiency), males only • 4 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 0.14, 0.7, 2.1 mg/kg bw/day*** (0, 1, 5, and 15 ppm F) • Vehicle - drinking water • 60 days of exposure Outcomes assessed • **Metabolism related (diabetes/ glucose or lipid metabolism) toxicity** • Specific outcomes: - Glucose homeostasis - Rate of fluoride uptake by bone tissue - Parameters of renal insufficiency	• Results: - Glucose homeostasis: **no significant differences either in glucose plasma levels sham and NX rats or among various F-treatment groups.** No significant change in the values of plasma glucose concentration after 120 min of the glucose load across F-treatment groups. However, plasma insulin levels were significantly increased with F levels in DW - Rate of fluoride uptake by bone tissue: significantly higher in NX rats than in sham-operated rats - Parameters of renal insufficiency: no significant differences in these parameters between NX rats and sham-operated rats or with different F-treatment levels	The intake of fluoridated water from water supply modifies plasma insulin levels without changes in plasma glycemia, both in controls and in rats with renal disease, after 60 days.	2
Lobo 2015 [194]				
Oral (drinking water) subchronic rat study • Weanling Wistar rats (diabetic D and nondiabetic ND; diabetes was induced with streptozotocin), males only • 9 animals/ group, 6 groups	Exposure • Sodium fluoride (NaF) • **0, 1.4, 7 mg/kg bw/day*** (0, 10, 50 ppm F) • Vehicle – water • 22 days of exposure Outcomes assessed • **Metabolism (diabetes/ glucose or lipid metabolism) related toxicity** • Specific outcomes: - Insulin tolerance test - Plasma Glucose - Plasma Insulin	D-R relationship: **F exposure significantly lowered plasma insulin levels in Diabetic animals, but not in non-Diabetic counterparts, with no dose-response trend** • Results: - Plasma glucose: Plasma glucose concentration was significantly higher in Diabetes animals compared with non-Diabetes animals but was not influenced by the treatment with Fluoride. - Diabetic animals had significantly lower plasma insulin levels compared with non-Diabetic counterparts. Exposure	"After 22 days of treatment, no alterations in glycemia, insulinemia, KITT, and HOMA2-IR (homeostasis model assessment 2 of insulin resistance) were seen for ND. F-exposure of D rats led to significantly lower insulinemia, without alterations in glycemia"	2

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
	- Insulin resistance	to F did not alter plasma insulin levels in non-Diabetes animals. However, in Diabetes animals, plasma insulin concentrations were significantly reduced upon exposure to F, but no dose-response relationship was observed. - Glucose Disappearance Rate was lower in Diabetic animals compared with their non-Diabetic counterparts, despite the difference being significant only for the animals treated with water containing 0 or 10 ppm fluoride. In addition, Kitt was not significantly changed upon exposure to fluoride, both in non-Diabetic and Diabetic animals. - Exposure to F significantly increased %S in Diabetic animals, and this effect was more pronounced for the rats treated with water containing 10 ppm fluoride.		
Malvezzi 2019 [205]				
Oral (drinking water) subchronic mice study • 35–60-day-old non-diabetic (NOD) mice, males only • 8 animals/ group, 3 groups	Exposure • Sodium fluoride (NaF) • **0, 0.9, 4.5 mg/kg bw/day*** (0, 10, 50 ppm NaF) • Vehicle – water • 21 days of exposure Outcomes assessed • **Metabolism related (diabetes/ glucose or lipid metabolism) toxicity** • Specific outcomes: - Evaluation of plasma glucose and insulin levels and insulin resistance (IR) - Proteomic analysis of liver and gastrocnemius muscle	**D-R relationship: Low F exposures reduced plasma glucose levels** • Results: - 10 ppm group had a significant reduction in the plasma glucose levels and a significant increase in the β-cell function (%B). No significant difference among the treatment groups were seen regarding plasma insulin or HOMA2-IR. - Proteomic analysis: in the muscle tissues of 10 ppm F group, increased expression of proteins involved in energy metabolism, and in the 50 ppm F group, increased expression of proteins related to muscle contraction, differentiation of brown adipose tissue and apoptosis were found. Similarly, in the liver tissue of the 10 ppm F group, increase in proteins involved in energy metabolism and protein synthesis, and in the 50-ppm group, proteins related to ROS metabolism and energetic metabolism were altered.	In summary, our results suggest that early treatment with low F concentration seems to prevent or at least delay the onset of T1D, probably by increasing the antioxidant defense. However, it should be noted that despite rodent models have greatly contributed to our understanding of T1D, differences between humans and rodents must be acknowledged when	1

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
		Additionally, western blotting confirmed an increase in isoforms of Glutathione S transferase in 100 ppm group liver tissues.	interpreting the results obtained using animal models.	
		Genotoxicity		
Chattopadhyay 2008 [234]				
Oral (drinking water) subchronic mice study • 2-3 months old, Swiss albino mice, males only • 4-6 animals/ group, 6 groups	Exposure • Sodium fluoride (NaF) • **0, 0.7, 1.4, 2.7, 9, 13.6 mg/kg bw/day*** (0, 7.5, 15, 30, 100, and 150 mg/L NaF**)** • Vehicle - drinking water • 30 or 90 days of exposure Outcomes assessed • **Genotoxicity** • Specific outcomes: - Organ weights - Mitotic inhibition, - Chromosomal aberrations - Chromatid breaks - Femur bone marrow cell count	**D-R relationship: inconsistent** • Results: - No treatment related changes in the percentage of - mitotic indices (MI) of bone marrow cells - a significant increase in the percentage of aberrant metaphases and chromatid breaks in all treatment groups with highest in 15 mg/L group. - The total number of nucleated cells per femur or percentage of bone marrow cells at different phases didn't change across any treatment groups	"F in vivo is actually more genotoxic at certain lower concentrations (15mg/L) than at higher concentrations (100 or 150 mg/L)."	3
Leite Ade 2007 [177]				
Oral (gavage) acute rat study • Adult Wistar rats, males only • 5 animals/ group, 7 groups	Exposure • Sodium fluoride (NaF) • **0, 10, 20, 40, 60, 80 and 100 mg/kg bw** • Vehicle – deionized water • Single dose (killed after 2 hours of administration) Outcomes assessed • **Genotoxicity** • Specific outcomes: - DNA damage in blood, liver, kidney, thyroid gland and urinary bladder	• Results: - **No DNA damage observed** in blood, liver, kidney, urinary bladder and thyroid gland cells, regardless of the fluoride dose administered.	"In conclusion, even acute lethal doses of fluoride administered to rats were unable to induce genotoxicity in all cell types tested, as depicted by the single cell comet assay. Since DNA damage is an important step in events leading from carcinogen exposure to cancer, this study represents a relevant contribution to the correct evaluation of the potential health risk	2

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
			associated with chemical exposure."	
		Neurotoxicity		
Nadei 2019 [215]				
Oral (drinking water) chronic rat study • PND42 Wistar rats, males only • 10 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 0.7, 2.8, 7 mg/kg bw/day*** (0, 5, 20, 50 ppm F) • Vehicle – water • 12 months of exposure Outcomes assessed • **Neurotoxicity** • Specific outcomes: - Short-term and long-term memory (using novel object recognition (NOR) test) - Spatial learning and memory (using Morris water maze test) - Expression of Calpain proteins in hippocampus	D-R relationship: **all three doses caused an impairment in the processes of spatial learning and formation of long-term memory** • Results: - Novel object recognition: in 1 hour session, a significant decline in DI (discrimination index), an index of recognition memory, in rats exposed to 50 ppm fluoride was noted; the decline noted in 5 and 20 ppm groups was not statistically significant. In 24 hours after training, the rats from all three fluoride groups were not able to discriminate between new and familiar object, with DI being a few times less than that of control rats for animals given 20 and 50 ppm fluoride. - Morris water maze test: Following everyday training, escape latency substantially decreased in all groups of animals. However, starting from day 3, efficiency for spatial learning was significantly lower for rats in 5 and 50 ppm whereas inconsistent in 20 ppm group. In spatial probe test (day 6), the rats from 20 ppm fluoride group had lesser number of visits to target quadrant and, accordingly, spent less time and swam shorter distance within this quadrant. The distance traveled in target zone by the animals exposed to 50 ppm fluoride was also shorter. No statistical difference in these parameters was revealed for rats from 5 ppm group.	"The results of our work have shown that long-term consumption of excessive F− doses exerts pronounced negative impact on cognitive capacities of rats and on their hippocampal cells. Although the formation of short-term memory was sensitive to 50 ppm F− only, all three F− doses induced the deficit of long-term memory." And, "altered expression of signaling molecules of calpain-1 cascade at background of stable activity of calpain-2 and its effectors, observed in rat hippocampus after long-term F− intoxication, suggests the disruption of link between early and late LTP phases, i.e., between induction and consolidation of memory, leading to decline in cognitive capacities of animals."	1

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
		- A dose-dependent decline of calpain-1 content in cytoplasm of hippocampus, but significant increase of its expression in membrane fractions in comparison to control		
Teng 2018 [266]				
Oral (drinking water) chronic rat study • Weanling SD rats, males only • 13 animals/ group, 4 groups	Exposure • Sodium fluoride (NaF) • **0, 0.9, 1.9, 3.8 mg/kg bw/day*** (0, 15, 30, 60 mg/L NaF) • Vehicle – water • 18 months of exposure Outcomes assessed • **Neurotoxicity** • Specific outcomes: - Ca2+ concentration in rats' hippocampus - CaMKIIα expression - c-fos expression - Histology and Immunochemistry of brain	**D-R relationship:** • Results: - **[Ca2+] increased in all treatment groups**, with significant increases noted in the 30 and 60 mg/L groups - CaMKIIα increased significantly in the 30 and 60 mg/L groups - c-fos increased significantly in the 30 and 60 mg/L groups	"In conclusion, our data showed fluorosis could lead to the enhancement of [Ca2+] and the expression level of CaMKIIα and c-fos in the rat hippocampal CA3 region. The results support the idea that fluorosis can exert neurotoxic effects by changing the [Ca2+] in nerve cells. Calcium overload in the hippocampus may be the initiating factor of neuronal apoptosis induced by fluoride. We deduce that Ca2+/ CaMKIIα/c-fos channel signal may be a molecular mechanism of central nervous system damage induced by chronic fluoride intoxication"	1
Zhang 2020 [304]				
Oral (drinking water) subchronic rat study • Four-weeks-old SPF-level Wistar	Exposure • Sodium fluoride (NaF) • **0, 3.5, 7, 14 mg/kg bw/day*** (0, 25, 50, 100 mg/L F) • Vehicle – distilled water • 90 days of exposure	**D-R relationship: An increase in learning impairment with increase in F exposure** • Results: - Learning Impairment ((Morris water maze): the average escape latency had an increasing trend with the increase	"This study shows that excessive intake of fluoride via drinking water would impair the learning ability of rats. The impairment of the ability of the	2

Study design	Exposure[28] & Outcomes	Results	Authors' conclusion	Quality
rats, males and females only • 10 /sex/ group, 4 groups	Outcomes assessed • **Neurotoxicity** • Specific outcomes: - Learning Impairment - Neuronal Autophagy	of fluoride exposure indicating fluoride-induced learning impairment. The escape latency of the rats in the 100 ppm group was significantly longer. - Neuronal Autophagy: the expression of Beclin-1 increased with the concentration of fluoride. Beclin-1 expression was significantly higher in the 50 and 100 ppm group. - Ultrastructural Abnormalities: lipofuscins increased in all groups with an increasing trend with the increase of fluoride exposure. Number of liposomes increased while the number of organelles decreased in the 100 ppm group.	hippocampus to collect and respond to external information may be related to a large amount of autophagy in the hippocampal CA1 and DG region neuron."	

Table 9: Study characteristics and results of included tier-2 animal studies

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
Owumi 2019 [225]			
Adult male Wistar rats (n=32; 10 weeks old)	Exposure • 0 (corn oil), 15 mg/L NaF (~5mg/kg bw F) in DW • 14 days Outcomes assessed • **Hepatotoxicity and Renal toxicity** • Liver and kidney function (serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)) • Levels of reactive oxygen (ROS) and nitrogen species (RONS); and antioxidant status of liver and kidneys (activity of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and GSH) Proinflammatory biomarkers (nitric oxide (NO), myeloperoxidase (MPO), TNF-α and IL-1β) and caspase-3 (CASP3) activity in liver and kidneys • Histopathology of liver and kidney	• F didn't induce any significant changes in body weight or relative tissue weights of liver or kidney • The serum ALT, AST, ALP, and LDH activities were significantly elevated in F-exposed rats • F caused a significant decrease in SOD, CAT, GPx, GST and GSH activities; and significantly increased in RONS and LPO levels in the liver and kidney • F exposure significantly increased the hepatic and renal MPO activity and NO, IL-1β, and TNF-α levels • F treated rats exhibited tubular desquamation, disseminated glomerular congestion with cellular infiltration by inflammatory cells in the kidney; and focal area of necrosis and mild infiltration by inflammatory cells were seen in liver	Rats exposed to fluoride (15 mg/L in drinking water) for 14 days demonstrated hepatorenal toxicity with the decrease in antioxidant enzyme activities, in elevation of ROS & RONS levels and histopathological damage via enhancement of oxido-inflammatory responses and caspase-3.
Podder 2011 [233]			

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
Male Swiss-albino mice (2–3 months old), 5 mice/group	Exposure • Group I (control): safe drinking water (0.1 mg/L F) Group II: NaF 15 mg /L for 30 days Group III: NaF 15 mg /L for 30 days + safe drinking water for 7 days Group IV: NaF 15 mg /L for 30 days + safe drinking water for 30 days Group V: NaF 15 mg /L for 30 days + safe drinking water for 90 days Outcomes assessed • **Genotoxicity** • Cell death, chromosomal aberrations (CAs) and chromatid breaks	• F-treatment was followed by safe drinking water for different time durations resulted in an increase in percentage of dead cells in bone marrow in groups II–IV compared with the control group • Significant increase in percentage of aberrant cells (cells with chromatid breaks) and chromatid breaks in groups II and III • Compared to group-II mice, group-III and IV showed significant decrease in percentage of aberrant cells and chromatid breaks although their values still remained significantly higher than the control	Treatment with 15 mg NaF/L for 30 days through drinking water followed by substitution with safe drinking water for 30–90 days significantly reduced chromosomal aberrations however these values remained significantly higher than the control group.
Ranjan 2009 [241]			
New Zealand white male rabbits (n=24; 4-6 weeks old); 6/group	Exposure • 0 (control), 50, 100, and 200 mg/L NaF • 90 days Outcomes assessed • **Oxidative stress** • Changes in oxidative stress indices in erythrocytes, liver, and kidneys	Lipid peroxide levels were positively, SOD and CAT levels were negatively correlated with the F exposure in RBC, liver and kidneys.	Excess F exposure is associated with oxidative damage in RBCs, liver and kidney tissues of rabbits.
Reddy 2014 [243]			

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
Male Wistar rats (n= 24; 4 months old), 6 per group	Exposure • 0, 20, 60 and 100 ppm • 90 days Outcomes • **Neurotoxicity and Immunotoxicity** • Brain F levels • Neurotransmitter levels in brain • Immunological effects (analysis of CD4 cells, IgG1 & NK cells in rat spleen and blood) • Oxidative stress in brain, blood and spleen	• An exponential increase in brain F content with an increase in F conc in DW • A significant change in various neurotransmitters (epinephrine, histamine, serotonin and glutamate) was observed • A significant dose-dependent reduction in CD4 cells, IgG1, NK of blood and spleen was observed • Similarly, a significant dose-dependent decrease in anti-oxidant enzymes (SOD, GPx, catalase) was noted	At higher exposures, NaF exhibited neuroimmunological and oxidative stress in rats. The results also showed that NaF may cause neurotoxicity.
Shashi 2017 [254]			
Young male Wistar rats; 6 per group	Exposure • 0, 100, 200, and 300 ppm NaF/kg bw/day by oral gavage • 40 days Outcomes assessed • **Reproductive toxicity** • Levels of gonadotropins and reproductive hormones (FSH, LH, testosterone, and intratesticular testosterone levels)	• A significant increase in serum level of FSH, LH; and significant decrease in both serum testosterone and intratesticular testosterone levels were observed at the end of 40 days in all F treatment groups	The present study demonstrates that excess fluoride exposure can induce endocrine hormone disruption over the hypothalamic-pituitary-testis axis by influencing the regulation of reproductive hormones, hence causing deleterious effects on spermatogenesis and

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
			alters sperm and semen quality.
Song 2014 [256]			
Male Sprague-Dawley rats, 12 rats/ group	Exposure • 0, 50, 100, and 200 mg/L of NaF in drinking water • 120 days Outcomes assessed • **Kidney toxicity** • Urinary F levels • Histology of kidneys • Apoptosis and DNA damage in kidneys • Immunohistochemistry of kidneys	• Urine fluoride levels were significantly higher in all of the F treated groups • NaF treated rats showed abnormal pathology in kidneys including hydropic degeneration of epithelial cells of tubule in renal cortex, interstitial fibrosis, chronic inflammatory cell infiltration and structure damage of tubular cells • The percentage of cells in early stages of apoptosis, the percentage of late apoptotic/dead of cells and the percentage of total apoptosis in the kidneys was significantly increased in all F-treated groups • A concentration-dependent increase in % tail DNA, an indicator of DNA damage, was observed	The current study demonstrated that NaF treatment exerts pronounced negative effects on renal cells, including histopathological changes, increased apoptosis, and DNA damage, as well as the increased expression of cytosolic Cyt C and cleaved caspases 9, 8, and 3 protein levels in a dose-dependent manner in rats.
Usuda 2016 [271]			
Male Wistar rats (9-weeks old), 5 per group	Exposure • Control: 0 mg F Low-dose NaF: 2.1 mg F	• Highest change in median UV value was noted in LG-NaF and MG-ZnF2 groups • The median NAG values in the high-dose HG-NaF, KF, and ZnF2 groups showed 2.0, 2.2,	Our results suggest the leakage of NAG into urine dose-dependent in NaF, KF, and ZnF2. The decline

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	Middle-dose NaF: 4.3 mg F High-dose NaF: 5.4 mg F Low-dose KF: 2.1 mg F Middle-dose KF: 4.3 mg F High-dose KF: 5.4 mg F Low-dose ZnF2: 2.1 mg F Middle-dose ZnF2: 4.3 mg F High-dose ZnF2: 5.4 mg F • Single dose Outcomes assessed • **Kidney toxicity** • Cumulative 24-h urine volume (UV), N-acetyl-β-D-glucosaminidase (NAG), and urine creatinine (Creatu) • Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum creatinine (Creats)	and 1.8 times higher than control, respectively ($p < 0.05$ with ≥90th percentile of control) • Highest change in median AST values was observed in MG-NaF and LG-KF groups • The median ALT level of all experimental groups was within the 10th - 90th percentile of controls • Excretion of fluoride was highest in HG-ZnF2 and MG-ZnF2 groups	of GFR for glomerular function disorder was remarkable in the high-dose ZnF2 group, which was placed at the top of the PIM [Probability-Impact Matrix] chart with the highest risk impact factor due to the tubular and glomerular damage it causes.
Wang 2019 [279]			
Female Sprague-Dawley rats (N= 48, 3-weeks-old), 12 per group	Exposure • 0, 25, 50, and 100 mg F/L (NaF salt) • 70 days Outcomes assessed • **Immunotoxicity**	• VH, CD, VH/CD of duodenum, jejunum and ileum were significantly reduced • the content of glycoproteins secreted by the goblet cells of duodenum, jejunum and ileum was significantly decreased in the F 100 group • IL-2, IL-6, TNF-α content was significantly decreased in all treatment groups	Excess F exposure induced morphological changes and immunity in small intestine of rats through decreasing its developmental parameters and the

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	• Small intestine morphology (villus height (VH), crypt depth (CD), and villus height to crypt depth ratio (VH/CD)) • Serum cytokine contents (IL-1β, IL-2, IL-6, and TNF-α)		distribution of immune cells, glycoprotein, and cytokine contents in the serum.
Wang 2017 [280]			
Female Kunming mice (30-day old) F0 generation; 21 per group into 4 groups	Exposure • F0 and F1 generation: 0, 50, 100, 150 mg F/L in DW (NaF salt) • 90 days (both F0 and F1 generations) • F0 females mated after 90 days exposure with healthy males by housing at 3:1 ratio F1: healthy F1 generation female mice (4 weeks old); 21 per group into 4 groups Outcomes assessed • **Reproductive toxicity** • Histology and ultrastructural changes in uteri tissues • Expression levels of MMP-9/TIMP-1, a member of matrix metalloproteinases (MMPs) and the tissue inhibitor of matrix metalloproteinases (TIMPs) families	• The rates of pregnancy in the F groups were decreased in a dose-dependent manner • The litter size and birth weight of F1 and F2 mice of both F100 and F 150 group were significantly decreased • Compared to controls, F150 group mice had endometrial epithelial cells irregularly arranged, intercellular space became large, and the boundary of endometrial epithelial cells was not clear; moreover, the following ultrastructural changes were observed: vague nucleus, microvilli reduction, increased lysosomes, a dilated endoplasmic reticulum, and mitochondrion vacuolization • the mRNA expression levels of MMP-9 in the F 150 group were consistently increased from the 2nd until the 5th days and then gradually decreased on the 6th and 7th days; similarly, the mRNA expression levels of TIMP-1 were significantly increased and peaked on the 5th	The results suggest that the excess F exposure in mice for 90 days causes ultrastructural changes in uterii and affect the embryo implantation process via interfering in the MMP-9/TIMP-1 system; may also reduce litter size in female mice.

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
		day. Also, corresponding protein levels of MMP-9 and TIMP-1 were significantly increased in the F 150 group on the 3rd and 5th days	
Wei 2016b [285]			
Wistar rats; 5 per sex per group	Exposure • 0, 50, 150, 250 mg/L NaF • 24 weeks Outcomes assessed • **Serum proteomics** • Serum protein expression profiles	• Expression levels of A2M, C4BPA, ORM1, C9, KNG2, SERPINA3N, CP, HPX, HP, and KNG1 showed an increasing trend in the 50 mg/L group, and in contrast decreasing trend in the 150 and/or the 250 mg/L group. Five proteins (A1BG, RGD1564515, F1LN61, F1LM30, and F1LPQ6) revealed a decreasing trend in the 50 and 150 mg/L groups • Most differentially expressed proteins belonged to: inflammatory response (46.9%), response to wounding (53.1%), acute inflammatory response (37.5%); suggesting inflammation and immune reaction proteins were involved in the pathogenesis of fluorosis.	The serum protein expression profile of F-treated mice suggests that the low-dose NaF may promote complement, inflammation, and immune responses, whereas moderate- and high-dose NaF may inhibit these responses; and the proteins identified in this study may serve as biomarkers for fluorosis.
Yan 2007 [293]			
Female B6 and C3H inbred mice	Exposure • 0, 50, 100 ppm F (NaF salt) • 3 weeks	• Significant increase in bone fluoride content with increasing fluoride exposure, in both strains of mice	This study demonstrates that increasing F doses at physiological levels has strain-specific effects on

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
(3-weeks old); 6 mice per group	Outcomes assessed • **Bone/skeletal related toxicity** • Bone F content • Osteoclastogenesis and hematopoietic colony-forming cell assays • Biomechanical testing of bones	• No change in serum osteocalcin levels in neither strain • In C3H mice, significant increase in osteoclast potential was correlated with: increased F exposure, serum PTH, serum RANKL, serum OPG, serum TRAP5b and bone osteoclast numbers • Tibia trabecular bone quantity and architecture were significantly different between the different F treatment groups for B6 mice only; No significant changes in femur cortical bone were observed between the F treatment groups for either mouse strain	bone physiology in mice such as the increase in intact PTH, changes in osteoclastogenesis and increase in CFU-M (monocyte/macrophage), CFU-GM (granulocyte and macrophage), and CFU-GEMM (multipotential) suggesting a role of F in the early stage of osteoclastogenesis.
Yan 2016 [295]			
Adult Wistar rats (5-weeks old); 10 per sex per group	Exposure • 0, 60, 120 ppm F (NaF salt) • 10 weeks Outcomes assessed • **Neurotoxicity** • F content in serum and brain • Ultrastructural changes in brain • Apoptosis in neurons • Bax and Bcl-2 Expressions in the Brain	• Dose-dependent increases of F levels in serum and brain tissues • In 60 ppm group, brain cells appeared cytomorphotic, with intranuclear heterochromatin margination condensation, mitochondrial outer membrane: part vague, rough endoplasmic reticulum: gently expanding, cellular membrane: part swollen. In 120 ppm group, brain cells appeared obviously intranuclear heterochromatin margination aggregated, cellular membrane	Based on the current results, the authors conclude that fluoride exposure induces neuron apoptosis and expression of inflammatory factors by activating microglia in rat brain.

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	• Inflammatory factor expressions in the Hippocampus and Cortex region	dissolved, with shrinkage of nuclear and cell volume, organelle dissolved, and apoptosis presented • Apoptotic cells (TUNEL-positive staining) increased with increasing fluoride concentrations • A dose-dependent correlation between expression of Bax and fluoride concentration and a negative correlation was found between Bcl-2 expression and fluoride concentration in the cortex • Indexes of Bcl-2/Bax in the hippocampus significantly lower than the control group, suggesting apoptosis in brain cells	
Zhao 2017 [316]			
Healthy Wistar pregnant rats; 11 per group	Exposure • 0, 1500 mg/L (prior to delivery all rats received distilled water alone; after delivery, maternal rats were given either 0 or 150 mg/L NaF continued to male puppies (same as to their maternal rats) after their weaning (3 weeks old) for 15 weeks) • 15 weeks Outcomes assessed	• An increasing trend in femur F content with an increase in duration of exposure • Sperm count and motility were significantly decreased in treated rats with exposure duration • In treated rats, the seminiferous tubules of each age were reduced in terms of diameter and thickness; the sperm cells were lost and shedding and finally disappeared after 9 weeks	NaF exposure altered organ coefficient, sperm quality, total protein content of testis and testicular histology, as well as the mRNA and protein expression levels of HSP27, 79, 90 and HSF in the testis of rats with an increase in the femur

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	• **Reproductive toxicity** • Femur fluoride determination • Organ coefficient of the testes and epididymis • Sperm quality evaluation • Testis histology • Immunohistochemical analysis of testis for expression of HSP27, HSP70, HSP90 and HSF	• Testicular morphological abnormalities were increased with exposure duration in treated rats • The relative mRNA expression levels of HSP27, 70, 90 and HSF in treated rats' testes were significantly changed	fluoride concentration. In addition, in terms of HSPs, significant differences following NaF exposure were observed in the puberty.
Zhou 2013 [320]			
Sexually mature (8-10 weeks old) SD rats, females only 20 animals/ group, 4 groups	Exposure • 0, 100, 150, 200 ppm NaF in water • 6 months of exposure Outcomes assessed • **Reproductive toxicity** - Fertility assessment - Relative weights of reproductive organs - Histopathological examination - Serum hormones - Immunohistochemistry	• Successful pregnancy: rates of successful pregnancy was declined in a dose-dependent manner • Organ coefficients: The ovarian organ coefficients were statistically lower in all treatment groups and the uterine organ coefficients increased statistically in 150 and 200ppm groups. • Serum hormones: Serum E2, P, and LH levels decreased in all treatment groups; serum T levels were statistically lower in100 and 200 ppm groups; serum FSH levels were statistically lower in 50 and 200ppm groups. • Uterine histology: the endometrial cells became larger, and the endometrial glands became hypertrophic. Blood vessels in the myometrium had altered shapes and sizes.	"In the present study, we demonstrated the following results. (1) The fertility of female rats may be inhibited after NaF exposure. (2) The secretion of E2, P, T, LH and FSH was suppressed in rats exposed to NaF. (3) NaF exposure decrease d ovarian and uterine weight. (4) The structures of the ovary and uterus were damaged in NaF-treated rats. These results indicate that the reproductive function of female rats exposed to NaF is

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
		• Ovarian histology: the total number of each type of follicle decreased in all treatment groups.	inhibited. The possible mechanism underlying NaF-induced fertility reductions is as follows: NaF hinders reproductive hormone synthesis and secretion, weakening its ability to regulate the ovary and maintain pregnancy. The ovarian and uterine structures may also be destroyed by NaF.”
Adedara 2017 [84]			
Adult male Wistar rats (8 weeks old) group size 8	Exposure • 0, 15 mg/L • 45 days of exposure Outcomes assessed • **Renal toxicity** • Oxidative damage and Thyroid dysfunction: glutathione, malondialdehyde, superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase	• Decreased glutathione, malondialdehyde, superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase	Chronically exposed to NaF induced renal toxicity in rats by increasing oxidative stress indices, decrease of antioxidant enzyme activities, and the functional status of the thyroid system
Ahmad 2012 [85]			

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
Male albino mice (3–4 months old) group size: 10	Exposure • 0, 50 ppm • 10 days of exposure Outcomes assessed • **Reproductive toxicity** • Toxicity in testis	• Loss of interstitial tissue, spermatogonia, and spermatogenesis. • Decrease in the average number of spermatogonia perspermatic cord. • Decline in the mean cross-sectional area (CSA) of the seminiferous tubules, whereas increase in the mean CSAs of spermatogonia and primary spermatocytes. • Decline in head length, breadth, tail length, and the length and diameter of the middle part of sperm.	NaF induced steroidogenesis and spermatogenesis in males
Baba 2016 [91]			
Wistar rats weighing, group size: 6	Exposure • 0, 1ppm, 10ppm • 28 days of exposure Outcomes assessed • **Renal toxicity** • Levels of glutathione, malondialdehyde, superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase	Increases in plasma protein, blood urea nitrogen, and creatinine levels	Concurrent exposure to fluoride increased the extent of renal damage, which is due to increased free radical formation and a reduced function of the antioxidant system in renal tissue.
Basha 2011 [96]			

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
Adult albino Wistar rats, male 200–250 g, female 170–200 g, group size: 8	Exposure • <1 ppm, 100 ppm, 200 ppm • Exposure through gestation period Outcomes assessed • **Endocrine and Thyroid related toxicity** • Serum thyroid hormones, brain histopathology, and learning memory: serum thyroid hormones (FT3 and FT4), acetylcholine esterase activity, spatial learning and memory	• Decreased serum-free thyroxine (FT4) and free triiodothyronine (FT3) levels and decreased acetylcholine esterase activity. • Presence of eosinophilic Purkinje cells, degenerating neurons, decreased granular cells, and vacuolations in discrete brain regions. • Poor acquisition and retention and higher latency In the T-maze experiments.	Fluoride ingestion continuously through multiple generations induced generational or cumulative effects on the development of the offspring.
Bondu 2017 [105]			
Male Sprague-Dawley rats ≈ 200g, group size 6	Exposure • <1 ppm, 15ppm, 50 ppm • On Vitamin D deficient (test groups) and adequate (control groups) diet • 180 days of exposure Outcomes assessed • **Renal or Kidney Toxicity** • serum osteocalcin, parathyroid hormone, C terminal telopeptide of type I collagen, creatinine, Cystatin C, bone mineral density	• Increased Alkaline Phosphatase and Osteocalcin • C terminal telopeptide levels increased with moderate fluoride exposure and decreased with high fluoride exposure	High fluoride intake deteriorates renal tubular function
Bondu 2019 [106]			

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
Male Sprague–Dawley rats, group size 6 **Bulduk 2020** [108]	Exposure • <1 ppm, 15ppm, 50 ppm • On Vitamin D deficient (test groups) and adequate (control groups) diet • 210 days of exposure Outcomes assessed • **Bone/ skeletal related toxicity** • bone damage: serum total 25OHD, PTH, Osteocalcin, CTx, ALP, calcium, phosphorus and creatinine, albumin, fluoride and urinary cystatin C. • Bone Mineral Density (BMD) and Bone Mineral content (BMC)	• Increased BMD, serum ALP, bone fluoride content, Osteocalcin, and urine fluoride in both control and test groups with increase in F concentration • Mild thickening and increased osteoid in 80% of the Vitamin D deficient rats. • Fluoride deposited in rat bone affects both osteoblastic and osteoclastic activity	Fluoride deposits in bone and affects bone remodeling.
Sprague Daley rats weighing 200-250 g (4 groups of 10 females, and 4 groups of 10 males)	Exposure • Groups of n=10 • 0 mg/L of NaF and 0 mg/L of resveratrol (control), 10 mg/L of NaF, 50 mg/L of resveratrol, 10 mg/L of NaF and 50 mg/L of resveratrol • 90 days of exposure Outcomes assessed • **Cardiovascular**	• For each gender, the most marked elevations in the blood pressures were seen in the NaF group. • In both the male and female groups, the chronic administration of resveratrol with NaF led to decreased blood pressures. • The contraction response resulting from phenylephrine administration was increased in the groups administered NaF, whereas it was	Resveratrol provides a protective effect against the increased blood pressure caused by NaF and the potential endothelial damage. The protective effect of resveratrol results from its capability to reduce fluorine induced oxidative

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	• The effect of resveratrol therapy on the contraction-relaxation responses of the thoracic aorta rings and, on the blood pressure of rats exposed to chronic fluorosis • Serum fluorine level • Blood pressure • Contraction response	decreased in the groups administered NaF and resveratrol.	stress and endothelial tissue damage.
Cao 2019 [110]			
APP/PS1 double-transgenic mice, B6.Cg-Tg (APPswe, PSEN1dE9) with a 85Dbo/Mmjax background, 3 months old, both male and female, group size 10	Exposure • 100 mg/L, 1000mg/L • 84 days of exposure Outcomes assessed • **Neurotoxicity** • Morris water maze test of spatial learning and memory • Senile plaques, ionized calcium binding adaptor molecule 1 (Iba-1), and complement component 3 (C3) expression, Aβ42, synaptic proteins and enzymes that cleave APP, malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px).	• Decline in learning and memory in shorter time. • Increased senile plaques and level of Aβ42, Iba-1, and BACE1, while reducing the level of ADAM10 in their brains. • Decreased synaptic proteins and enhanced oxidative stress in the hippocampus of APP mice.	Exposure to fluoride, even at lower concentration, can aggravate the deficit in learning and memory and neuropathological lesions of the mice that express the high level of APP.
Chaudhary 2010 [117]			
Adult male albino rats, group size 10	Exposure • 14.29 mg/L	• Increase in serum AST and ALT.	Fluoride exposure increase serum AST, ALT, total

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	• 30,45,60 days of exposure Outcomes assessed • **Diabetes/ glucose or lipid metabolism related toxicity** • Enzyme profile and lipid profile: serum ALT, AST, LDH, total cholesterol, triglycercide, LDL, HDL, VLDH	• Increase in serum total cholesterol, LDL, VLDL, TG, and decrease in HDL.	cholesterol, LDL, VLDL, TG, and decrease in HDL
Chen 2013 [118]			
8-weeks-old male Sprague–Dawley rats (weighing 200–210 g), group size 6	Exposure • 20 mg/L • 84 days of exposure Outcomes assessed • **Bone/ skeletal related toxicity** • Bone health: bone mineral density, biomechanical test of femur	• Slightly increased vertebral bone mineral density • Negatively affected bone biomechanical property and bone microstructure.	Fluoride slightly increased vertebral bone mineral density but negatively affected bone biomechanical property and bone microstructure.
Choudhary 2020 [120]			
Swiss albino female mice. Four groups of eight animals each	Exposure • Group 1: 0 ppm NaF • Group 2: 100 ppm NaF • Group 3: 250 ppm NaF • 18 days exposure Outcome assessment:	• Significant decrease in maternal bodyweight in 100ppm and 250 ppm groups • Significant differences in live fetuses, dead fetuses, fetal weight and fetal size in NaF groups compared to the control	• -"NaF treated mice showed decrease in weight as compared to control" • -"Number of dead fetuses in high dose of NaF treated group got

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	• **Bone/Skeletal related toxicity** Skeletal examination • Reproductive toxicity Fertility tests: number of implantations, number of resorptions, number of viable fetuses and dead fetuses, number of stunted fetuses, maternal body weight and placental • Other: Weight, fetal body weight and size.	• Reduced ossification, higher prevalence of rib defects, and skeletal malformation in NaF treatment groups	increased when compared to the control group" • -"The treatment of NaF in this study also affected the average body weight of pups and the placental weight." •
Chu 2020 [126]			
Male BALB/c mice (4 weeks old, n=64), 16 mice/group	Exposure • 0 (control), 25, 50, and 100 mg F/L • 3 months of exposure Outcomes assessed • **Bone/skeletal related toxicity** • Bone histopathology • Dental fluorosis • Bone F concentration • Serum biomarkers (ALP, OCN) for bone differentiation • Protein expression in bone tissue (Wnt/b-catenin signaling pathway)	• In the high F group, tibial trabecula enlarged and merged into large pieces with the adjacent bone trabeculae, F increased cancellous bone formation, and there was thickened cortical bone in the femur of mice exposed to F, especially in high F group • Prevalence rates of dental fluorosis in the three fluoride groups were 43.75% (25 mg F/L), 93.33% (50 mg F/L) and 100% (100 mg F/L). Mice in high F group showed severe dental fluorosis, characterized by white spots, cloudy splotches and pitting • There was a dose-dependent positive association with F concentration in drinking water and F in spinal bone.	“Fluoride up-regulates the expression of Wnt/b-catenin signal transduction molecules and Runx2, contributing to aberrant osteoblasts activity and osteogenesis, and b-catenin plays a pivot role in fluoride-induced viability and differentiation of osteoblasts.”

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
		• Serum concentrations of ALP and OCN (biomarkers of bone differentiation) were increased in middle and high F groups • Wnt3a (ligand of Wnt/b-catenin signaling pathway) was significantly up-regulated in the 50 and 100 mg/L F- groups. F gradually increased the protein expression of Gsk3b phosphorylation, b-catenin and its downstream target gene Runx2, which was accompanied by translocation of b-catenin into the nucleus induced by fluoride. F exposure was correlated with increased Wnt3a, b-catenin, the ratio of p-Gsk3b (Ser9) to Gsk3b and Runx2 protein levels	
Dey 2021 [132]			
Male Swiss albino mice (n=54, ~ 20g, 1 month old), 9 mice/group	Exposure • Group I (control): < 0.5 ppm F • Group II: 6.8 ppm F for 4 months • Group III: 6.8 ppm F for 8 months • Group IV: 6.8 ppm F for 4 months, then fresh drinking water (containing < 0.5 ppm of F) for next 4 months • Group V: 6.8 ppm F for 4 months, then drinking water (containing < 0.5 ppm of F) supplemented with calcium and vitamin D (2.5-g calcium kg−1	• Teeth whitening distinctly evident after 1 month of F treatment. In group II, complete whitening of lower incisors was observed after 4 months of F treatment. Teeth became chalky white with enamel erosions after 8 months of treatment in group III. • Groups II and III exhibited osteosclerosis/increased hardening of the bone. Mild calcification of pelvic bone was observed in group III.	"Prolonged exposure to environmentally relevant concentration of F accumulates in the teeth and bone leading to development of dental and skeletal fluorosis." Exposure to F altered the metal profile of bone and worsened skeletal health.

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	diet and 1000 IU vitamin D kg−1 diet) for next 4 months • Group VI: 6.8 ppm F and supplemented with calcium and vitamin D (2.5-g calcium kg−1 diet and 1000 IU vitamin D kg−1 diet) for 4 months • 4-8 months of exposure Outcomes assessed • **Bone/skeletal related toxicity** • Dental fluorosis • Skeletal fluorosis • Bone elemental content • F content in bone • Behaviour • Locomotion	• F exposure significantly decreased Ca, Zn, Mn, K, Ni, and S levels in the bone while increased magnesium Mg and Fe was observed. In Group IV, Ca, Zn, Mn, and K levels increased compared to Groups II and III. • F content in the bone was significantly higher in all treated groups, except in group V where F content was comparable to control group levels. F content was highest in group II, followed by group III, VI, and IV. • No signs or symptoms of behavioral changes. • Mice in groups II and III showed slight restrictions in activities like walking and movement of the head and limbs. Changes in locomotion were not observed in other groups.	
Dhurvey 2016 [133]			
Adult female albino rats, weighing about 180–200 g, group size 6	Exposure • 0, 5, 10, 15, and 20 mg NaF/kg body weight/day • 30 days of exposure Outcomes assessed • **Reproductive toxicity** Estrous cycle and ovarian hormones: serum follicle-stimulating hormone (FSH), luteinizing	• Reduced body weight in the rats ingesting 10, 15, and 20 mg NaF/kg bw/day • Reduced ovarian weight in the rats ingesting 15 and 20 mg NaF/kg bw/day. • Increased duration of the proestrous phase in the 10, 15, and 20 mg NaF/kg bw/day group.	Exposure of female albino rats to NaF in drinking water might have some immediate harmful effects on the reproductive system.

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	hormone (LH), and estrogen	• Decreased diestrous, estrous, and metaestrous phases in the 15 and 20 mg NaF/kg bw/day groups. • Decreased hormonal concentrations of luteinising hormone in the 15 and 20 NaF/kg bw/day groups, follicle-stimulating hormone in the 10, 15, and 20 NaF/ kg bw/day groups, and estrogen in the 10, 15 and 20 NaF/kg bw/day groups.	
Ferreira 2021 [138]			
Female pregnant wistar rats (n=6, 150-200 g, 90 days old) and their male offspring (sample size not reported)	Exposure • 0 (control), 10, 50 mg F/L • 42 days of exposure Outcomes assessed • **Mechanistic** • F plasma concentrations • Oxidative stress • BDNF expression in hippocampus • Hippocampal proteome	• F exposure increased plasma F concentration in treatment groups compared to control group • Oxidative biochemistry analyses showed that F caused a decrease of ACAP in 10 mg/L group and in 50 mg/L group compared with control group. There was also a marked increase in MDA levels and nitrite levels for both treated groups. • mRNA analysis of whole hippocampus indicated that there was an increase BDNF expression in both exposure groups compared to controls. • In the 10 mg F/L group, there were changes in proteins associated to axogenesis, positive regulation of neuron projection development,	"Exposure to both F concentrations during pregnancy and lactation increased the F bioavailability, triggered redox imbalance featured by a decrease of ACAP, increase of LPO and NO−2 levels, BDNF overexpression and changes in the hippocampus proteome."

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
		glycolytic process and regulation of calcium ion transport. In the 50 mg F/L group, proteins associated with morphogenesis of neuronal projection processes, regulation of neuron projection development, axogenesis, glycolytic process and regulation of ERK 1 and 2 cascade.	
Geng 2014 [143]			
female Sprague-Dawley rats, group size 10	Exposure • 100 or 200 mg/L • 180 days of exposure Outcomes assessed • **Reproductive toxicity** Female fertility: ovarian apoptosis, ROS, SOD, CAT and GSHPx activities and MDA content	• NaF induced ovarian apoptosis, with concomitant activation of oxidative stress. • Exposure to NaF activated extracellular regulated protein kinase (ERK) and c-Jun NH2 kinase (JNK), disrupting the ERK and JNK signaling pathways, while p38 and PI3K remained unchanged	Oxidative stress may play a key role in NaF-induced ovarian dysfunction by activating the apoptotic ERK and JNK signaling pathways.
Hosokawa 2015 [151]			
4–5-weeks-old male BALB/c mice weighing 23.2±0.2 g, group size 6	Exposure • 1, 5, 25, and 125 ppm • 30 days of exposure Outcomes assessed • **Immunotoxicity**	• Reduced intake of food or water per body weight in the 125-ppm group. • Reduced relative weights of spleens in the 1- and 5-ppm groups. • Decline in mRNA expression of TNFα in the macrophages in the 125- ppm group.	The F concentration in the blood in this study may not be sufficiently high (as in vitro studies) to affected mRNA expression in vivo.

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	Immunotoxic effects: TNFα, IL-1β, β-actin, IFN-γ and IL-2		
Inkielewicz-Stepniak 2012 [153]			
Wistar Han rats (6-weeks old male and female rats weighing ~220 and ~170 g), group size 10	Exposure • 0, 12 mg/L • Days of exposure not reported Outcomes assessed • **Hepatic and renal toxicity** Liver and kidney function: nitric oxide level, thiobarbituric acid reactive substances, advanced oxidation protein products, total antioxidant status, glutathione, protein content in post nuclear supernatant fractions of the liver and kidney	• Fluoride enhanced oxidative and nitrosative stress in investigated tissues. • No gender difference was observed.	F enhanced oxidative and nitrosative stress in investigated tissues.
Kaya 2012 [165]			
Tuj sheep weighing 31±2 kg, group size 10	Exposure • 4 ppm • 270 days of exposure Outcomes assessed • **Bone/skeletal related toxicity** • Calciotropic hormone: serum parathyroid hormone (PTH) and calcitonin (CT) activity levels	• Decreased serum PTH levels • Increased serum CT levels	Fluorosis in sheep incurred a decrease in the PTH levels and an increase in the CT levels, which may be the result of a temporary rise in serum Ca.

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
Khan 2019 [166]			
Weanling male A/J and 129P3/J mice	Exposure • 15ppm, 50ppm • 42 days of exposure Outcomes assessed • **Hepatotoxicity** Liver proteome profiles	• Fold change in liver proteins more pronounced in lower F treatment group. • Most of the proteins with fold change upon treatment with 15 ppm F were increased in the A/J mice compared with their 129P3/J counterparts. • Most proteins with fold change were decreased in the A/J mice compared with their 129P3/J counterparts, upon treatment with 50 ppm F.	Male A/J mice attempt to fight the deleterious effects of F at low concentration. A/J animals have higher susceptibility to the deleterious effects of F.
Kido 2017a [169]			
11–12-weeks-old ICR-derived glomerulonephritis (ICGN mice), male ICR mice, group size 5	Exposure • 0, 50, 100, and 150 ppm • 28 days of exposure Outcomes assessed • Renal/ Kidney toxicity • Renal function: blood urea nitrogen (BUN), the serum creatinine (CRE), the level of urinary protein, and the creatinine clearance	• For the ICGN mice, at the end of the experimental period, BUN in the 150 ppm group was significantly higher than 0 and 50 ppm groups • For the ICR mice, after 3 days, the BUN in the 150 ppm group was higher than the 0 and 100 ppm groups.	Serious toxic effects of ≥100 ppm F in the drinking water for mice with impaired kidney function.
Kido 2017b [170]			
6-weeks-old male Sprague-Dawley	Exposure • 0, 75, and 150 ppm	Increase in areas or number of cells that stained with Masson trichrome, or with antibodies	M2 macrophage-TGF-β1-fibroblast/myofibroblast-

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
rats with unilateral ureteral obstruction operation, 250–280 g, group size 13	• 14 days of exposure Outcomes assessed • Renal/ Kidney toxicity: transforming growth factor beta 1 (TGF-β1) transcription	against collagen type I, alpha-smooth muscle actin (α-SMA, a myofibroblast marker), ED1, ED2, and ED3 (macrophage markers), and TGF-β1.	collagen synthesis pathway is related to fluoride exacerbated tuburointerstitial nephropathy from UUO.
Kuang 2017 [176]			
ICR mice, group size 60	Exposure • 0, 12, 24, 48 mg/kg bw/day • 42 days of exposure Outcomes assessed • **Immunotoxicity** • Splenic development: splenic growth index, histopathological lesions, T and B-cell subsets and CD4+/CD8+ ratio, cytokine expression levels, IgA, IgG, and IgM contents, cyclins/cdks protein expression	• Decline in growth index and lymphocytes in the white and red pulp • Increased cell percentages of the G0/G1 phase and decreased cell percentages of the S phase • Decline in T cells and B cells as well as IgA, IgG, and IgM contents. • Decreased expression levels of cytokines including interleukin-2 (IL-2), transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and cyclin (E/D and CDK2/4 • Increased protein expression level of interleukin-10 (IL-10)	NaF in 12 mg/kg and over causes toxic effects on the splenic development in mice. Cell cycle arrest is the molecular basis. Cellular and humoral immunity were impaired due to the reduction of T, B cell numbers and activities.
Leite Ade 2007 [177]			
male Wistar rats with 75 days and weighing	Exposure • 0, 10, 20, 40, 60, 80 and 100 mg/Kg bw/day • 2 hours of exposure	No change in the level of DNA strand breaks in all organs at all doses in the mean tail moment.	Oral exposure to NaF did not result in systemic genotoxic effect in multiple

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
approximately 270 g, group size 5	Outcomes assessed • **Genotoxicity** DNA damage		organs related to fluoride toxicity
Li 2017 [178]			
3-weeks-old male C57BL/6 mice, group size 10	Exposure • 0, 100mg/L • 105 days of exposure Outcomes assessed • **Bone/ skeletal related toxicity** Bone homeostasis: bone osteoclasts numbers, osteoclasts ultrastructure, osteoclastogenesis, NFATc1 and ATP6v0d2 mRNA expression in osteoclasts	• Impaired bone resorption. • Decline in mRNA expression of nuclear factor of activated T-cells 1 (NFATc1), ATPase H+ transporting V0 subunit D2 (ATP6v0d2) and osteopetrosis-associated transmembrane protein 1 (Ostm1)]	The consumption of fluoride resulted in severe fluorosis and in an impaired OC function.
Lima Leite 2014 [184]			
Male Wistar rats (60 days old), group size 6	Exposure • 0, 10, or 50 ppm • 22 days of exposure Outcomes assessed • **Diabetes/ glucose or lipid metabolism related toxicity** Diabetes: protein functions and protein interaction	• Quantitative intensity analysis of the proteomic data revealed differential expression between diabetic/nondiabetic rats, and between different F concentrations. • The GO annotations with the most significant terms were muscle contraction, carbohydrate catabolic processes, generation of precursor metabolites and energy, NAD metabolic processes and gluconeogenesis.	The presence of the two stress proteins indicates an increase in insulin resistance, which might worsen diabetes.

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
		• Proteins with fold changes interacted with GLUT4. GLUT4 interacting proteins, such as MDH and the stress proteins HSPB8 and GRP78, exhibited decreased expression when D animals were exposed to F.	
Liu 2012 [186]			
SD rats 4 weeks old, group size 20	Exposure • 0, 50mg/L, 100mg/L, and 200mg/L • 150 days of exposure Outcomes assessed • **Endocrine and Thyroid related toxicity** Thyroid function: structural changes in the thyroid gland, expression of vascular endothelial growth factor (VEGF) mRNA, expression and deposition of VEGF	• Increased average relative weight of the thyroid glands. • Proliferation and dilatation of capillary blood vessels enlarged follicles with excessive colloid, and obvious nodules in the thyroid glands. • Increased expression of VEGF mRNA in the thyroid gland and the serum NO levels. • Increased deposition of VEGF in epithelial and follicular cells of the thyroid gland.	Abnormal expression of VEGF induced by fluoride can lead to the proliferation of vascular endothelial cells in the thyroid gland.
Liu 2020 [192]			
Four-week-old male Wistar rats (20 rats per group, 4 groups)	Exposure • 0, 25mg/L, 50mg/L, and 100mg/L of NaF • 12 weeks of exposure Outcomes assessed • **Bone/Skeletal** • Skeletal fluorosis	• Urine fluoride concentrations showed a dose-dependent and statistically significant increase in different fluoride-exposed groups, compared to control group. • The ratio of 2-degree and 3-degree dental fluorosis increased with increasing levels of fluoride exposure.	Urine fluoride concentrations, 2nd and 3rd-degree dental fluorosis, serum sKlotho levels, and sKlotho expression in the kidney and small intestine showed

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	• Kidney and small intestine were isolated for detection of Klotho with immunohistochemistry (IHC). • Femoral artery blood was sampled to measure the serum levels of sKlotho.	• In rats, serum sKlotho levels was significantly higher in F-exposed groups than that in the control group. • Immunohistochemistry results showed that the Klotho expression in the kidney and small intestine increased with increased doses of NaF treatment	a dose-dependent increase
Lu 2014 [198]			
Kunming male mice (8 weeks old, weighing about 20 g), group size 65	Exposure • 0, 50, 100, 150 mg/L • 56 days of exposure Outcomes assessed • **Reproductive toxicity** Sperm chemotaxis: sperm chemotaxis, Ca2+ concentration, adenylate cyclase (AC) content and mRNA expression of mACIII, mACVIII, Golf alpha, CatSper1, CatSper2	• The percentage of chemotactic sperm decreased with NaF in a dose-dependent manner. • Decreased Ca2+ concentration and AC content in the 100 and 150 mg/L groups. • Decreased mRNA expression of CatSper1 in the 100 and 150 mg/L groups.	Excessive fluoride adversely affects sperm chemotaxis. The alteration of Ca2+ concentration, AC content and CatSper1 mRNA expression level may play a key role in the mechanism underlying the affection.
Ma 2020 [201]			
male Wistar rats (3 weeks old; weighing 114.8–180.0 g), group size 20	Exposure • 0, 25, 50, or 100 mg/L • 30 or 90 days of exposure Outcomes assessed • **Bone/ skeletal related toxicity**	• Increased protein expression of BMP-2 and BMP-7 in plasma at 1 month and 3 months. • Increase in BMP-2 expression with an increase of fluoride exposure time.	Fluoride has a dose-response effect on BMP-2 in fluorosis rats, and fluoride-induced hypomethylation of specific CpGs may play an

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
	• Skeletal fluorosis: expression and DNA methylation level of the promoter region off Bone Morphogenetic Proteins (BMP)-2 and BMP-7	• Hypomethylation was observed in 2 CpG sites (CpGs) of BMP-2 and 1 CpG site of BMP-7 promoter regions.	essential role in the regulation of BMP-2 and BMP-7 expression in rats.
Miao 2013 [209]			
male Sprague-Dawley rats (weight = 70–90 g), group size 10	Exposure • <0.1, 50 mg/L • 180 days of exposure Outcomes assessed • **Hepatotoxicity** Liver function: apoptosis and Fas/FasL expressions	• Increased protein and mRNA levels of Fas, and FasL. • Decreased activity of GSH-Px, and SOD. • Increased activity of MDA.	Fluoride induced apoptosis in the liver, thereby causing liver damage in the rats.
Mrvelj 2020 [212]			
Twenty male Sprague-Dawley rats. Four groups of two to seven animals per group	Exposure and Outcomes: • Group 3 received 1.2 ppm F drinking water; Group 4 received 0 ppm water. Groups 1 and 2 were not relevant. • 4 weeks exposure Outcomes assessed: • **Endocrine toxicity** - Dark and light cells per unit area in pineal gland - Total cell numbers in pineal gland	• Pineal glands from Group 3 showed significantly fewer cell counts than Group 4 in both light and dark cells	"In sum, our findings suggest that the removal of dietary fluoride promotes growth of the pineal gland in aged rats. This growth initially involves an increase in supporting cell numbers, followed by subsequent increases in the numbers of both light and dark pinealocytes."

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
Oka 2020 [219]			
C57BL/6 mice (10-week-old, male, body weight 29.4 ± 0.8 g), 2 groups of 6 animals per group.	Exposure • 0 (control), 5 mM NaF treated group • 46 weeks of exposure Outcomes assessed • **Mechanistic** Cell viability and cell apoptosis after exposure to NaF	• Reduced expression of Osterix and Runx2 • The expression levels of ATG5 and Beclin1 were both suppressed by 5 mM NaF in cementoblasts and in periodontal ligament cells • 5 mM NaF induced a high expression of the HIF1-a/p- NFkB axis, which suppressed autophagy and promoted apoptosis. • Treatment with 5 mM NaF enhanced the alveolar bone resorption in both the upper jaw and the lower jaw compared to the control group induced the expression of Cathepsin K and RANKL in periodontal tissues • Upregulated the expression of autophagy related proteins (ROS, p-NFkB, HIF1-a), suppressed the expression of cementoblast markers and induced apoptosis via the downregulation of ATG5 and Beclin1 expression.	• 5 mM NaF reduces autophagy in cementoblasts and increases the expression of HIF-1α. • The oxidative stress activation by 5 mM NaF was also observed with the suppression of autophagy through 5 mM NaF-mediated apoptosis. • Decreased levels of autophagy-related proteins in cementoblasts and the periodontal ligament after 5 mM NaF ingestion. • The inhibition of cell proliferation and increased apoptotic rates after treatment with 5 mM NaF suggests that excessive NaF may be cytotoxic.

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
			• Five mM NaF-treated autophagy was not succent to counteract the NaF-induced cellular damages in HCEM2 cells
Sanchez-Gutierrez 2019 [245]			
Male CD-1 mice aged 45 days old, group size 6	Exposure • 0, 45.2 mg/L • 60 days of exposure Outcomes assessed • **Reproductive toxicity** Spermatozoa Quality, Spermatozoa Mitochondrial Membrane Potential, Caspase 3/7 Enzymatic Activity, Histology Analysis	• Decreased sperm quality (motility, viability, and concentration). • Spermatozoa presented a significant decrease in ψm and a significant increase in activity caspase 3/7. • Decreased urinary fluoride excretion.	Subchronic fluoride exposure of mice with STZ-induced diabetes aggravated testicular damage and the spermatozoa function.
Zhang 2013 [313]			
Oral (drinking water) subchronic mice study • Adult Sprague-Dawley rats, both sex	Exposure • Sodium fluoride (NaF) • **0, 2.2, 4.5, 9 mg/kg bw/day*** (0, 25, 50, 100 mg/L NaF) • Vehicle – distilled water • From pre-pregnancy to PND 56 Outcomes assessed (in male offspring)	D-R relationship**: higher F doses altered testicular histology** • Results: - Testicular histopathology: the testes showed atrophy of seminiferous tubule, injury of spermatogonia and decrease of spermatocytes, as well as absence	Authors conclude that "developmental exposure of rats to fluoride results in testicular ER stress and inflammatory response, as well as oxidative stress and germ cell apoptosis, with defects in

Study Design	Exposure & Outcomes	Results	Authors' Conclusion
• 20 animals/sex/ group, 4 groups (male offspring = 5/group)	• **Reproductive toxicity** • Specific outcomes: - Testicular Histopathology - Testicular ultrastructure - Germ cell apoptosis - Oxidative stress markers in testis (MDA and SOD activity)	of elongated spermatids in the severely damaged seminiferous tubules, indicative of impaired spermatogenesis and loss of germ cells in 50 and 100 mg/L groups; - Testicular ultrastructure: many spermatogonia and spermatocytes displayed the characteristic features of apoptosis, including condensation and margination of nuclear chromatin in 50 and 100 mg/L groups - Germ cell apoptosis: TUNEL-positive cells were notably increased in testes of 50 and 100 mg/L NaF; apoptotic cells accounted for degenerating spermatogonia and spermatocytes - Oxidative stress markers: marked increase of MDA levels in the 50 and 100 mg/L groups; significant reduction in enzymatic activities of SOD in all treated groups	spermatogenesis and accompanying decrease in germ cell count. Furthermore, the present study has also provided important new insights into the roles of ER stress and inflammation in the aggravation of testicular damage.”.

Assessment of quality of the identified animal studies (Tier-1)

The quality of included studies was assessed using the OHAT risk of bias tool [5] as summarized in Table 10. Fifty-six percent of studies (n=20) were of high quality (Q=1), compared to 39% percent that were of acceptable quality (n=14).

Table 10: Quality assessment for animal studies (Tier-1) using OHAT risk of bias tool

Study	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Were there any other potential threats to internal validity	Quality
Cao 2016 [104]	+	NR	++	NR	++	+	+	++	+	1
Cárdenas-González 2013 [106]	+	NR	+	+	+	+	+	+	+	1
Chaithra 2019a [108]	NR	NR	+	NR	+	–	–	++	++	2
Chaithra 2019b [109]	–	NR	NR	NR	++	+	++	++	+	2
Chattopadhyay 2011 [110]	+	NR	++	NR	++	+	+	++	+	1
Gutierrez-Salinas 2010 [134]	+	NR	+	NR	++	+	+	++	+	2
Hosokawa 2010 [136]	NR	NR	+	NR	+	+	+	++	++	2
Hosokawa 2016 [150]	NR	NR	+	NR	– –	–	+	++	–	3

Study	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Were there any other potential threats to internal validity	Quality
Kobayashi 2014 [158]	+	NR	++	NR	++	+	+	++	+	1
Kobayashi 2009 [160]	+	NR	+	NR	+	+	+	++	+	1
Leite Ade 2007 [163]	+	NR	+	++	++	+	+	+	+	2
Li 2021a [180]	+	NR	++	NR	++	+	+	+	+	1
Liang 2020a [166]	+	NR	+	NR	++	–	+	++	+	2
Liang 2020b [167]	+	NR	+	NR	+	–	+	++	+	2
Liu 2016 [171]	+	NR	+	NR	++	+	+	++	+	1
Lobo 2015 [176]	+	NR	NR	NR	–	–	+	+	+	2
Lopes 2020 [196]	+	+	+	+	++	+	+	++	+	1
Lupo 2011 [180]	+	NR	+	NR	+	+	+	++	–	2
Malvezzi 2019 [185]	+	NR	++	NR	+	+	+	++	+	1
Martin-Pardillos 2014 [186]	NR	NR	+	NR	+	–	–	+	–	2
McPherson 2018 [187]	+	NR	++	NR	+	++	++	++	++	1
Min 2021 [210]	+	+	++	+	+	–	+	++	+	2

Study	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Were there any other potential threats to internal validity	Quality
Nadei 2019 [192]	+	NR	+	NR	++	+	+	+	+	1
Perera 2018 [206]	+	NR	+	NR	++	++	+	++	+	1
Podder 2008 [208]	NR	NR	+	NR	+	–	+	++	–	3
Ran 2021 [240]	+	+	++	+	++	+	+	++	+	1
Song 2011 [225]	+	NR	+	NR	++	+	+	++	–	2
Sun 2010 [229]	+	NR	++	NR	++	+	+	++	+	1
Sun 2012 [325]	+	NR	+	NR	++	+	+	++	+	1
Teng 2018 [232]	NR	NR	+	++	++	+	+	++	+	1
Turkekul 2020 [270]	NR	NR	NR	NR	+	+	+	+	+	2
Wang 2018 [277]	+	NR	+	NR	++	–	+	++	+	1
Wasana 2015 [282]	+	NR	++	NR	+	+	++	++	–	1
Wei 2016a [284]	+	NR	+	NR	+	+	+	++	+	1
Wu 2019 [288]	+	NR	++	NR	+	+	+	++	+	1
Zhang 2020 [304]	+	NR	+	NR	++	–	+	++	+	2

Legend:	Definitely low risk of bias	++	Probably low risk of bias	+	Probably high risk of bias	– / NR	Definitely high risk of bias	– –

VII. Literature review summary

Summary of evolving human evidence

Out of a total of 39 endpoints reported in the RSI review, the RSI literature search identified new human evidence relating to 16 endpoints, which were not reported in either NHMRC [9, 10] or CADTH [2, 3] reports. CADTH had initially reported on 23 endpoints, for which the RSI review updated the evidence on 13 of those endpoints and found no new evidence on the remaining 10 endpoints. This section describes the evolving evidence reported in NHMRC 2016, CADTH 2019, and the current updated review of the literature. A summary is provided for all endpoints in Table 11. Where no earlier evidence was reported, the CADTH 2019 conclusion was described as 'N/A'. While no limit was used to restrict studies based on fluoride exposure levels as an exclusion criterion for the literature review, synthesis of evidence was predominantly based on studies generally relevant to the Canadian context. Although these studies may involve fluoride water concentration higher than those in Canadian drinking water supplies, they are relevant to the evaluation of causality and exposure-response assessment. Some studies reported results based on serum/urinary fluoride levels (detailed in Section 3 of the Supplementary Material). Where available, fluoride levels in drinking water were listed in the following section for the purpose of comparison across studies.

All-cause mortality

NHMRC 2016 [9, 10] identified one major study with acceptable quality that reported a small decrease in all-cause mortality incidence in association with CWF. No further evidence was identified by CADTH 2019 [2] or RSI literature search in relation to all-cause mortality.

RSI evidence synthesis: In the absence of new studies, the CADTH summary of evidence remains unchanged: Insufficient evidence for an association between water fluoridation at the current Canadian levels and all-cause mortality.

Bone health

Bone, cancer

NHMRC [9, 10] identified three studies of acceptable quality and three of low quality, where all except one of low quality concluded no association between water fluoridation and bone cancer. NHMRC also identified two systematic reviews where one reported a positive association only in males, and the other reported no clear association. CADTH 2019 [2] identified two further studies of acceptable quality and reported that the evidence up to that date was largely in support of no association between fluoride and bone cancer. The RSI literature search identified 1 case control study [33] and 2 ecological studies [35, 43] of high/acceptable quality that were conducted in South Korea [35], and the US [33, 43]. Two of these studies concluded the absence of association between bone cancer and water fluoridation at a fluoride exposure range between 0.04-0.8 ppm [33, 43]. The third study [35] that concluded the absence of association did not report a water fluoride exposure level.

RSI evidence synthesis: Based on the available literature to date, there is consistent evidence of no association between bone cancer and fluoride exposures relevant to current Canadian drinking water levels.

Bone, density and quality

NHMRC 2016 [9, 10] identified one systematic review and one study of low quality, which concluded the absence of association between bone quality or osteoporosis (low mineral bone density) and exposure to fluoride. No further evidence was identified by CADTH 2019 [2]. The RSI literature search identified 5 new studies including 4 studies of high quality [21, 32, 35, 39] and a fifth study with acceptable quality [65]. One cohort study from Sweden [21] and 2 cross-sectional studies from China [39] and Ethiopia [32] concluded a positive association between bone quality disruption and fluoride in drinking water (≤1 ppm) [21] or ground water (6.8 ppm) [32]. The Chinese study [39] did not report on the examined fluoride exposure level. Alternatively, 2 studies reported no association between fluoride exposure/intake and bone quality disruption. One ecological study was conducted in South Korea [35] on residents from all ages (no water fluoride level reported). Another US cohort study [65] was conducted on

adolescents (17 years of age) and reported no association between bone quality disruption and life-long fluoride intake from all sources (0.7-0.9 ppm) [65].

RSI evidence synthesis: Based on the available literature to date, there is inconsistent evidence for the association of bone quality and fluoride exposures relevant to current Canadian drinking water levels.

Bone, hip fracture

NHMRC 2016 [9, 10] reported two systematic reviews and two studies of acceptable quality, which showed no clear association between fluoride and hip fractures. The later review by CADTH 2019 [2] identified one additional study of acceptable quality and concluded that evidence was consistent for no association with fluoride exposure. The RSI literature search identified 1 ecological study [35] of high quality that was conducted in South Korea and concluded the absence of an association between fluoride exposure and the risk of hip fracture.

RSI evidence synthesis: Based on the available literature to date, there is consistent evidence of no association between hip fracture and fluoride exposures relevant to current Canadian drinking water levels.

Bone, musculoskeletal pain

NHMRC 2016 [9, 10] identified two studies of low quality, which reported a positive association between musculoskeletal pain and higher fluoride levels (not applicable to the Canadian context). No further evidence was identified by CADTH 2019 [2] or in the current updated literature review in relation to musculoskeletal pain.

RSI evidence synthesis: In the absence of new studies, the CADTH summary of evidence remains unchanged: Insufficient evidence for an association between water fluoridation at the current Canadian levels and musculoskeletal pain.

Cancer, total incidence and mortality

The earlier review by NHMRC 2016 [9, 10] reported conflicting evidence for the association of cancer incidence and mortality based on examination of two systematic reviews and three individual studies of acceptable quality. CADTH 2019 [2] identified an additional study of acceptable quality that reported an inverse association and concluded that there was consistent evidence of no association. The RSI literature search did not identify any new evidence relating to cancer incidence and mortality.

RSI evidence synthesis: In the absence of new studies, the CADTH summary of evidence remains unchanged: Consistent evidence for no association between water fluoridation at the current Canadian levels and the overall incidence of cancer or cancer-related mortality.

Cognition

Cognition, ADHD

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019a [3] that reported on the association of fluoride with ADHD. The RSI literature search identified two studies of high quality which examined the association between fluoride exposure and ADHD in youth [52] and in children due to maternal exposure to fluoride during pregnancy [58]. The first one [52] was a cross-sectional study conducted on Canadian youth 6-17 years old from the Canadian Health Measures Survey (Cycles 2 and 3). The study reported a significantly positive association between tap water fluoride (mean of 0.04 mg/L in non-fluoridated regions to 0.49 mg/L in fluoridated regions) and the risk of ADHD including symptoms of hyperactivity and inattention, especially among adolescents. The second study [58] was a cohort study conducted in Mexico, which enlisted 213 mother-child pairs as part of the ELEMENT study. This study reported an association between higher maternal fluoride levels (measured during pregnancy, mean MUFcr was 0.85 mg/L (SD=0.33)) and “more behavioral symptoms of inattention, but not hyperactivity or impulse control” measured in the children at 6-12 years old. In the study, water fluoride levels of 0.15-1.38 ppm were extrapolated from an earlier study on the ELEMENT cohort in 2017 [71].

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of ADHD and fluoride exposures relevant to current Canadian drinking water levels.

Cognition, dementia

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019a [3] that reported on the association of fluoride with dementia. The RSI literature search identified one large, high quality cohort study [37] that was conducted in Scotland and included all people born in 1921 who were at school in Scotland in June 1932, and took part in a comprehensive national intelligence test at a mean age of 11 years (Scottish Mental Survey 1932, SMS1932). The study reported a positive association between the risk of dementia and higher fluoride levels in men and women who consumed water with low fluoride levels (0.05 ppm).

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of dementia and fluoride exposures relevant to current Canadian drinking water levels.

Cognition, Down syndrome

NHMRC 2016 [9, 10] reported on two systematic reviews which did not show a clear association between fluoride and Down syndrome, compared to another study with a large population size and acceptable quality that showed no such association. CADTH 2019a [3] identified an additional, large-size observational study with acceptable quality that reported no association of fluoride exposure and Down syndrome. The RSI literature search did not identify any new evidence relating to Down syndrome.

RSI evidence synthesis: In the absence of new studies, the CADTH summary of evidence remains unchanged: Limited evidence for no association between water fluoridation at the current Canadian levels and Down syndrome.

Cognition, IQ

Based on one systematic review and eleven studies (1 high, 2 acceptable, and 8 low quality), the NHMRC 2016 [9, 10] reported mixed findings regarding the association of fluoride exposure

with lower IQ scores in children. A subsequent report by CADTH 2019a [3] identified a Canadian cohort study [326] that used data from the MIREC birth cohort, which was conducted on mother-child pairs from six major Canadian cities. The study reported a positive association between maternal exposure to fluoride and reduction of IQ levels in children 3-4 years old. Despite describing the evidence as weak based on this single cohort study, CADTH 2019a [3] suggested that results should be part of the efforts to further explore the possible association of fluoride exposure and neurological development in children. In a 2020 update to their 2019 review of neurological and cognitive effects, CADTH [327] identified two additional studies of low quality in relation to IQ, and concluded there was insufficient evidence for an association between IQ levels and "fluoride exposure at the Canadian water fluoride levels (optimum at 0.7 mg/L)".

A 2020 draft report[29] [328] by the US National Toxicology Program (NTP) concluded that exposure to fluoride *"is presumed to be a cognitive neurodevelopmental hazard"* in children, with only limited evidence in support of cognitive effects in adults. This statement was modified in 2022 [420] in response to another NASEM review: "This review finds, with moderate confidence, that higher fluoride exposure (e.g., represented by populations whose total fluoride exposure approximates or exceeds the World Health Organization Guidelines for Drinking-water Quality of 1.5 mg/L of fluoride) is consistently associated with lower IQ in children. More studies are needed to fully understand the potential for lower fluoride exposure to affect children's IQ." According to NTP, for effects on children's IQ at exposure levels below 1.5 mg/L, the supporting studies provided less consistent results and were mostly at higher risk of bias. ""

The RSI literature search identified a total of 22 studies including 18 studies (12 high [40, 41, 59, 69, 71, 405, 409, 413, 415-416, 418-419] and 6 acceptable quality [64, 70, 76, 401, 414 ,417]) that reported a positive association between fluoride exposure and reduced IQ scores and school performance in children. Studies reporting positive association include the Till and Colleagues [40] study, using MIREC birth cohort data, which reported that an increment of 0.5 mg/L in

[29] NTP disclaimer: "This DRAFT Monograph is distributed solely for the purpose of pre-dissemination peer review under the applicable information quality guidelines. It has not been formally disseminated by NTP. It does not represent and should not be construed to represent any NTP determination or policy. The September 6, 2019 draft monograph was peer reviewed by a committee convened by the National Academy of Sciences, Engineering, and Medicine (NASEM). This current draft incorporates changes in response to that review and is being submitted to the same NASEM committee for an additional round of peer review."

water fluoride concentration corresponded to A 9.3- and a 6.2-point reduction in performance IQ in formula-fed and breastfed children, respectively. Such an association remained significant upon controlling for fetal fluoride exposure.

Results from a recent study [418] that used data from the Mexican Cohort ELEMENT suggests that maternal urinary fluoride exposure may affect visual-spatial and perceptual cognitive domains more so than verbal. The study reported a drop of 2 points in IQ scores for each 0.5 mg/L increase in maternal urinary fluoride. Another recent and high-quality analysis of critical time windows of exposure using the Canadian MIREC cohort, reported an association between children's performance IQ and fluoride exposure during the perinatal period and into early childhood. Results suggest that prenatal exposure may be more critical for effects in boys but infancy (over the first year) as the more critical exposure window for girls [409]. A third study [419] examined prenatal fluoride exposure in a small mother-child birth cohort in Spain: Results in boys suggest improved scores in cognitive domains with maternal urinary concentrations. Another study [69] reported that each increment of 0.5 mg/L in water fluoride corresponds to a 40% reduction in the odds of having excellent IQ in those exposed to low fluoride levels (0.20-1.40 mg/L). In 2020, Wang and Colleagues [41] reported a significant IQ score reduction for each 1 mg/L increase in water fluoride concentration [β: −1.59 (−2.61, −0.57), p=0.002].

Another high-quality study [59] reported an association with reduced IQ scores only in children carrying the dopamine receptor-2 (DRD2) Taq 1A- TT genotype, with no similar association with the other DRD2 Taq 1A genotypes. In a high-quality study [71] from Mexico (using data from the ELEMENT cohort), the authors reported a positive association of maternal exposure to fluoride during pregnancy with lower GCI (IQ) scores in children at approximately 4 years old, and with lower Full-Scale IQ scores at 6–12 years old. And finally, a cross-sectional study conducted by Kousik and Colleagues in 2016 [76] reported a positive and significant correlation between exposure dose and IQ ($r = -0.343$, $p < 0.01$).

These studies reported a reduction of IQ scores in association with water fluoride concentrations of 0.01-2.07 ppm [64], 0.1–1.6 ppm [414], 0.1–15.8 ppm [405], 0.15-1.38 ppm [71], 0.20–2.49 ppm [59], 0.20–3.90 ppm [413], 0.58 ppm [40], >1.0 ppm [417], 1.39 ppm [41], >1.5 ppm [70], 1.53–2.84 ppm [416], 2.0 ppm [69], 2.11 ppm [76], and 2–5 ppm [401]. Three studies with

acceptable quality reported no effect of fluoride on children's IQ at fluoride exposures of 0.3-3.0 ppm [75], 1.22 ppm ±1.09 [54], or 2.04 ppm [398].

RSI evidence synthesis: Based on the available literature to date, the accumulating body of evidence suggests a positive association of reduced IQ scores for children and fluoride exposures relevant to current Canadian drinking water levels.

Cognition, memory loss

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019a [3] that reported on the association of fluoride with memory loss. The RSI literature search identified only one case-control study [70] with acceptable quality that was conducted in India and examined men and women with dental and skeletal fluorosis. The study reported a positive association between fluoride exposure (>1.5 ppm) and several biomarkers (decreased activity of the membrane bound enzymes, AChE and ATPase). These results indicate defects in signaling and energy metabolism, and can predict potential memory loss (unmeasured in the study) in fluorosis patients.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of water fluoride and memory loss.

Cognition, trouble working

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019a [3] that reported on the association of fluoride with trouble working. The RSI literature search identified a large cross-sectional study [75] with acceptable quality that was conducted using data on >500,000 US adolescents and adults from the National Health and Nutrition Examination Survey III (NHANES III). The study reported no association between exposure to water fluoride (0.3-3 ppm) and trouble working.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of trouble working and fluoride exposures relevant to current Canadian drinking water levels.

Cardiovascular Diseases (CVD)

A number of studies examining individual cardiovascular endpoints were reported in earlier reviews [2, 9] as well as by the current RSI review. Whereas the evidence for each individual endpoint is supported by few studies, and given the fact that these endpoints are closely interrelated, the evolving evidence merits further investigations to properly assess the association of fluoride exposure with cardiovascular diseases.

CVD, atherosclerosis

Based on a single study with low quality, NHMRC 2016 [9, 10] reported a significantly higher risk of carotid artery atherosclerosis in adults in areas with high fluoride levels (>1.2 ppm). No further studies were identified by CADTH 2019 [2]. The RSI literature review identified 3 additional studies that examined the association of fluoride with cardiovascular disease biomarkers. A cross-sectional study [27] with acceptable quality in Ukraine reported that children in the fluorosis area (>1.5 ppm) showed higher blood TBARS levels, while the acyl hydroperoxide levels were non-significantly increased in comparison with healthy children living in the non-fluorosis area. Another cross-sectional study with high quality [45] that was conducted in 2015 on 5-12 years old Mexican school children. The study reported significant association of fluoride exposure (0.3 ppm) with alterations in some cardiovascular disease biomarkers, suggesting fluoride exposure may be atherogenic and may increase the likelihood of cardiovascular diseases later in life. A third study of case-control design with acceptable quality [70] was conducted on patients with dental and skeletal fluorosis in India. The study reported variable associations of fluoride (>1.5 ppm) with different cardiovascular disease biomarkers, which support the chances of cardiovascular-related complications in fluorosis patients.

RSI evidence synthesis: Based on the available literature to date, there is limited evidence for an association of atherosclerosis and fluoride exposures relevant to current Canadian drinking water levels.

CVD, hypertension

Five studies were reported by NHMRC 2016 [9, 10] (n=3) and CADTH 2019 [2] (n=2) provided mixed findings on the association of fluoride with risk of hypertension. All studies were of low quality and were derived from countries with higher fluoride levels compared to those

implemented in Canada. The RSI literature review did not identify any additional studies in relation to this endpoint.

RSI evidence synthesis: In the absence of new studies, the CADTH summary of evidence remains unchanged: Insufficient evidence for an association between water fluoridation at the current Canadian levels and hypertension.

CVD, myocardial infarction

There were no earlier studies identified in NHMRC 2016 [9, 10]. With only one study with large population size and low quality that reported no association between fluoride and myocardial infarction, CADTH 2019 [2] concluded there was insufficient evidence for this association. The RSI literature review did not identify any additional studies in relation to myocardial infarction.

RSI evidence synthesis: In the absence of new studies, the CADTH summary of evidence remains unchanged: Insufficient evidence for an association between water fluoridation at the current Canadian levels and myocardial infarction.

Diabetes mellitus

As reported by CADTH 2019 [2], there were only two earlier studies with low quality that provided mixed evidence for the association of exposure to fluoride with risk of diabetes mellitus (DM). No earlier studies were identified by NHMRC 2016 [9, 10]. The RSI literature search identified 1 study of high quality that was conducted in India and concluded that the increase in serum Fluoride increases diabetes mellitus and diabetic nephropathy [34]. Serum fluoride levels ranged from 0.5128 ±0.30 (DM with CKD) to 0.6318 ±0.59 (DM without CKD).

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of diabetes mellitus and fluoride exposures relevant to current Canadian drinking water levels.

Eye diseases and conditions

Eye, select diseases

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride with eye diseases. The RSI literature review identified one study [56] of high quality that was conducted on Chinese adults aged ≥40 years old, with no congenital eye disease or ocular trauma, for examining the association between water fluoride exposure (>1.2 ppm) and seven eye diseases. The study reported significant positive associations with pterygium and arteriosclerotic retinopathy, a significant inverse association with cataracts, and non-significant associations with primary angle closure glaucoma, diabetic retinopathy, age-related macular degeneration, and strabismus.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of any of the examined eye diseases and fluoride exposures relevant to current Canadian drinking water levels.

Eye, refractive errors

An earlier review by CADTH 2019 [2] identified a single low-quality study and concluded that evidence was insufficient for an assessment of this association. There were no earlier studies identified in NHMRC 2016 [9, 10] or RSI literature search in relation to the association of fluoride exposure with the prevalence of refractive errors (myopia, hyperopia, astigmatism) [2].

RSI evidence synthesis: In the absence of new studies, the CADTH summary of evidence remains unchanged: Insufficient evidence for an association between water fluoridation at the current Canadian levels and refractory errors.

Fluorosis

Fluorosis, dental

Earlier evidence on the association of fluoride with dental fluorosis was reported by NHMRC 2016 [9, 10] (three systematic reviews) and CADTH 2019 [2] (21 studies: 1 acceptable, 19 low; N= 35,374), which reported consistent findings for an association between fluoride and dental fluorosis. The RSI literature search identified 33 cross-sectional studies, including 15 studies of high quality [19, 22, 25, 57, 62, 72, 77, 402-403, 405-406, 408, 411-413] and 18 studies of acceptable quality [17, 25, 26, 30, 31, 38, 47, 54, 74, 78, 396-397, 399-400, 404, 407, 410, 414] that were not included in earlier reviews. Thirty-two of those studies reported a positive/possible association with dental fluorosis at a

wide range of fluoride concentration in drinking water (both tap and ground). Out of those 32 studies, 14 were of high quality that were conducted in India [62, 72, 399, 400, 404], China [57, 397, 408, 413], Brazil [402, 410, 412], and 1 in each of Canada [403], Egypt [406], Indonesia [414], Iran [77], Malaysia [25], Pakistan [405], Peru [396], Sri Lanka [407], Thailand [411] , and USA [19].

The study by Dong et al. (2021) [19] included children and adolescents (age 6 to 19 years), and reported the odds (95%CI) of dental fluorosis (Dean's Fluorosis Index (DFI) ≥1) as 1.48 (1.13, 1.96), 1.92 (1.44, 2.58), and 2.30 (1.75, 3.07) times greater at water fluoride levels of 0.31 – 0.50 mg/L, 0.51 – 0.70 mg/L, and >0.70 mg/L, compared to ≤0.30 mg/L. Mohd Nor et al. (2021) [25] conducted a study on children (age 9 to 12 years) and reported that compared to those exposed to non-fluoridated water, the odds of dental fluorosis (DFI ≥ 2) (95% CI) were 5.97 (3.32, 10.72) times greater among children with a lifetime exposure to 0.5 ppm, and 9.12 (5.15, 16.14) times greater among those exposed to 0.7 ppm during the first two years of life, followed by a level of 0.5 ppm.

The study by Zhou and Colleagues [57] included children (age 7 to 13 years) from rural areas with low-to-moderate levels of fluoride and reported that each 1mg/L increase of water fluoride was associated with a 1.47 (1.40, 1.55), 1.85 (1.63, 2.11), 1.68 (1.57, 1.79), and 3.85 (3.01, 4.92) increased odds (95% CI) of total, very mild, mild and moderate dental fluorosis, respectively [57]. The study by Kumar et al. (2018) [62] included adolescents (age 12 to 15 years), and reported a correlation coefficient between water fluoride and dental fluorosis severity of 0.97 (p-value <0.05). In the bivariate analysis, the study reported 1.76 (1.31, 2.38) times greater odds (95% CI) of dental fluorosis (any fluorosis, measured using the Modified Dean's Index) among participants exposed to water fluoride levels >1.2 ppm compared to ≤1.2 ppm.

Verma et al. (2017) [72] included adolescents (age 12 to 17 years) and demonstrated a positive correlation (rho = 0.57) between the Community Fluorosis Index (CFI) and levels of fluoride in drinking water. The study by Sabokseir et al. (2016) [77] included children (age 9 years), and reported the frequency of participants with genuine fluorosis (excludes fluorosis-resembling defects) as 42 (47.7%), 39 (20.6%), and 3 (3.3%) in areas with high, optimal, and low levels of fluoride, respectively. Compared to areas with high levels of fluoride, the odds of genuine dental fluorosis were 70.8% (OR= 0.29, 95% CI: 0.17, 0.51) and 96.3% (OR= 0.04, 95% CI: 0.01, 0.13) less in areas with optimal and low levels of fluoride, respectively.

In general, studies identified by the RSI literature search reported a wide range of fluoride concentrations ranging from 0.06 ppm in Brazil [31] to >4 ppm in Iran [77]. Further to a study conducted in 2022 in Canada [403] where the reported fluoride levels in tap water was 0.1 – 1.0 ppm, other examples of fluoride concentrations relevant to the Canadian context were reported from Ireland (tap water, 0.6 – 1.0 ppm) [22], China (tap water, 0.89 – 0.91ppm) [78], Mexico (tap water, 1.22 ±1.09 ppm) [54], and India (tap water, 0.67-0.83 [404], 1.1–2.92 [399] and 1.27 ±0.46 ppm) [62]. Only 2 studies [22, 38] reported non-significant (possible) association between high drinking water fluoride (>6 ppm) and dental fluorosis.

Although no meta-analysis was conducted for the current RSI review, an earlier review by Iheozor-Ejiofor et al [395] included a dose-response meta-analysis of 40 studies at high risk of bias (published up to that time). The results suggested that the odds of dental fluorosis increased by 2.9 for each unit increase of fluoride exposure, and at 0.4 ppm fluoride, 10% of a population (95% CI: 6%-15%) would be expected to have dental fluorosis of aesthetic concern (defined as ≥3 TFI, ≥2 TSIF, or mild or worse DFI).

RSI evidence synthesis:

Several newer studies have been published since the CADTH 2019 review, adding to the large body of literature on fluoride and dental fluorosis effects. Evidence in these new studies is consistent with previously published work for the prevalence of dental fluorosis in populations with varying levels of fluoride in drinking water.

Fluorosis, skeletal

Earlier evidence on the association of fluoride with skeletal fluorosis was reported by NHMRC 2016 [9, 10] (one systematic review at 3.8 to 8 ppm and two studies of low quality at <4, 4 to 6, and >6 ppm for one study, and 1.51 to 3.71 ppm for the other study) and CADTH 2019 [2] (two studies of low quality at Canadian CWF levels). Evidence was collectively reported by CADTH 2019 as insufficient to conclude an association. The RSI literature review identified 3 cross-sectional studies with high/acceptable quality that were conducted in China [51], Ethiopia *[18]*, and India [23] on individuals aged 10 years or older. Whereas only 1 study *[18]* reported a positive association between fluoride exposure and skeletal fluorosis, the 2 other studies of acceptable quality reported a possible impact [23, 51]. Reported ground water fluoride levels

included a mean of 6.8 ppm ±4.3 SD in one study, and a wide range ≤1 – >4.0 in another study. No water fluoride levels could be extracted, or extrapolated from the third study [51].

RSI evidence synthesis: Based on the available literature to date, there is limited evidence for an association of skeletal fluorosis with fluoride exposures relevant to Canadian drinking water levels.

Genotoxicity

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure and genotoxicity. The RSI literature search identified 2 cross-sectional studies that were conducted in China with high [24] or acceptable quality [57]. Whereas the first study [24] suggested a possible association of fluoride exposure (1.1 – 4.1 ppm) with disrupting DNA methylation, the second study [57] concluded a positive association of low-moderate water fluoride exposure (0.60 ppm) and disrupting circulating mitochondrial DNA (mtDNA) levels.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of genotoxicity and fluoride exposures relevant to current Canadian drinking water levels.

Growth and development

BMI

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure and BMI. The RSI literature search identified an ecological study [76] with acceptable quality that was conducted on Indian children and adolescents (6-18 years old), which reported a positive and non-significant correlation between water fluoride levels (range: 0.25-9.4 ppm; average: 2.1 ppm) and BMI.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of BMI and fluoride exposures relevant to current Canadian drinking water levels.

Childhood obesity

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure with childhood obesity. The RSI literature search identified a single cross-sectional study [48] with high quality that was conducted on Chinese children and adolescents aged 7–13 years old from ground water-supplied areas. The study reported an association of low-to-moderate exposure to fluoride (0.83 ppm) with overweight status and obesity in children.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of childhood obesity and fluoride exposures relevant to current Canadian drinking water levels.

Newborn's height and weight

A single study of low quality was reported by NHMRC 2016 [9, 10], which reported that mothers exposed to drill water with a fluoride level of 4.7 ppm were more likely to have low birth weight newborns. Another study with low quality, as reported by CADTH 2019 [2], showed a positive correlation between drinking water fluoride and infant height and weight. The RSI literature review did not identify any additional studies for this endpoint.

RSI evidence synthesis: In the absence of new studies, the CADTH summary of evidence remains unchanged: Insufficient evidence for an association between water fluoridation at the current Canadian levels and newborns' weight or newborns' height.

Kidney diseases

Kidney, dysfunction

There were no earlier studies identified in NHMRC 2016 [9, 10] that reported on the association of fluoride and kidney dysfunction. In 2019, the review by CADTH 2019 [2] identified a single study with low quality and concluded that there was insufficient evidence on the association between CWF and kidney dysfunction.

The RSI literature search identified 4 studies with high quality and 2 studies with acceptable quality, which examined the association of fluoride exposure with kidney dysfunction [36, 44, 49,

[60, 45, 73]. Four out of these 6 studies reported results consistent with a possible association [36, 44, 49, 60]. The first study [49] was cross-sectional in design that was conducted on US adolescents (12-19) as part of the NHANES survey, which suggested a possible association with complex changes in kidney functions. A second cross-sectional study [60] was conducted on Mexican adults (18-77 years old) who were exposed to high drinking water fluoride levels. The study reported a possible fluoride-associated kidney tubular dysfunction, with a likely impact on future development of chronic kidney dysfunction. A third cross-sectional study with acceptable quality [36] was conducted on men diagnosed with CKDu, and concluded a possible association with serum fluoride. A fourth Sri Lanka-based study of case-control design with acceptable quality [44] was conducted on 19-76 years old, non-dialysis, biopsy-proven CKDu adult cases. Study suggested a possible association between fluoride exposure and CKDu. These 4 studies reported kidney dysfunction at water fluoride concentrations of 0.48 ppm [49], 1.33 ppm [44], 1.5 ppm [60] and 0.68 ppm (±0.48) [36].

One cross-sectional study with high quality was conducted on 5-12 years old Mexican school children [45], and reported an inconclusive association with kidney disease of unknown origin (CKDU) at a fluoride concentration of 0.3 ppm. Another cross-sectional study conducted on Mexican children (5-12 years old) reported no association between kidney injury biomarkers and fluoride [73] at a concentration of 2.47 ppm.

RSI evidence synthesis: Based on the available literature to date, there is limited evidence for an association of kidney dysfunction (mainly CKDu) and fluoride exposures relevant to current Canadian drinking water levels.

Kidney, stones

The review by CADTH 2019 [2] concluded there was limited evidence for an inverse association between fluoride exposure and development of kidney stones based on a single study with acceptable quality. There were no studies identified in NHMRC 2016 [9, 10] or RSI literature search that reported on the association of fluoride exposure with kidney stones.

*RSI evidence synthesis: I*n the absence of new studies, the CADTH summary of evidence remains unchanged: Limited evidence for an inverse association between water fluoridation at the current Canadian levels and the incidence of kidney stones.

Kidney, ultrastructural

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure with ultrastructural changes in the kidney. The RSI literature search identified a single study of acceptable quality, which was conducted in India on children (4-12 years old) with nephrotic syndrome minimal change disease (NS-MCD). Although the study was case-control in design, only cross-sectional analysis results relevant to the RSI review were included. The study reported a positive association between fluoride exposure and ultrastructural changes and apoptosis in human renal tubules [66]. However, no water fluoride levels were extracted or extrapolated from this identified study.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of ultrastructural changes in the kidney and fluoride exposure.

Liver dysfunction

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure with liver dysfunction. The RSI literature search identified two studies, which examined the association of fluoride exposure with liver dysfunction. The first [49] was cross-sectional in design, that examined data on US adolescents (12-19) as part of the NHANES survey, and suggested a possible association between water fluoride (0.48 ppm) and complex changes in liver functions in adolescents. Another case-control study with acceptable quality [70] was conducted on patients with dental and skeletal fluorosis in India, which reported that prolonged water fluoride (>1.5 ppm) could overwhelm the regenerative capacity of liver tissues leading to liver damage.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of liver dysfunction and fluoride exposures relevant to current Canadian drinking water levels.

Neurologic

Neurologic, Headache

The NHMRC 2016 [9, 10] examined two studies with low quality, which did not provide a clear conclusion on the association between fluoride and headache symptoms. No studies were identified in CADTH 2019a [3]. RSI literature search identified 1 study [18] that reported a possible association between headache and paresthesia at ground water fluoride level of 6.8 ±4.3 ppm.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of headache and paresthesia and fluoride exposures relevant to current Canadian drinking water levels.

Neurologic, Sleep-related Outcomes

The NHMRC 2016 [9, 10] examined two studies with low quality, which did not provide a clear conclusion on the association between fluoride exposure and insomnia. CADTH 2019a [3] literature search did not identify any new studies. The RSI literature search identified a single cross-sectional study with high quality [50] that was conducted on US adolescents (16-19) as part of the NHANES survey (2015–2016). This study reported positive and significant associations between water fluoride levels (0.39 ppm) and sleep apnea, bedtime and wake time; non-significant positive associations with the recommended sleep duration and daytime sleepiness; possible and significant inverse association and snoring among males; and no association with trouble sleeping.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of sleep-related outcomes and fluoride exposures relevant to current Canadian drinking water levels.

Reproduction

Reproduction, abortion and fertility

Two studies of low quality were identified earlier by CADTH 2019 [2], which did not provide a clear conclusion between water fluoride level and rates of abortion. There were no studies

identified in NHMRC 2016 [9, 10] or RSI literature search that reported on the association of fluoride exposure with abortion.

RSI evidence synthesis: In the absence of new studies, the CADTH summary of evidence remains unchanged: Insufficient evidence for an association between water fluoridation at the current Canadian levels and reproduction in women.

Reproduction, preterm births

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure and preterm births. The RSI literature search identified a single cross-sectional study of high quality, that was conducted on US women with a live birth (2009- 2016) who responded to the PRAMS survey (Pregnancy Risk Assessment Monitoring System) [55]. The study reported that women who had dental cleaning during pregnancy and lived in a community with water fluoridation had lower prevalence of preterm births. However, no water fluoride levels could be extracted, or extrapolated from this identified study.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of preterm births and fluoride exposure.

Reproduction, sex hormones

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure and disruption of male sex hormones. The RSI literature search identified 2 cross-sectional studies of high quality that examined US children and adolescents 6–19 years old (NHANES survey) [28], and male farmers from Henan Province in China [42]. Results from the first study [28] indicated a gender- and age-specific inverse associations of fluoride in plasma and water with sex steroid hormones of total testosterone, estradiol and SHBG in U.S. children and adolescents, with a mean water fluoride level of 0.36 ppm (0.30 – 0.42). The second study [42] reported a significant inverse association between water fluoride level and serum sex hormone binding globulin (SGBH) levels but not with androgen binding protein (ABP) levels. The average fluoride concentration in villages in the high exposure group (HEG) was 2.44 ± 1.88 mg/L, and in the control, low

exposure villages (LEG) was 0.37 ± 0.15 mg/L. RSI also identified a relevant abstract [15] that reported a possible association with altering the hypothalamic testicular axis hormones in human males residing in high fluoride regions. There were insufficient details on the study in the published abstract.

RSI evidence synthesis: Based on the available literature to date, there is limited evidence for an association of levels of sex hormones and fluoride exposures relevant to current Canadian drinking water levels.

Thyroid dysfunction

Evidence on the association of fluoride with thyroid gland dysfunction was reported on by NHMRC 2016 [9, 10] (3 studies of low quality) and CADTH 2019 [2] (1 study of acceptable and three studies of low quality), which concluded mixed findings, flagging insufficient evidence for this association.

The RSI literature review identified seven relevant studies, which were all of cross-sectional design, and were conducted on children and adolescents. Three studies were conducted in India [53, 61, 67], 3 in China [20, 29, 41], and 1 in Canada [63]. Four studies of high [20, 41] or acceptable quality [61, 67] reported a positive association with thyroid dysfunction, 1 study of high quality reported a possible association [63], and 1 study of acceptable quality that reported a non-significant association [29] with thyroid dysfunction. These studies identified disruption of thyroid hormones at water fluoride concentrations of 0.22 ppm [63], <1ppm [67], 1.39 ppm [41], and 2.88 ppm [61]. A seventh study of acceptable quality reported no association between disruption of thyroid functions and drinking water fluoride levels (0.01-2.0 ppm) [53].

RSI evidence synthesis: Based on the available literature to date, there is limited evidence to evaluate the association of thyroid hormone disruption and fluoride exposures relevant to current Canadian drinking water levels.

Other outcomes

Arsenic methylation

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure and arsenic methylation. The RSI literature search

identified a single cross-sectional study of high quality that was conducted on Mexican adults. The study reported a positive association between water fluoride exposure (1.6 ppm) and increasing arsenic (As) toxicity in adults, which has been linked to adverse health outcomes such as cancer, cardiovascular diseases, diabetes and cardiometabolic risk [46].

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of arsenic methylation and fluoride exposures relevant to current Canadian drinking water levels.

General health

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure and general health. The RSI literature search identified a large cross-sectional study [75] with acceptable quality that was conducted using data on >500,000 US adolescents and adults from the National Health and Nutrition Examination Survey III (NHANES III). The study reported a lack of evidence for an effect of water fluoridation (0.3-3 ppm) on general health.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of general health and fluoride exposures relevant to current Canadian drinking water levels.

Other non-skeletal manifestations of fluoride toxicity

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure with non-skeletal manifestations of fluoride toxicity (referred to as non-skeletal fluorosis by some authors). The RSI literature search identified 2 cross-sectional studies with acceptable quality that were conducted in Ethiopia [18] and India [68]. The first one [18] reported a possible association between fluoride exposure from ground water and multiple manifestations including loss of appetite, constipation, and fatigue. Participants were 10–70 years old from rural areas who were exposed to groundwater fluoride levels with a mean concentration of 6.8 ±4.3 mg/L (range: 0.3–15.5 mg/L). The second study [68] compared individuals living in areas with high-fluoride exposure to those in normal fluoride exposure areas. The study reported that non-skeletal manifestations of fluoride toxicity

(dyspepsia, fatigue and muscle weakness) may be due to fluoride exposure through water (>1.5 ppm) or other sources like food.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of the listed non-skeletal manifestations of fluoride toxicity and fluoride exposure.

Suicide

There were no earlier studies identified in NHMRC 2016 [9, 10] or CADTH 2019 [2] that reported on the association of fluoride exposure and suicide. The RSI literature search identified a single relevant abstract [16], which reported a possible association between fluoride exposure and reduction in suicide rates. However, no water fluoride levels were extracted or extrapolated from this identified abstract.

RSI evidence synthesis: Based on the available literature to date, there is insufficient evidence to evaluate an association of suicide and fluoride exposure.

Table 11: Summary of evolving evidence on health effects

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
All-cause, mortality	1 study (N= 208,570,962, acceptable) *A small reduction in incidence rate of all-cause mortality in CWF areas. Difference in rate was: -1.3% (95% CI: -2.4%, 0.1%).*	No studies	Insufficient evidence for an association at the current Canadian CWF levels.	No new studies	Insufficient evidence for an association at the current Canadian CWF[30] levels.
Bone, cancer	2 Systematic Reviews (SR) (2 Not Reported (NR)); 6 studies (3 acceptable, 3 low) • 1 SR (8 studies; N=NR) *No clear association between water fluoridation and the incidence rate of osteosarcoma* • 1 SR (1 study; N= 318) *Higher exposure to fluoridated water was associated with a higher risk of developing osteosarcoma in males, but not in females* • 5 studies (N > 253,768,952, partial applicability to the Canadian context) *No significant difference in the incidence rate of*	2 studies (2 acceptable; N=1,663 and N=710,260,000 person-years) *Two studies with partial applicability to the Canadian context showed no significant difference in incidence rate of osteosarcoma in children and adults between high and low fluoride level areas.*	Consistent evidence for no association at the current Canadian CWF levels.	3 studies (1 high, 2 acceptable) • 1 study (N=645, acceptable). *No association between CWF and bone cancer [33]* • 1 study (N=4,406,021, high). *No association between CWF and bone cancer. Risk was a little high due to smaller sample size compared to the other examined bone diseases [35].* • 1 study (N=24,661, acceptable). *No association with secondary bone cancer [43].*	Sufficient evidence for no association at fluoride exposures relevant to current Canadian DWL[31].

[30] CWF: Community water fluoridation
[31] DWL: Drinking water levels

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
	osteosarcoma in children and adults between high and low exposure to water fluoridation • 1 study (N=20) *A conclusion could not be drawn from a low-quality study from India with high risk of bias*				
Bone, density and quality	1 SR (NR); 1 study (low) • 1 SR (27 studies; N=NR) *Addition of fluoride to drinking water at the level of 1 ppm did not associate with a decrease in bone mineral density compared with non-fluoridated water.* • 1 study (low, N= 675) *Prevalence of osteoporosis was not significantly different between groups. (Limited)*	No studies	Insufficient evidence for an association at the current Canadian CWF levels.	5 studies (4 high and 1 acceptable) • 1 study (N=4,306, high) *Positive association with increasing bone mass density in older women [21]* • 1 study (N=4,406,021, high) *No association between CWF and osteoporosis. [35].* • 1 study (N=722, high) *Positive association with decreasing BMD in women [39]* • 1 study (N=341, high) *Negative association with bone quality [32].* • 1 study (N=380, acceptable) *No association with bone quality [65].*	Inconsistent evidence for an association at the current Canadian CWF levels.
Bone, hip fracture	2 SRs (2 NR); 2 studies (acceptable) • 2 SRs (19 studies; N=NR) *No clear association between water fluoridation and hip fracture incidence in adults.*	1 study (acceptable; N=477,610,000 person-years) *A weak association between water fluoridation and hip fracture observed in*	Consistent evidence for no association with CWF levels.	1 study (high) • 1 study (N=4,406,021, high). *No association between CWF and risk of hip fractures in older women [35].*	Sufficient evidence for no association at fluoride exposures relevant to current Canadian DWL.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
	• 2 studies (acceptable; N=313,329,725) *No increased risk of hip fracture from water fluoridation exposure.*	*females, but not in males.*			
Bone, musculoskeletal pain	2 studies (2 low; N=3,266) *Increased risk of lower back pain and joint pain associated with higher fluoride levels. The results were from studies of low quality and from countries where socio-economic parameters differed than those in Canada.*	No studies	Insufficient evidence for an association at the current Canadian CWF levels.	No new studies	Insufficient evidence for an association at the current Canadian CWF levels.
Cancer, total, incidence and mortality	SRs (2 NR); 3 studies (acceptable) • 2 SR (13 studies; N=NR) *No clear association between water fluoridation and overall cancer incidence* • 1 study (N=208,770,962) *No significant difference in the incidence of all cancer between CWF and non-CWF* • 1 study (N=555,127,448) *Significantly lower incidence rate of invasive bladder cancer in CWF. Difference in*	1 study (acceptable; N=827,660,000 person-years) *Incidence of bladder cancer was lower in fluoridation areas. Odds ratio was 0.94 (95% CI, 0.90 to 0.98).*	Consistent evidence for no association at the current Canadian CWF levels.	No new studies	Sufficient evidence for no association at the current Canadian CWF levels.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
	rate= –8.0% (95% CI: –9.9%, –6.0%) • 1 study (N=NR) *An inverse correlation between the percentage of the population receiving fluoridated water and incidence of eye cancer (r= –0.45; P=0.002).*				
Cognitive, ADHD	No studies	No studies	N/A	• 1 study (cycle 2: N=2,520; cycle 3: N=2,667, high). *Positive association with ADHD among Canadian youth, particularly among adolescents [52].* • 1 study (N=213, high). *Positive association with inattention, but not hyperactivity or impulse control (ADHD) in children due to prenatal exposure to fluoride [58].*	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.
Cognitive, dementia	No studies	No studies	N/A	• 1 study (N=6,980, high). *Positive association with dementia risk [37].*	Insufficient evidence for an association
Cognitive, Down syndrome	2 SRs (2 NR); 1 study (acceptable) • 2 SRs (N= NR) *No clear association between water fluoridation and Down syndrome.* • 1 study (N=2,272,300)	1 study (acceptable; N=2,020,259) *No significant difference in the incidence rate of Down syndrome by fluoridation status*.	Limited evidence for no association at the current Canadian CWF levels.	No new studies	Limited evidence for an association at fluoride exposures relevant to current Canadian DWL.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
	No significant difference in the incidence rate of Down syndrome between CWF and non-CWF.				
Cognitive, IQ	1 SR (NR); 11 studies (1 high, 2 acceptable, 8 low) • 1 SR (2 studies; N=NR) *No evidence of sufficient quality to make any conclusions for a relationship between water fluoridation and IQ in children or cognitive impairment in adults.* • 1 study (N=942; acceptable quality and partial applicability to the Canadian context) *No difference in mean IQ scores of children and adults between fluoridated water (0.7 ppm-1.0 ppm) and naturally occurring water fluoride (0.0 ppm-0.3 ppm).* • 10 studies (N= 1,445) *Mixed findings on the relationship between water fluoridation and IQ or cognitive function from low quality studies with limited applicability to the Canadian context.*	6 studies (1 acceptable, 5 low) • 1 study (N=NR, acceptable) *No effect of water fluoride on cognitive ability, non-cognitive ability, and math test in participants aged ≥ 16 years in Sweden.* • 1 study (N=2,220, low) *No clear association between fluoride exposure and reported learning disability among Canadian children aged 3 to 12 years.* • 4 studies (N=1,341, low) *Mixed findings from studies of low quality and with limited applicability to the Canadian context.*	Limited evidence for no association at the current Canadian CWF levels.	22 studies (12 high, 10 acceptable) • 1 study (N=386, high) *Positive association of prenatal exposure to fluoride with sustained impacts on IQ* [418]. • 1 study (N=316, high) *Results in boys suggest improved scores in cognitive domains with maternal urinary concentrations* [419]. • 1 study (N=148, high) *Positive association between high fluoride exposure and lower IQ levels* [405]. • 1 study (N=596, high) *Positive association of IQ with prenatal and postnatal fluoride exposure, which may be modified by sex (further evidence needed)* [409]. • 1 study (N=709, high) *Positive association between low-to-moderate fluoride exposure with alteration of IQ* [413]. • 1 study (N=952, high) *Positive association between intelligence and fluoride, and possibly with the interaction of fluoride with mitochondrial function-related SNP-set, genes and pathways* [415].	Sufficient evidence for a positive association with lowering IQ scores in children at fluoride exposures relevant to current Canadian DWL.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
				• 1 study (N=567, high) *Positive association of exposure to drinking water fluoride and IQ in children. Dopamine-related genes polymorphism may modify the effects of such exposure* [416]. • 1 study (N=571, high). *Positive association with alterations in childhood thyroid function that may modify the association between fluoride and intelligence (IQ scores)* [41]. • 1 study (N=398, high). *Positive association with diminished non-verbal intellectual abilities (performance IQ)* [40]. • 1 study (N=299, high). *Positive association of prenatal exposure with lower GCI (IQ) scores in children at approximately 4 y old, and with lower Full-Scale IQ scores at 6–12 y old* [71]. • 1 study (N=2,886, high). *Negative association with IQ scores* [69]. • 1 study (N=323, high). *Negative association with IQ scores in children carrying the dopamine receptor-2 (DRD2) Taq 1A- TT genotype. No association with the other DRD2 Taq 1A genotypes* [59]. • 1 study (n=90, acceptable)	

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
				Positive association between excess drinking water fluoride exposure and IQ reduction [401] • 1 study (n=100, acceptable) *Positive association of intelligence of children with prevalence of fluorosis, with the intelligence level of those in high-exposure areas being lower than those in low-exposure areas* [414]. • 1 study (n=683, acceptable) *Positive association of IQ reduction with fluoride exposure, as well as with fluoride's interaction with MTHFD1 polymorphisms* [417]. • 1 study (N=498, acceptable) *Some non-significant frequency differences between urinary fluoride levels and reducing IQ scores* [29] • 1 study (N=498, acceptable) *No association between fluoride and IQ scores* [54] • 1 study (N >500.000, acceptable). *No evidence of an effect of water fluoridation on retardation in children* [75]. • 1 study (N=775, acceptable). *Possible inverse association with schooling performance (IQ)* [64]. • 1 study (N= 508, acceptable). *Decreased activity of the membrane bound enzymes, AChE and ATPase, indicates lower IQ scores as well as*	

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
				defect in signaling and energy metabolism in fluorosis patients [70]. • 1 study (N=149, acceptable). *Negative correlation with IQ* [76].	
Cognitive, memory loss	No studies	No studies	N/A	• 1 study (N= 508, acceptable). *Decreased activity of erythrocyte membrane bound enzymes, AChE and ATPase, which indicates defects in signaling and energy metabolism, and can predict memory loss (unmeasured)* [70].	Insufficient evidence to evaluate an association with memory loss.
Cognitive, trouble working	No studies	No studies	N/A	• 1 study (N >500.000, acceptable). *No evidence of an effect of water fluoridation on trouble working for children or adults* [75].	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.
CVD, atherosclerosis	1 study (N= 500, low) *Significantly higher risk of carotid artery atherosclerosis in adults in areas with high fluoride levels (>1.2 ppm).*	No studies	Insufficient evidence for an association at the current Canadian CWF levels.	3 studies (1 high, 2 acceptable) • 1 study (N=31, acceptable) *Children in the fluorosis area had higher blood TBARS levels, while the acyl hydroperoxide levels were non-significantly increased in comparison with healthy children living in the non-fluorosis area* [27]. • 1 study (N= 374, high). *Significant positive association of urinary fluoride with cardiovascular diseases'*	Limited evidence for an association at fluoride exposures relevant to current Canadian DWL.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
				markers VCAM-1, ICAM-1 and cIMT, significant negative association with sCys-C, and no significant association with ET-1 [45]. • 1 study (N= 508, acceptable). *Positive correlation with erythrocyte TBARS (p <0.01), plasma TBARS (p <0.05), cholesterol (p <0.01) and LDL (p <0.01). Significant inverse association with PON1, ARE, and lactonase. No significant association with TGL and VLDL. No observed correlation with serum HDL; however, serum fluoride modulates the activities of PON1, ARE and lactonase, which might be useful for predicting the risk of atherosclerosis in fluorosis patients* [70].	
CVD, hypertension	3 studies (low; N>160,637) *Mixed findings from studies of low quality and derived from countries where fluoride levels were many times higher than the current Canadian levels*	2 studies (2 low; N=3,224) *Mixed findings from studies of low quality and from countries where fluoride levels were many folds higher than the current Canadian levels (2 studies*	Insufficient evidence for an association at the current Canadian CWF levels.	No new studies	Insufficient evidence for an association at the current Canadian CWF levels.
CVD, myocardial infarction	No studies	1 study (low; N=474,217) *No significant difference in the risk of myocardial infarction and water*	Insufficient evidence for an association at the current Canadian CWF levels.	No new studies	Insufficient evidence for an association at the current

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
		fluoride levels in Sweden.			Canadian CWF levels.
Diabetes Mellitus	No studies	2 studies (2 low) • 1 study (N=NR) *No convincing evidence for an association between water fluoride levels and incidence of type 1 diabetes in Canada* • 1 study (N=NR) *A positive relationship between added fluoride in drinking water, even at optimum level, and the incidence and prevalence of diabetes in the US.*	Insufficient evidence for an association at the current Canadian CWF levels.	• 1 study (N=92, high) *The increase in serum Fluoride increases diabetes mellitus and diabetic nephropathy* [34]	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.
Eye, diseases	No studies	No studies	N/A	• 1 study (N= 1, 813, high). *Possible (significant) positive association of water fluoride levels with pterygium and arteriosclerotic retinopathy, and significant inverse association with cataract. Non-significant associations with primary angle closure glaucoma, diabetic retinopathy, age-related macular degeneration, and strabismus* [56].	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.
Eye, refractive errors	No studies	1 study (low; N=1,415) *No difference in prevalence of refractive errors*	Insufficient evidence for an association at the	No new studies	Insufficient evidence for an association at the current

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
		(myopia, hyperopia, astigmatism) between high and low water fluoride levels.	current Canadian CWF levels.		Canadian CWF levels.
Fluorosis, dental	3 SRs (2 NR, 1 high) • 1 SR (88 studies; N= NR) *Prevalence increased with water fluoride levels. Prevalence of dental fluorosis of any level of severity at 1 ppm was 48% (95% CI, 40 to 75), of which 12.5% (95% CI, 7.0 to 21.5) had fluorosis of aesthetic concern.* • 1 SR (10 studies; N= NR) *A fourfold higher risk in the development of overall dental fluorosis and fluorosis of aesthetic concern in optimal water fluoridation compared with non-CWF. The absolute increase in prevalence was 26% and 5%, respectively.* • 1 SR (90 studies; N= 59,630) *Prevalence of any level at 0.7 ppm was 40%, of which 12% had fluorosis of aesthetic concern*	21 studies (1 acceptable, 20 low; N= 35,374) *In all studies, dental fluorosis prevalence and its severity increased with increased water fluoride levels (21 studies;). The majority of evidence (17 out of 21 studies) derived from countries where water fluoride levels were many folds higher than the current Canadian levels.*	Consistent evidence for an association at the current Canadian CWF levels.	33 studies (15 high, 18 acceptable) • Thirty-two studies (15 high, 17 acceptable) *These studies reported a positive association with dental fluorosis at a wide range of fluoride concentration in drinking water (both tap and ground).* • Only 1 study [38] (acceptable, N= 1,397) *Study reported no significant association between high drinking water fluoride and dental fluorosis.*	Sufficient evidence for a positive association at fluoride exposures relevant to current Canadian DWL.
Fluorosis, skeletal	1 SR (NR); 2 studies (2 low) • 1 SR (1 study; N=NR)	2 studies (2 low; N=1,595)	Insufficient evidence for an association at the	3 studies (1 high, 2 acceptable) • 1 study (N=316, high)	Limited evidence for an association with

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
	Skeletal fluorosis found only in areas of high fluoride levels (3.8 ppm to 8.0 ppm). • 2 studies (2 low, N=2,816) *No clear relationship between water fluoride level and prevalence of skeletal fluorosis (<4, 4 to 6, and >6 ppm for one study, and 1.51 to 3.71 ppm for the other study).*	*Mixed findings from studies of low quality and from countries where fluoride levels were many folds higher that the current Canadian levels.*	current Canadian CWF levels.	*Positive association between fluoride and skeletal fluorosis [18].* • 1 study (N=3,268, acceptable) *Possible association between fluoride and skeletal fluorosis [23]* • 1 study (N=302, acceptable). *Possible impact on some of the genetic biomarkers of skeletal fluorosis [51].*	fluoride exposure relevant to Canadian DWL.
Genotoxicity	No studies	No studies	N/A	2 studies (1 high, 1 acceptable) • 1 study (N=281, acceptable) *Possible association of fluoride exposure with disrupting DNA methylation [24].* • 1 study (N=616, high) *Positive association of low-moderate water fluoride exposure and disrupting circulating mitochondrial DNA (mtDNA) levels [57].*	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.
Growth & development, BMI	No studies	No studies	N/A	• 1 study (N=149, acceptable). *Positive, non-significant correlation with BMI [76].*	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.
Growth & development, childhood obesity	No studies	No studies	N/A	• 1 study (N=2,340, high).	Insufficient evidence for an association at

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
				Low-to-moderate fluoride exposure is associated with overweight and obesity in children. Gender and paternal education level may modify the relationship [48].	fluoride exposures relevant to current Canadian DWL.
Growth & development, Newborn's height & weight	1 study (low; N=324) *Mothers exposed to drill water with a fluoride level of 4.7 ppm had higher risk to have low birth weight newborns.*	1 study (low; N= 492) *A positive correlation between babies' height (r = 0.69; P < 0.001) or babies' weight (r = 0.44; P < 0.001) and drinking water fluoride.*	Insufficient evidence for an association at the current Canadian CWF levels.	No new studies.	Insufficient evidence for an association at the current Canadian CWF levels.
Kidney, dysfunction	No studies	1 study (low; N= 824) *No conclusion could be drawn due to significant methodological limitations and lack of statistical analysis.*	Insufficient evidence for an association at the current Canadian CWF levels.	6 studies (4 high, 2 acceptable) • 1 study (N=311, acceptable) *Possible association with chronic kidney disease of unknown origin (CKDU)* [36] • 1 study (N=4,470, high). *Possible association with kidney functions (lower estimated glomerular filtration rate and blood urea nitrogen concentration, and slightly elevated serum uric acid concentration) in adolescents* [49]. • 1 study (N= 239, high). *Possible association with kidney tubular dysfunction* [60]. • 1 study (N=193, acceptable). *Possible association with chronic kidney disease of unknown origin (CKDU)* [44]. • 1 study (N= 374, high).	Limited evidence for an association at fluoride exposures relevant to current Canadian DWL.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
				Inconclusive association of fluoride exposure with kidney injury (increased eGFR, decreased uCys-C, and no significant association with KIM-1) [45]. • 1 study (N= 83, high). *No association was found between kidney injury biomarkers and fluoride* [73].	
Kidney, stones	No studies	1 study (acceptable; N=47,610,000 person-years) *Lower incidence of emergency admissions for kidney stones in CWF areas in England. Incidence rate ratio was 0.90 (95% CI, 0.82 to 0.98).*	Limited evidence for an inverse association at the current Canadian CWF levels.	No new studies	Limited evidence for an inverse association at the current Canadian CWF levels.
Kidney, ultrastructural	No studies	No studies	N/A	• 1 study (N=156, acceptable). *Positive association with ultrastructural changes and apoptosis in human renal tubules* [66].	Insufficient evidence for an association with fluoride exposure.
Liver dysfunction	No studies	No studies	N/A	2 studies (1 high, 1 acceptable) • 1 study (N=4,470, high). *Possible association between fluoride exposure and complex changes in liver functions* [49]. • 1 study (N= 508, acceptable). *Increased LDH5 isoenzyme (liver synthesized) activity is an indication of possible liver damage in fluorosis patients.*	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
				Therefore, prolonged fluoride ingestion overwhelmed the regenerative capacity of liver tissues. Serum fluoride modulates the activities of PON1, ARE and lactonase, which might be useful for predicting the risk of liver diseases in fluorosis patients [70].	
Neurologic, Headache	2 studies (2 low; N=5,342) *No conclusion could be drawn due to significant methodological limitations and lack of statistical analysis.*	No studies	Insufficient evidence for an association at the current Canadian CWF levels.	• 1 study (N=316, acceptable) *Possible association between fluoride and headache and paresthesia [18].*	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.
Neurologic, Sleep-related Outcomes	2 studies (2 low; N=5,342) *No conclusion could be drawn for the association of fluoride exposure and insomnia due to significant methodological limitations and lack of statistical analysis.*	No studies	Insufficient evidence for an association at the current Canadian CWF levels.	• 1 study (N= 419, high). *Fluoride exposure may contribute to changes in sleep cycle regulation and sleep behaviors among older adolescents in the US. Positive and significant association between water fluoride levels and sleep apnea, bed time and wake time. Non-significant positive association with recommended sleep duration and daytime sleepiness. Possible (significant) inverse association between water fluoride levels and snoring among males. No association with trouble sleeping [50].*	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
Reproduction, abortion and fertility	No studies	2 studies (2 low; N=5,993) *No clear relationship between water fluoride level and rates of abortion and fertility due to lack of controlling for confounders, from studies of low quality and of limited applicability to the Canadian context.*	Insufficient evidence for an association at the current Canadian CWF levels.	No new studies	Insufficient evidence for an association at the current Canadian CWF levels.
Reproduction, preterm births	No studies	No studies	N/A	• 1 study (N=9,234, high). *Women who had dental cleaning during pregnancy and lived in a community with water fluoridation had lower prevalence of preterm birth (significant). Water fluoridation alone was inversely associated (non-significant) with prevalence of preterm births [55].*	Insufficient evidence for an association with fluoride exposure.
Reproduction, sex hormones	No studies	No studies	N/A	2 studies (2 high), 1 abstract (N/A) • 1 study (N=3,392, high) *Significant inverse associations of fluoride in plasma and water with sex steroid hormones of total testosterone, estradiol and SHBG in U.S. children and adolescents.* • 1 study (N= 348, high). *Significant inverse association between urinary fluoride levels and serum sex hormone*	Limited evidence for an association at fluoride exposures relevant to current Canadian DWL.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
				binding globulin levels: SHBG (significant) and ABP (non-significant) [42]. • 1 abstract (N= 100, N/A). *Possible association with altering the hypothalamic testicular axis hormones in human males residing in high fluoride regions (insufficient study information)* [15].	
Thyroid function	3 studies (3 low) • 4 studies (N=789) *Mixed findings from studies of low quality and with limited applicability the Canadian context.*	4 studies (1 acceptable, 3 low) • 1 study (N=5,201) *No association between fluoride exposure and impaired thyroid functioning in the Canadian population.* • 1 study (N=7,935 GP practices) *Significantly higher odds of GP practice recording high levels of hypothyroidism in areas with fluoridation compared with areas without fluoridation in the US* • 2 studies (N=1,037) *No clear relationship between water fluoride and thyroid function from studies*	Insufficient evidence for an association at the current Canadian CWF levels.	7 studies (3 high, 4 acceptable) • 1 study (N=446, high) *Positive association with thyroid dysfunction (TSH, Tvol)* [20]. • 1 study (N=498, acceptable). *Non-significant frequency differences between urinary fluoride levels and TSH* [29] • 1 study (N=6,914,124, high). *Possible association with thyroid hypofunction [*[63]. • 1 study (N=571, high). *Positive association with alterations in childhood thyroid function that may modify the association between fluoride and intelligence (IQ scores)* [41]. • 1 study (N=400, acceptable). *Positive association with alteration in thyroid hormones activity* [61]. • 1 study (N=100, acceptable).	Limited evidence for an association at fluoride exposures relevant to current Canadian DWL.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
		of low quality and with limited applicability to the Canadian context.		*Positive association with increased thyroid hormone levels* [67]*.* • 1 study (N=293, acceptable). *No association with thyroid functions in children with normal nutritional status and optimal iodine intake* [53]*.*	
Other outcomes, arsenic methylation	No studies	No studies	N/A	• 1 study (N=236, high). *Positive association with increasing arsenic (As) toxicity in adults, which has been linked to adverse health effects such as cancer, cardiovascular diseases, diabetes and cardiometabolic risk* [46]*.*	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.
Other outcomes, general health	No studies	No studies	N/A	• 1 study (N >500.000, acceptable). *No evidence of an effect of water fluoridation on general health* [75]*.*	Insufficient evidence for an association at fluoride exposures relevant to current Canadian DWL.
Other outcomes, other non-skeletal manifestations of fluoride toxicity	2 studies (2 low; N=5,342) *No conclusion could be drawn due to significant methodological limitations and lack of statistical analysis.*	No studies	Insufficient evidence for an association at the current Canadian CWF levels.	2 studies (acceptable) • 1 study (N=316, acceptable) *Possible association between fluoride and Loss of appetite, constipation, and fatigue* [18]*.* • 1 study (N=903, acceptable). *Compared to low-fluoride group, persons in the high-fluoride group reported dyspepsia (75.0%), fatigue (59.4%), and muscle weakness (69.2%)* [68]*.*	Insufficient evidence for an association with fluoride exposure.

Outcome	NHMRC 2016	CADTH 2019 new evidence	CADTH 2019 conclusion	RSI 2021 new evidence	RSI 2021 conclusion
Other outcomes, suicide	No studies	No studies	N/A	• 1 abstract (N=201, N/A). *Possible association with decrease in suicide rates (insufficient study information)* [16].	Insufficient evidence for an association with fluoride exposure.

Summary of evolving animal evidence

The RSI search identified new animal evidence relating to twelve primary endpoints, updating the evidence reported in two previous authoritative reviews of animal studies: Health Canada 2010 [1] and the NTP 2016 [4] draft report on neurocognitive outcomes. A summary of evidence for each outcome, based on the most recent critical review and the current review, is provided in the following sections, with emphasis on effects occurring at or below exposures (i.e., 20 ppm) relevant to current fluoride levels in Canadian drinking water.

Neurological and cognitive outcomes

Summary based on the NTP 2020 draft report [328]: NTP systematically reviewed experimental animal studies published up until 2019 that investigated the effect of fluoride on neurodevelopmental and cognitive outcomes with priority given to learning and memory. This review also focused on mechanistically linked outcomes such as measures of thyroid function. Each included individual study was assessed for study quality using OHAT risk of bias tool. This review concluded that the evidence based on experimental animal studies was 'inadequate' to assess whether exposure to fluoride could affect learning and memory, particularly at human-relevant exposure levels. The primary rationale provided for this conclusion was "the inability to separate the learning and memory effects from the effects on motor activity or motor coordination". The majority of the studies were of poor quality in which either the assessment of learning and memory was not accompanied with evaluation of motor activity to determine whether the observed changes in learning and memory could be due to motor dysfunction or to determine whether results had been affected by adverse effects on general health [392]. However, those studies that did examine both cognitive and motor deficits, "mainly found an association between fluoride exposure and both types of neurological outcomes or found no effect of fluoride exposure on either type of neurological outcome irrespective of the dose range or duration of dosing".

RSI updated evidence synthesis: In the current review, a total of 3 low risk-of-bias studies with at least one test concentration ≤20 ppm (tier-1 study) and published since 2019 were identified. Although 1 study [215] found an impairment in the processes of spatial learning and memory in rats from long term fluoride exposure at 50 ppm, it possesses the same study limitations (i.e., no concurrent assessment of motor activity) identified in studies reviewed in

the draft NTP 2020 [328]. Two other low risk of bias tier 1 studies found no significant effects below 20 ppm [196, 240].

Endocrine including thyroid outcomes

Summary based on Health Canada 2010 report: No studies were found examining adverse effects on thyroid at exposure concentrations below 20 ppm. Only studies under very high fluoride exposures (600 mg/L) and/or iodine imbalance (excess or deficiency) conditions were identified.

RSI updated evidence synthesis: A total of two lower risk-of-bias tier-1 studies [187, 208] that assessed changes in thyroid related to fluoride exposure at or below 20 ppm fluoride in drinking water were identified. Both studies were conducted in rats (Wistar or Long-Evans hooded) with exposure concentration ranging from 2.3 to 20 ppm fluoride and for 2 to 8 months. One study [208] did not find a significant association between increase in fluoride concentration (up to 20 ppm) and change in thyroid hormone levels (TSH, T3, or T4); the other study [187] reported statistically significant – though inconsistent across two time points – changes in thyroid hormonal levels (serum T4 increased at 2 months but not at 8 months; serum TSH unchanged at 2 months but decreased at 8 months; serum T3 unchanged; apoptosis of thyroid cells increased) in rats exposed to 4.5 and 9 mg/L fluoride. Additionally, one tier-2 study [84] found significant decline in plasma T3 and T4 levels in rats exposed to 15 mg/L fluoride for 45 days.

RSI updated evidence synthesis: Overall, the studies included in the current review suggest no or inconsistent evidence of thyroid dysfunction in animals exposed to fluoride in drinking water at concentrations relevant to current fluoride levels in Canadian drinking water.

Renal or Kidney related outcomes

Summary based on Health Canada 2010 report: No studies found examining adverse effects on kidney at exposure concentrations below 20 ppm. Wistar mice exposed to very high fluoride concentrations (226 mg/L) during pregnancy through to the 14th day post-delivery demonstrated increase in urinary fluoride excretion (mothers and pups), increase in plasma creatinine and decrease in urinary creatinine levels. *RSI updated evidence synthesis:* Six low

to medium risk of bias animal studies [112, 116, 149, 174, 232, 282] were identified that evaluated fluoride effects on kidney function at test concentrations 20 ppm or below. These studies investigated the impact of fluoride exposure in rodents (mice or rats) at different exposure durations (chronic or sub-chronic) given a range of drinking water fluoride concentrations (ranged from 0.05 – 150 mg/L); specific outcomes measured include kidney dysfunction markers such as blood urea nitrogen (BUN) or creatinine (CRE) levels or histological analysis. Three out of six studies found some histopathological changes in kidneys (such as proximal tubule injury) but none reported any significant changes in kidney dysfunction markers such as BUN or CRE at or above test concentrations relevant to humans; except one study [232] found slight but significant increase in CRE levels after long term exposure at 20 ppm fluoride concentrations.

Reproductive/ Developmental outcomes

Summary based on Health Canada 2010 report: Numerous good quality animal studies reported adverse effects on reproductive function however these effects occurred only at very high concentrations that are not relevant to current fluoride levels in Canadian drinking water or at levels that known to cause dental and/or skeletal fluorosis. Further, high quality multigeneration guideline studies didn't find effects on reproductive function from continuous exposure to fluoride in drinking water.

RSI updated evidence synthesis: Twelve low to medium risk-of-bias tier-1 studies were identified that evaluated adverse effects on reproductive system in experimental animal studies from fluoride exposure at or below 20 ppm [109, 114, 115, 182, 183, 210, 262, 263, 277, 284, 288, 313]. These studies reported that fluoride exposure could induce changes in the organ coefficient of the testis, sperm count, sperm abnormalities, sperm motility, sperm survival, sperm hyperactivation, fertility, testosterone levels, testicular histology and fertility indices. These effects were observed at a range of fluoride exposure concentrations (5 – 100 ppm fluoride in drinking water), different exposure durations (49 to 211 days) and in multiple rodent species (rats and mice); only one study examined effects from exposures during premating, mating, gestation. The lowest concentration tested showing significant reduction in sperm quality was 5 mg/L fluoride. Overall, there was evidence of effects on male fertility, primarily decrease in

sperm quality and increased testicular damage, from fluoride exposures at concentrations relevant to current fluoride levels in Canadian drinking water.

Cancer

Summary based on Health Canada 2010 report: In a cancer bioassay, no malignant tumors related to fluoride exposure were observed, in Sprague-Dawley rats or CD-1 mice exposed to 25 mg/kg bw/day NaF for 95-99 weeks, or in F344 rats exposed to 250 mg/L NaF.

RSI updated evidence synthesis: No animal studies evaluating the association between fluoride exposure and cancer outcomes were found.

Skeletal/bone related outcomes

Summary based on Health Canada 2010 report: In a comprehensive chronic toxicity/ carcinogenicity bioassay, with F344/N rats and B6C3F1 mice exposed to drinking water containing up to 75 mg/L NaF for 2 years, the estimated NOAELs were 2.7 and 4.1 mg/kg bw/day for the female and male rats, respectively, and 5.7 and 4.9 mg/kg bw/day for the female and male mice, respectively.

RSI updated evidence synthesis: Three lower risk-of-bias tier-1 studies [150, 172, 258] were identified to assess the bone/skeletal related toxicity of fluoride exposure at or below 20 ppm fluoride in drinking water. Hosokawa et al. 2016 [150] reported no significant increase in any bone indexes in male and female nephrotic mice model (ICGN) exposed to NaF for 4 weeks. Kobayashi et al., [172] reported that fluoride in drinking water for 8 weeks didn't induce any significant changes in bone mineral density or bone modeling. Song et al., [258] reported increase in serum ALP, but no change in serum bone alkaline phosphatase activity, in Wistar rats exposed to 10 ppm fluoride for 15 and 30 days. Turkekul et al. [270] reported severe thinning of the epiphyseal growth plate and trabecular thickness, as well as fat accumulation in the bone marrow in a dose-dependent manner (5-50 ppm fluoride). In a few tier-2 studies, rats and mice exhibited, upon long term (84-210 days) fluoride exposure (15-50 ppm fluoride), elevated bone mineral density and serum ALP and worsened skeletal health (osteosclerosis, mild calcification of pelvic bone) [105, 106, 118, 132].

Diabetes or Glucose or Lipid Metabolism related outcomes

Summary based on Health Canada 2010 report: No animal evidence on diabetes, or any metabolism related outcomes occurred at or below 20 ppm fluoride exposure concentrations was identified.

RSI updated evidence synthesis: Three lower risk-of-bias tier-1 studies [194, 200, 329] were identified to assess association between diabetes or any metabolism related outcomes and fluoride exposure at or below 20 ppm fluoride in drinking water. Lupo et al. 2011, [200] reported that intake of fluoridated water from water supply (up to 15 ppm for 60 days) modified plasma insulin levels without affecting plasma glycaemia in Sprague-Dawley rats. No change in glycaemia, insulinemia, KITT, and HOMA2-IR were found in Wistar rats exposed to 10 ppm NaF for 22 days [194]. In another study [205], non-diabetic mice exposed to 10 ppm NaF had a significant reduction in the plasma glucose levels and a significant increase in the β-cell function.

Cardiovascular outcomes

Summary based on Health Canada 2010 report: In a multigeneration rodent study, Wistar rats exposed to 0.45, 4.5, 22.5, 45 mg/L in drinking water showed significant histopathological changes in the myocardial tissues (at ≥ 22.5 mg/L) accompanied by increase in markers of oxidative stress such as superoxide dismutase, GSH peroxidase, and catalase.

RSI updated evidence synthesis: The single tier-1 study identified [207] in this review found that after being exposed to NaF for up to 15 mg/L for 4.5 months, Wistar rats with chronic kidney dysfunction had significantly increased medial vascular calcification (MVC). No experimental studies on animals with normal kidney function were identified. In one tier-2 study [108] an observed increase in blood pressure and potential endothelial damage occurred at 10 mg NaF/L in healthy Sprague Dawley rats.

Respiratory outcomes

Summary based on Health Canada 2010 report: No animal evidence on respiratory outcomes were identified.

RSI updated evidence synthesis: No tier-1 or tier-2 study was identified.

Hepatic system related outcomes

Summary based on Health Canada 2010 report: No animal evidence on hepatotoxicity was identified.

RSI updated evidence synthesis: Two lower risk-of-bias tier-1 studies [116, 232] were identified to assess the hepatotoxicity of fluoride exposure at or below 20 ppm fluoride in drinking water. Chattopadhyay et al. 2011, [116] reported increasing GPT level, decreasing GST levels, and extensive vacuolar degeneration in the cytoplasm and loss of integrity in the epithelium lining of central vein, on 8 weeks old Swiss albino mice, exposed at 15ppm NaF exposure for 30 to 90 days. Perera et al. 2018, [232] reported a dose-response increase in serum AST and ALP on male adult Wistar rats, exposed to up to 20 ppm NaF for 60 days. Another tier-2 study, Owumi et al., [225] showed that male Wistar rats exposed to fluoride (15 mg/L in drinking water) for 14 days had decreasing antioxidant enzyme activities, evidenced by elevated ROS & RONS levels and histopathological damage via enhancement of inflammatory responses.

Immune system related outcomes

Summary based on Health Canada 2010 report: No animal evidence on immunotoxicity was identified.

RSI updated evidence synthesis: Two low risk-of-bias tier-1 studies were identified. Gutierrez-Salinas et al. [147] assessed the immunotoxicity of fluoride exposure at or below 20 ppm fluoride in drinking water; however, the observed changes (decreased metabolic activity or increase in apoptotic markers in macrophages) occurred only at higher concentrations (i.e., 50 mg/L). Li et al. [180] also observed immunotoxicity of fluoride exposure changes at 11.25 ppm F and above, as well as histopathological changes of the spleen (an unclear junction between the splenic cortex and medulla, and irregularly shaped cells). Another two tier-2 studies were identified [151, 279]. Wang et al. 2019 [279] reported that serum cytokine contents (IL-1β, IL-2, IL-6, and TNF-α) was significantly decreased in Sprague-Dawley rats exposed to NaF 25 and 50 mg/L for 70 days. Hosokawa et al. 2015 [151] showed a decline in relative weights of spleens, in male BALB/c mice exposed to 1 and 5 ppm for 30 days.

Genotoxicity

Summary based on Health Canada 2010 report: “Inconsistencies in the overall results of the studies on the genotoxicity/mutagenicity potential of fluoride do not allow for firm conclusions to be made regarding the genotoxic potential of fluoride although the balance of evidence for genotoxicity of fluoride does not support the view that fluoride is genotoxic in humans.”

RSI updated evidence synthesis: One lower risk-of-bias tier-1 study [234] was identified to assess the genotoxicity of fluoride exposure at or below 20 ppm fluoride in drinking water. It showed that increase in the percentage of aberrant metaphases and chromatid breaks was more salient in animals treated with 15 mg/L fluoride, than higher doses. One tier-2 study [233] observed significant increase in percentage of dead cells in bone marrow and in percentage of aberrant cells (cells with chromatid breaks) and chromatid breaks, in male Swiss-albino mice exposed to NaF 15 mg/L for 30 days.

Intestinal outcomes

Summary based on Health Canada 2010 report: No animal evidence on intestinal outcomes was identified.

RSI updated evidence synthesis: No tier-1 or tier-2 studies were identified to assess the intestinal toxicity in animals exposed to fluoride at or below 20 ppm in drinking water.

Summary of in vitro evidence

Evidence from in vitro models of humans and non-human animals

Data abstraction from original in vitro studies is not considered; however, literature reviews covering original studies investigating fluoride induced toxicity in various in vitro models would be reviewed and summarized, for weight of evidence assessment along with animal and human data.

RSI reviewed in vitro studies to understand the mechanisms of action of fluoride in exposed animals or humans. The evidence collected from literature reviews on this subject was discussed by type of mechanism, and we summarized concentration ranges in which fluoride

induced a positive effect, e.g., oxidative stress apoptosis, ER-stress pathway activation, [Ca^{2+}] increase etc.

Oxidative stress

As described, “oxidative stress is a recognized mode of action of fluoride exposure that has been observed in vitro in several types of cells and also in vivo in soft tissues such as the liver, kidney, brain, lung, and testes in animals and in people living in areas of endemic fluorosis” [330].

Reactive Oxygen species (ROS) can be generated from a variety of sources classified as exogenous and endogenous; UV irradiation, ozone and polyaromatic hydrocarbons (PAH) are key examples for exogenous sources. There are multiple sources considered as endogenous such as mitochondrial oxidative phosphorylation, xenobiotic metabolism, active peroxisomes and inflammation. Numerous studies demonstrated that one of the downstream effects of increase in release of ROS and subsequent oxidative stress is induction of cytotoxicity by activating apoptotic pathways. At cellular level, fluoride appeared to induce oxidative stress, cell cycle arrest, and apoptosis through various pathways such as inhibition of metalloproteins, organelle disruption, altered pH, and electrolyte imbalance. For example, excess NaF (up to 3 mM) showed to cause DNA damage, oxidative stress, mitochondrial agglutination and cytoskeleton damage to neuronal cell lines [331]. Several in vitro studies that demonstrated oxidative stress upon fluoride exposure were identified by searching through published review studies on this association and are summarized in

Table *12*. In summary, based on these studies, fluoride (mostly as NaF salt) caused cytotoxicity or another apical endpoint via induction of oxidative stress pathway (measured as ROS levels, SOD activity, LDH release etc.) in a range of cell lines at concentrations ranging between 0.005 mM and 6 mM. These concentrations may be compared to human plasma fluoride levels.

Table 12: Characteristics of studies on oxidative stress

Reference	Cellular system	Fluoride Exposure	Endpoints assessed (positive effect)
Chen et al., 2017 [332]	Neuro-2A (mouse neuroblastoma cell line)	1 – 6 mM	Cell viability Lactate dehydrogenase (LDH) release
Zhang et al., 2015 [333]	PC 12 cells (pheochromocytoma cells)	0.005 mM	Intracellular ROS increase Apoptotic cells Cytotoxicity
Chen et al., 2017 [334]	BV-2 microglia cells	0.5 - 2 mM	Increase of IL-6 concentration Decrease of cell viability Decrease in SOD activity Increase of TNF-α level
Shuhua et al., 2012 [335]	BV-2 microglia cells	0.024 mM	SOD activities decreased NOS (synthesizing NO) increased
Xu et al. 2013 [336]	Human neuroblastoma SH-SY5 Y cells	0.48 - 0.95 mM	LDH levels higher
Ma et al., 2017 [337]	Human umbilical vein endothelial cells (HUVECs)	0, 4.2, and 8.4 mg/L	Oxidative stress and impaired NO production are involved in their pro-inflammatory and pro-apoptotic effects.
Grzegorzewska et al., 2020 [338]	Chicken embryonic gonads	75, 150, 300, and 600 mg/L	Increased expression of antioxidant enzymes (CAT and SOD) and nuclear respiratory factors (Nrfs)
Peng et al., 2019 [339]	F9 embryonic carcinoma cells	0, 40, 80, and 160 mg/L	Decreased Sirtuin 1 (Sirt1) protein expression, promoted the acetylation of manganese superoxide dismutase (SOD2), increased mitochondrial reactive oxygen species (mROS) production, and stimulated cytotoxicity
García-Montalvo et al., 2009 [141]	Mouse pancreatic beta-cells (betaTC-6)	0.15, 0.4, 3, 20, and 40 mg/L	Decreased SOD activity, in a dose-dependent manner, increase in the generation of O (2) (-), and decreased mitochondrial membrane potential
Zhang et al., 2007 [340]	Primary rat hippocampal neurons	20, 40, and 80 mg/L	Increased malondialdehyde levels, decreased glutathione levels and glutathione peroxidase activities, reduced superoxide dismutase activity
Gao et al., 2008 [341]	Neuroblastoma (SH-SY5Y) cells	1 to 100 mg/L	Lipid peroxidation and protein oxidation in a dose-response manner

Apoptosis

Apoptosis is genetically programmed cell death, an irreversible process of cell senescence with characteristic features different from other cellular mechanisms of death such as necrosis. There are three pathways related to fluoride exposure induced apoptosis: mitochondrion-mediated, endoplasmic reticulum (ER) stress-mediated, and death receptor-mediated pathways [330, 342-346].

Mitochondrial dysfunction

Mitochondrial dysfunction has been shown to contribute to the occurrence of apoptosis and it is central to the apoptotic pathway [347]. There are three key types of proteins involving in this pathway: Bcl-2 family proteins, caspases, and mitochondrial pro-apoptosis proteins a rising level of intracellular reactive oxygen species (ROS), as a signal of oxidative stress, can also activate the apoptosis signaling pathway Mitochondrion is both a target of ROS and a source of the additional ROS generation [345].

Evidence show that fluoride exposure induces apoptosis by regulating the mitochondrial pathway (decreased MMP and increased ROS) in H9C2 cardiomyocytes [348], human thyroid cells [349]), and umbilical vein endothelial cells [337]. Fluoride exposure can trigger apoptosis via increasing mRNA or protein levels of Cyt c, caspase-3, and caspase-9 in HL-60 cells [350], Leydig cells [351], H9C2 cardiomyocytes [352], and human lung BEAS- 2B cells [353]. Another study found that fluoride induced cell apoptosis is accompanied by increased Bax mRNA expression level and reduced Bcl-2 expression level in PC12 cell [312].

Table 13: Characteristics of studies on mitochondrial dysfunction

Reference	Cell lines	Sodium fluoride concentrations	Findings and conclusion
Yan et al., 2015 [348]	H9c2	0, 2, 4, 8, and 16 mg/L	Induced apoptosis by increasing intracellular reactive oxygen species and downregulating mitochondrial membrane potential
Liu et al., 2014 [349]	human thyroid c ells (Nthy-ori 3-1)	0, 0.4, 4.2, and 12.6 mg/L	Decreased cell viability improves the lactate dehydrogenase leakage rate, and reactive oxygen species level.
Ma et al., 2017 [337]	human umbilical vein endothelial cells (HUVECs)	0, 4.2, and 8.4 mg/L	Induced endothelial activation and apoptosis. Oxidative stress and impaired NO production are involved in their pro-inflammatory and pro-apoptotic effects.
Anuradha et al., 2001 [350]	HL-60	8.4 mg/L	Induced apoptosis by oxidative stress-induced lipid peroxidation, causing loss of deltaPsi(m), and thereby releasing cytochrome c into the

Reference	Cell lines	Sodium fluoride concentrations	Findings and conclusion
			cytosol and further triggering the caspase cascade.
Song et al., 2014 [351]	Leydig cells	0, 5, 10, and 20 mg/L	Increased expression levels of stress response factors, signal transduction components, and apoptosis-related proteins, including caspase-3/caspase-9, B-cell lymphoma 2 (Bcl-2), and Bax
Yan et al., 2017 [352]	H9c2	0, 5, 10, 20, and 40 mg/L	Increased mRNA levels of caspase-3, caspase-9, and cytochrome c. Induced apoptosis through the mitochondrial pathway.
Ying et al., 2017 [353]	human lung BEAS-2B	0, 1, 2.1, 4.2, 8.4, and 16.8 mg/L	Induced apoptosis through mitochondria-mediated signal pathways. Increased bax, caspase-3, caspase-9, p53, and the cytoplasmic CytC, decreased bcl-2 and mitochondrial CytC. Increased ROS and decreased membrane potential of mitochondria.
Liao et al., 2017 [312]	PC12	0, 2.1, and 21 mg/L	Decreased cell activity, enhanced cell apoptosis, increased c-fos, CAMKII, and Bax mRNA expression. Decreased Bcl-2 expression.

Endoplasmic reticulum dysfunction

The endoplasmic reticulum is the main site for the folding and maturation of transmembrane, secretory, and ER-resident proteins. Accumulation of misfolded and unfolded proteins will cause ER stress, leading to the activation of self-protecting mechanisms called unfolded protein response (UPR). UPR is responsible for either relieving ER stress or inducing apoptosis [354, 355].

Fluoride exposure could induce apoptosis by triggering ER stress through upregulated GRP78, PERK, phosphorylation-eukaryotic initiation factor 2α (p-eIF2α), and CHOP in Sertoli cells ([296]) and human thyroid follicular epithelial cells [349]. Studies on mouse ameloblast-derived LS8 cells showed that fluoride exposure could induce caspase-dependent apoptosis through overexpression of PERK, eIF2α, IRE1, activation of Xbp-1, BiP/GRP78, GADD153/CHOP, and JNK, which in turn inducing ER stress and UPR [356-358].

Table 14: Characteristics of studies on endoplasmic reticulum dysfunction

Reference	Cell lines	Sodium fluoride concentrations	Findings and conclusion
Yang et al., 2015 [296]	Sertoli cells	0, 6, 12 and 24 mg/L	Decreased cell viability and induced apoptosis. Increased ER stress by up-regulating glucose-regulated protein 78 kDa (GRP78), PKR-like ER kinase (PERK), phosphorylation of eukaryotic translation initiation factor 2α (p-eIF2α) and CCAAT/enhancer-binding protein-homologous protein (CHOP)
Liu et al., 2014 [349]	human thyroid cells (Nthy-ori 3-1)	0, 4.2 mg/L	Induced cytotoxicity related to IRE1 pathway-induced apoptosis.
Sharma et al., 2008 [356]	ameloblast-derived LS8	0, 5.1, 10.5, 21, and 42 mg/L	Induced ER stress response interfering with protein synthesis and secretion. Extracellular secretion of SEAP decreased in a linear, dose-dependent manner.
Sharma et al., 2010 [357]	ameloblast-derived LS8	0, 2.1 mg/L	Increased cell stress by phosphorylating stress-related proteins, PERK, eIF2α, JNK and c-jun
Kubota et al., 2005 [358]	ameloblast-derived LS8	0, 21, 42, 63, and 84 mg/L	Inhibited cell growth at low dose, whereas higher doses induced ER stress and caspase-mediated DNA fragmentation.

Death receptor-mediated pathways

Fluoride can induce apoptosis by regulating Fas ligand (FasL)/Fas signaling pathway and tumor necrosis factor-α (TNF-α)/tumor necrosis factor-αrecpter-1 (TNF-R1) signaling pathway, which belongs to the death receptor pathways.

Fluoride exposure could induce apoptosis by upregulating the protein expression of FasL, Fas, caspase-8, caspase-3, and cleaved PARP in the primary rat ameloblasts [359], human neuroblast cells [360], and human gingival fibroblasts [361]. Studies using mice splenic lymphocytes show that fluoride exposure cause ER stress and UPR [362] and decreasing mitochondria transmembrane potential, up-regulating Bax, Bak, Fas, FasL, caspase 9, caspase 8, caspase 7, caspase 6 and caspase 3, and down-regulating Bcl-2 and Bcl-xL [363].

Table 15: Characteristics of studies on death receptor-mediated pathways

Reference	Cell lines	Sodium fluoride concentrations	Findings and conclusion
Wang et al., 2016 [359]	primary rat ameloblast	0.4, 0.8, 1.6, 3.2, and 6.4 mmol/L	Induced apoptosis via activation of FasL/Fas signaling pathway and diminished secretion of AMBN
Deng et al., 2016 [362]	mice splenic lymphocytes	0, 4.2, 8.4, and 16.8 mg/L	Induced apoptosis and caused ER stress by up-regulating protein expression levels of glucose-regulated protein 78 (BiP) and glucose-regulated protein 94 (GRP94), and by activating unfolded protein response (UPR).
Deng et al., 2016 [363]	mice splenic lymphocytes	0, 4.2, 8.4, and 16.8 mg/L	Induced apoptosis by decrease of mitochondria transmembrane potential, up-regulation of Bax, Bak, Fas, FasL, caspase 9, caspase 8, caspase 7, caspase 6 and caspase 3 protein expression, and down-regulation of Bcl-2 and Bcl-xL protein expression.

Reference	Cell lines	Sodium fluoride concentrations	Findings and conclusion
Lee et al., 2008 [361]	human gingival fibroblasts	5, 10, 20, 30, and 40 mmol/L	Induced apoptosis through the Bcl-2 family and death receptor-mediated pathway. Increased cytochrome c release from the mitochondria into the cytosol, enhanced the caspase-9, -8 and -3 activities, the cleavage of poly (ADP-ribose) polymerase (PARP), and up-regulated the voltage-dependent anion channel (VDAC).
Xu et al., 2011 [360]	human neuroblastoma (SH-SY5Y)	0, 20, 40, and 80 mg/L	Induce apoptosis by increasing caspase-3 and mRNA expression levels for Fas, Fas-L, and caspases (-3 and -8).

Na, K-ATPase

Sodium, potassium-activated adenosine triphosphatase (Na, K-ATPase) is a member of the P-type family of active cation transport proteins, which maintains sodium and potassium homeostasis in animal cells by transporting Na+-ions to the outside and K+-ions to the inside of the cell, at the expense of ATP. Na, K-ATPase is responsible for the electrochemical gradient across the plasma membrane and the regulation of the cellular ionic homeostasis. In addition, Na, K-ATPase activity plays a crucial role in the function of neurotransmitter transporters, which are essential for regulating neurotransmitter signaling and homeostasis. [364, 365].

Fluoride exposure inhibits the activity of Na, K-ATPase through multiple pathways. In summary, fluoride has been shown to upregulate PKC, cAMP, cGMP, NO, Pi, PLA2, AA, PGE2, dopamine, glucose and PTH. The formation of these biomarkers inhibits Na, K-ATPase activity.

Table 16: Characteristics of studies on Na, K-ATPase

Reference	Cell lines	Sodium fluoride concentrations	Findings and conclusion
Gutowska et al., 2010 [366]	human monocytic line THP-1	0.4, 1.2, 2.4, 4.0 mg/L	decreased the amount of synthesized cellular ATP and increased formation of ROS and apoptosis in a dose-dependent pattern
Agalakova & Gusev 2012 [367]	rat erythrocytes	0, 4, 20, 80, 400 mg/L	Dose- and time-dependent decline of ATP a, diminishing to extremely low levels within 24h.
Cittanova et al., 2002 [368]	rabbit kidney thick ascending limb cells	0, 40, 200, and 400 mg/L	Depletion of Na-K-ATPase activity and renal mitochondrial dysfunction

Inflammatory response

Inflammation is the body's immune system's response to an irritant, e.g., infection or tissue damage. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair. Chronic inflammation plays an important role in the development of chronic conditions, e.g., diabetes, atherosclerosis, cardiovascular disease, allergies, and COPD [330, 342, 343].

Studies have shown that fluoride exposure can promote inflammatory response via increasing oxidative stress and ROS in human umbilical vein endothelial cells (Ma 2017), human monocytic line THP-1 [366], and RAW 264.7 murine macrophage line [369]. Fluoride related phosphorylation of c-Jun NH (2)-terminal kinase (JNK) was involved in the pro-inflammatory response in the MDPC-23 odontoblast-like cells ([370]) and human ameloblast lineage cells ([371]).

Table 17: Characteristics of studies on inflammatory response

Reference	Cell lines	Sodium fluoride concentrations	Findings and conclusion
Ma et al., 2017 [337]	human umbilical vein endothelial cells (HUVECs)	0, 4.2, and 8.4 mg/L	Endothelial activation and apoptosis. Oxidative stress and impaired NO production are involved in their pro-inflammatory and pro-apoptotic effects.
De la Fuente et al., 2016 [369]	RAW 264.7 murine macrophage line	5, 10, 25, and 65 mg/L	Increased ROS, redox imbalance, lipid peroxidation, and cytokines Il6 and Mip2
Gutowska et al., 2010 [366]	human monocytic line THP-1	0.4, 1.2, 2.4, 4.0 mg/L	Depleted ATP and increased ROS and apoptosis in a dose-dependent pattern
Karube et al., 2009 [370]	MDPC-23 odontoblast-like cells	200 mg/L	phosphorylation of c-Jun NH(2)-terminal kinase (JNK) and p38, exhibited caspase-3 activation, cleavage of poly(ADP-ribose) polymerase, DNA fragmentation, and an increase in cytoplasmic nucleosomes
Zhang et al., 2007 [371]	human ameloblast lineage cells	0.4 mg/L	Decreased MMP-20 protein levels and related to suppression of JNK/c-Jun phosphorylation.
Zhang et al., 2007 [340]	primary rat hippocampal neurons	20, 40, and 80 mg/L	Induced S-phase cell-cycle arrest, up-regulation of NF-kappaB and DNA damage

VIII. Candidate endpoints and Bradford Hill considerations

Selection of new candidates for the most sensitive endpoint(s)

In reviewing evidence for the link between fluoride in drinking water and all health endpoints, a number of provisional candidate endpoints were considered as the basis for updating the HBV, based on identifying a most sensitive endpoint). In evaluating the body of evidence identified in the RSI literature review, the following hierarchical approach was used for

selection of candidate endpoints. A health endpoint was chosen for further examination (using Bradford Hill's considerations for causality) [8] based on the following criteria:

1. In the human stream of evidence for a specific endpoint:
    a. The endpoint was of concern (either serious or severe)
    b. There was consistent evidence of an association across studies
    c. The association occurred in the studies at a level below that of current municipal water supplies or close to this exposure level (not higher than 2 ppm)
    d. Studies were of reasonable quality (high or acceptable)
2. If the human stream of evidence was inconclusive for a specific endpoint, but in the animal stream of evidence:
    a. There was consistent evidence of an association in Tier-I animal studies
    b. The association occurred in the studies at an exposure level (less than 20 ppm F) relevant to current fluoride levels in Canadian drinking water

After consideration of all evidence identified in the updated literature review, consolidated with earlier reviews from Health Canada, CADTH, NTP, several authoritative reviews, and numerous published peer-reviewed systematic reviews, four endpoints were selected based on considerations of the overall evidence and whether these effects were plausibly occurring at exposure levels close to fluoride exposure levels in the Canadian context.

The four endpoints identified as candidates for most sensitive endpoint (with the exception of dental fluorosis) were:

- Cognitive dysfunction (specifically, reduced IQ score)
- Thyroid dysfunction
- Kidney dysfunction
- Male sex hormone alterations

Bradford Hill considerations for causality

For each of the selected candidate endpoints, relevant human, animal, and in vitro evidence was organized and evaluated along the nine Bradford Hill considerations [8]. While avoiding

any misapplication of these considerations as hard rules of evidence, the review attempted to qualify how credible the associations were to support a claim of causality. As Hill remarked, “What [the nine viewpoints] can do, with greater or less strength, is to help us to make up our minds on the fundamental question—is there any other way of explaining the set of facts before us, is there any other answer equally, or more, likely than cause and effect?” [8]. The considerations include the following: strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy. In weighing evidence of causality, the review drew from human, tier-I and tier-II animal, and in vitro streams of evidence. Only evidence from original studies of high and acceptable quality was included in the evaluation of each endpoint. To support each of the considerations, evidence was cited where available from the NHMRC 2016, [9, 10], CADTH 2019 [2, 3], and the current RSI update. The consideration for “strength of association” was only assessed based on studies reporting positive or possible associations. For the “consistency” consideration, all relevant studies were included irrespective of the nature of the reported association.

Reducing IQ scores

NHMRC 2016 [9, 10] identified a study of acceptable quality [372], which reported a statistically significant negative correlation between drinking water fluoride and IQ. CADTH 2019 identified one study of acceptable quality (no association), and 5 studies of low quality (mixed findings). RSI identified 18 studies of high or acceptable quality that reported a positive/possible association between reduced IQ scores and water fluoride [40, 41, 64, 69, 70, 76, 401, 405, 413-416], or urinary fluoride levels [59, 71, 409, 417-419]. Based on the available literature to date, the accumulating body of evidence suggests a positive association of reduced IQ scores for children and fluoride exposures relevant to current Canadian drinking water levels.

The available evidence demonstrated a **moderate to strong magnitude (strength) of association** between fluoride and neurocognitive effects with **consistent** evidence across studies for the impact on children’s IQ at fluoride exposures relevant to current Canadian drinking water levels. Fluoride appears to play a role in the induction of a range of adverse health outcomes, and is **not specific** to a single health effect. Although a **temporality** cannot be evaluated in the available cross-sectional studies of the potential health effects of fluoride, this condition is satisfied in two large cohort studies showing reduction in children’s IQ scores.

Significant **increasing exposure-response relationships** between fluoride in drinking water and reduction in IQ scores were noted in seven epidemiologic studies. Fluoride is known to dysregulate the activity of certain enzymes in relation to IQ [70]. However, at this time **no specific mechanisms** could be determined for fluoride effects on learning and memory or other neurodevelopmental or cognitive outcomes. Results from this assessment of the included studies are summarized in Section 6 of the Supplementary Material.

Table 18: Hill's consideration of causality for fluoride and IQ reduction

Criterion	Summary of recent evidence
Strength of association	• A study of acceptable quality examined by NHMRC [372] reported a significant negative correlation between IQ and drinking water fluoride levels. A reduction of 6.7, 11.2, 10.2 in performance, verbal and full IQ scores, respectively, was observed per increase in log fluoride values [372]. • Although CADTH did not consider the Canadian cohort study conducted by Green et al [326] to be of acceptable quality, the study was subsequently assessed by the 2020 [238] and 2022 [420] NTP draft reports as having an overall low risk of bias. This study showed a positive association between higher maternal fluoride intake and reduction in IQ. A reduction of 3.66 points was reported per 1 mg increase in daily maternal intake of fluoride. • RSI identified 18 new studies [40, 41, 59, 64, 69-71, 76, 401, 405, 409, 413-419], which provided statistically significant results supporting a positive/possible association of reduced IQ levels in response to increasing exposure to water fluoride. Two studies reported no association between reduction of IQ scores and drinking water [398], or non-significant frequency differences among urinary fluoride levels [29]. • Three studies [40, 69, 418] reported that an increment of 0.5 mg/L in water fluoride concentration corresponded to: ▪ A 9.3- and a 6.2-point reduction in performance IQ in formula-fed and breastfed children, respectively. Such an association remained significant upon controlling for fetal fluoride exposure [40]. ▪ A 40% reduction in the odds of having excellent IQ in those exposed to low fluoride levels (0.20-1.40 mg/L) [69]. ▪ A drop of 2 points in full-scale IQ scores [418]. • A more recent study that used the same cohort (MIREC) reported an association between children's performance IQ and fluoride exposure during the perinatal period and into early childhood. Such an association was reported to differ

	between boys and girls across the different exposure periods, though more validation is proposed by the authors [409]. • Another recent study [419] examined prenatal fluoride exposure in a small mother-child birth cohort in Spain: Results in boys suggest improved scores in cognitive domains with maternal urinary concentrations.
Consistency	• A total of 15 RSI-identified studies [40, 41, 59, 69, 71, 76, 401, 405, 409, 413-419] and 1 earlier study [372] of high/acceptable quality concluded a positive association with reduced IQ[32] levels in children/adolescents. • Two RSI-identified studies [64, 70] concluded a possible positive association, and 1 reported a non-significant association [29] with reducing IQ scores. • Three RSI-identified studies [54, 75, 398] and 3 earlier [373-375] studies of high/acceptable quality reported no association • Within the 18 RSI-identified studies showing a positive/possible association, the directionality of association did not differ by study design (12 studies were cross-sectional, 5 were cohorts and 1 was a case-control study), geographic location (6 in China, 3 in India, 2 in each of Canada and Mexico, and 1 in each of Indonesia, Pakistan, Spain and Sudan), studied population (12 studies in children and/or adolescents, 5 in mother-child pairs and 1 that examined patients of all ages), or sampling time-frame.
Specificity	Fluoride appears to play a role in the induction of a range of adverse health outcomes, and reduction in IQ levels can be caused by a number of risk factors including exposure to toxic factors other than fluoride.
Temporality	Out of those that showed positive or possibly positive association, there were different follow up durations reported in two cohort studies • Four cohort studies [71, 409, 418, 419] reported an association of maternal fluoride exposure and reduced IQ levels in their offspring, where the long follow up period (at ages 1, 4 then at age 6-12) allowed a reasonable assessment of temporality • Another cohort study [40] reported on the association of water fluoride exposure in newborns using breast milk or formula, where IQ was measured at 3-4 years old. • Fifteen other studies [29, 41, 59, 64, 69, 70, 76, 372, 401, 405, 413-417] that showed positive or possibly positive association were cross sectional, whereby an inference of causality cannot be concluded.

[32] IQ: involving scores of different IQ tests, or the use of proxies to IQ such as school performance, school tests

Biological gradient (exposure-response)	• Nineteen studies [40, 41, 59, 64, 69-71, 76, 372, 401, 405, 409, 413-419] provided statistically significant exposure-response relationship supporting a positive/possible association between exposure to fluoride and lower IQ levels, with varying categories of exposure (continuous vs. categorical). • One study [70] reported a change in the serum levels of two enzymes (AChE and ATPase/Na+ K+ ATPase), which upregulate IQ-related neurological operations, in response to fluoride exposure levels as measured by the severity of fluorosis diagnosis (mild, moderate, severe) ▪ AChE: Controls: 6.29 ± 0.68; Mild: 4.64 ± 0.54; Moderate: 4.11 ± 0.4; Severe: 3.78 ± 0.35 ▪ ATPase/Na+ K+ ATPase: Controls: 2.41 ± 0.34; Mild: 2.56 ± 0.31; Moderate: 2.64 ± 0.29; Severe: 2.87 ± 0.4 • Another study by Wang 2020 [41] reported a change in IQ scores per 1 mg/L increment of water fluoride (continuous) or compared to a reference quartile. ▪ Continuous: β= −1.587 (−2.607, −0.568), p-value= 0.002 ▪ Quartiles: IQ scores, β= (95% CI), p-value o Quartile 1 (≤0.70): reference o Quartile 2 (0.70–1.00): β= −0.506 (−3.764, 2.753), p-value= 0.761 o Quartile 3 (1.00–1.90): β= −3.065(−5.636, −0.493), p-value= 0.020 o Quartile 4 (> 1.90): β= −3.471(−6.108, −0.835), p-value= 0.010
Biological plausibility	• Fluoride has reportedly been capable of crossing the blood brain barrier with the subsequent accumulation in brain tissues [69]. Animal evidence showed fluoride in excess is capable of reducing the “fluidity of the synaptic membrane of the hippocampus in rats” leading to hippocampal neuronal impairment and neurodegenerative changes, resulting in impaired learning and memory [69]. Such neurologic/cognitive effects are more pronounced in children compared to adults due to the former’s relatively less brain capacity to clear the toxicant (fluoride) burden [69]. • Other proposed biological mechanisms include a direct fluoride effect on the activity of PON1 enzyme via oxidative stress, and/or inactivation of various enzymes (PON1, Na+-K+ ATPase and ALAD) [70]. • Human studies also reported on the possibility of fluoride to cross the placental barrier from the mother to her developing fetus [71], or via breastfeeding to her newborn [40]. • Infants who are fed formula reconstituted with fluoridated water have approximately three to four times greater exposure to fluoride than adults on a per

	body-weight basis. Formula-fed infants residing in fluoridated areas have an approximate 70-fold higher fluoride intake than exclusively breastfed infants [40] • However, based on the draft 2020 NTP [328] review of evidence on mechanism of action related to fluoride induced neurocognitive effects, the available data is "too general" or "cannot necessarily be attributed to effects on learning and memory or other cognitive functions". Therefore, at this time no specific mechanism could be determined for fluoride effects on learning and memory or other neurodevelopmental or cognitive outcomes.
Coherence	Coherence with previous evidence cannot be assessed based on the findings: • No specific mechanisms were directly linked to fluoride and IQ • Non-human evidence was inconclusive/inadequate
Experimental evidence	• There has been no experimental evidence generated from human studies. • The most recent systematic review of experimental evidence[33] found that the animal data were inadequate to evaluate the effects of fluoride on learning and memory due to reasons such as difficulty in parse out the observed learning and memory effects from the effects on motor activity or motor coordination and concerns of study quality. Additionally, one of the highest quality experimental studies [208] found no fluoride treatment related effects on learning, memory or motor activity in rats provided with up to 20 ppm concentration in drinking water • This review concluded that "the experimental animal body of evidence does not contribute to confidence in conclusions derived from human epidemiological studies with respect to effects on human IQ. Although the evidence supports an association between fluoride exposure and neurodevelopmental effects, the data are not sufficient to support the primary effect evaluated in children (i.e., IQ) nor is it sufficient to support a conclusion on cognitive effects in adults especially in the absence of additional adult human data"
Analogy	No suitable analogies identified

[33] US NTP (2020) conducted a systematic review (including risk of bias assessment) of evidence on F induced neurodevelopmental and cognitive effects in experimental studies (non-human animals)

Thyroid dysfunction

CADTH 2019 [2] identified a study of acceptable quality [376], which reported no association between drinking water fluoride and thyroid function. The RSI examination of eligible studies identified 5 studies of high or acceptable quality that reported a positive/possible association of thyroid dysfunction with water fluoride [41, 61, 63, 67] or urinary fluoride levels [20]. Cui and Colleagues [29] reported a non-significant association between thyroid dysfunction and urinary fluoride levels. A seventh study reported no association between thyroid dysfunction and water [53]. Based on the available literature to date, there is limited evidence to evaluate the association of thyroid hormone disruption and fluoride exposures relevant to current Canadian drinking water levels.

The scarce human evidence demonstrated a **moderate magnitude (strength) of association** between fluoride and dysregulation of thyroid hormones at fluoride exposures relevant to current Canadian drinking water levels. Fluoride appears to play a role in the induction of a range of adverse health outcomes, and is **not specific** to a single health effect. **Temporality** cannot be evaluated as the available evidence is entirely based on cross-sectional studies. **Exposure-response relationships** between fluoride and dysregulation of thyroid hormones were reported in four studies with variable levels of statistical significance. Whereas multiple mechanisms were discussed in the identified studies, **no specific mechanisms** could be confirmed for explaining the impact of fluoride on thyroid hormone dysregulation. Scarce **experimental evidence** reported inconsistent results for thyroid dysfunction. Results from the included studies are summarized in Section 6 of the Supplementary Material.

Table 19: Hill's consideration of causality for fluoride and thyroid dysfunction

Criterion	Summary of Evidence
Strength of association	• Six studies of high/acceptable quality reported results demonstrating a positive/possible association between thyroid dysfunction and water [41, 61, 63, 67] or urinary fluoride [20]. • One study [61] reported a higher percent thyroid hormone level derangement in fluorosis-endemic areas (67.5%) compared to non-endemic areas (54%), and a significant increase in mean TSH (endemic: 3.849 µlU/m; non-endemic: 2.588 µlU/m, p = 0.02). Non-significant derangements were reported for mean free T3,

and free T4 levels among participants from a fluorosis-endemic area compared to a fluorosis non-endemic area.

- Another study [67] reported on levels of free T3, free T4, and TSH by villages belonging to one of four groups based on the fluoride levels in drinking water. These levels range from <1 ppm (group 1), 1-1.9 ppm (group 2), 2-2.9 ppm (group 3) and >4 ppm (group 4). Derangement of TSH (range of normal values: 0.5–2.5 µIU/mL) has been shown in all groups. However, only group 1 (TSH: 0.4-2.99 µIU/mL) and group 2 (TSH: 0.29-3.76 µIU/mL) were relevant to Canadian water fluoride levels.
- The third study [41] reported a change in thyroid hormone levels per 1 mg/L increment of water fluoride (continuous) or compared to a reference quartile. Only hormones with significant results are listed below.

TT4 (µg/dL)

- Continuous: β= −0.083 (−0.181, 0.015), p-value= 0.097
- Quartiles: β= (95% CI), p-value
  - Quartile 1 (≤0.70): reference
  - Quartile 2 (0.70–1.00): β= −0.376(−0.686, −0.066), p-value= 0.017
  - Quartile 3 (1.00–1.90): β= −0.442(−0.687, −0.198), p-value= < 0.01
  - Quartile 4 (> 1.90): β= −0.271(−0.522, −0.020), p-value= 0.034
  - P-trend: 0.036

FT4 (ng/dL)

- Continuous: β= −0.010 (−0.021, 0.000), p-value= 0.054
- Quartiles: β= (95% CI), p-value
  - Quartile 1 (≤0.70): reference
  - Quartile 2 (0.70–1.00): β= −0.030 (−0.063, 0.003), p-value= 0.072
  - Quartile 3 (1.00–1.90): β= −0.027 (−0.053, −0.001), p-value= 0.042
  - Quartile 4 (> 1.90): β= −0.037 (−0.063, −0.010), p-value= 0.007
  - P-trend: 0.009

TSH (µIU/mL)

- Continuous: β= 0.127 (0.014, 0.241), p-value= 0.028
- Quartiles: β= (95% CI), p-value
  - Quartile 1 (≤0.70): reference
  - Quartile 2 (0.70–1.00): β= −0.154 (−0.517, 0.209), p-value= 0.404
  - Quartile 3 (1.00–1.90): β= 0.236 (−0.005, 0.522), p-value= 0.106
  - Quartile 4 (> 1.90): β= 0.306(0.012, 0.600), p-value= 0.041
  - P-trend: 0.019

	• A fourth study [63] reported that every 1mg/L increment of urinary fluoride (in iodine-deficient adults) was associated with a 0.35 mIU/L increase in TSH [95% CI: 0.06, 0.64, p-value: 0.01, one-tailed].
Consistency	• Four studies [41, 61, 63, 67] found a positive association between thyroid dysfunction and higher exposure to water fluoride. • Two additional studies reported a possible [63], or a non-significant association [29] between urinary fluoride levels and thyroid dysfunction. • Within studies showing a positive/possible association, the directionality of association did not differ by study design (all studies were cross-sectional), geographic location (two in India and one in each of Canada and China), studied population (three studies in children/adolescents and one included all ages), or sampling timeframe. • Two additional studies of acceptable quality identified by CADTH 2019 [376] and RSI [53] reported no association between fluoride and thyroid function.
Specificity	Fluoride appears to play a role in the induction of a range of adverse health outcomes, and dysregulating thyroid hormone levels can be caused by a number of risk factors including exposure to toxic factors other than fluoride.
Temporality	All of the four studies that suggest a positive/possibly positive association were of cross-sectional study designs, whereby an inference of causality cannot be inferred.
Biological gradient (exposure-response)	• Six studies reported results supporting a positive/possible association between thyroid dysfunction in association with either water fluoride levels as categories [61, 67], or on a continuous scale [41, 63], or using urinary fluoride levels [20, 29]. • One study reported on four groups of exposure, and identified a consistent increase in mean levels of free T3, free T4, and TSH per increase in concentrations of fluoride in water [67]. • Another study reported that each 1mg/L increment of water fluoride is associated with an increase in TSH, FT3 and TT3, and a decrease in FT4 and TT4. However, the effect estimate for TSH was only statistically significant before correcting for multiple testing [41]. • A third study [61] reported a higher thyroid hormone level derangement in fluorosis-endemic areas compared to non-endemic areas. This derangement was significant for TSH and non-significant for FT3 and FT4 levels. • The study by Malin et al. [63] reported that each 1mg/L increment of urinary fluoride (in iodine-deficient adults) is associated with a 0.35 mIU/L increase in TSH.

Biological plausibility	• As halogens of lower atomic weight are capable of displacing halogens of higher atomic weight, fluoride (fluorine) can displace iodine from the body. Such displacement thus interferes with iodine uptake by the thyroid gland, leading to its dysfunction and increased secretion of the pituitary TSH hormone to stimulate the thyroid to produce more thyroid hormones [63, 67]. • The effect of fluoride on thyroid function may depend on nutritional status and iodine deficiency. In the Canadian context, iodine deficiency is unlikely given that the intake exceeds 1mg/day due to the use of iodized salt [1]. However, the evidence also suggests that the effect of fluoride on the thyroid gland can occur independently of iodine [41]. • Interference of fluoride with Na/K-ATPase and iodothyronine deiodinase: two enzymes that are required for proper thyroid functioning [63]. • Fluoride may inhibit the prolactin hormone, which promotes thyroidal iodine uptake, lowers T4 secretion and inhibits stimulatory effects of exogenous TSH [63]. • Fluoride is a structural analog of thyroid stimulating hormone (TSH) and able to bind to TSH receptors leading to change in TSH levels and changes in secretion of thyroid hormones (by altering the regulation of hypothalamus-pituitary-thyroid axis) • The other plausible mechanism by which excess fluoride may affect thyroid structure and function is to induce endoplasmic reticulum stress pathways and subsequent apoptosis leading to cell death and changes in thyroid follicle morphology
Coherence	Coherence with previous evidence cannot be assessed based on the findings: • No specific mechanisms were directly linked to fluoride and thyroid dysfunction • Animal evidence was inconclusive
Experimental evidence	• There has been no experimental evidence generated from human studies. • Two experimental studies [187, 208] were identified, which evaluated fluoride effects on thyroid function (changes in thyroid hormone levels) at test concentrations required to achieve comparable blood fluoride levels in humans exposed to drinking water in Canada and are of lower risk of bias (more details are provided in the table containing results from selected animal studies) • Out of two studies (both were rat chronic studies), one study didn't find an association between increase in fluoride concentration and change in thyroid hormone levels; the other study reported inconsistent evidence (across two time points) on small but significant change in TSH and serum T4 levels.
Analogy	No suitable analogies identified

Kidney dysfunction

No studies from NHMRC were found, and only one study with low quality from CADTH 2019 was identified where no conclusion could be drawn due to methodological limitations and lack of statistical analysis. The RSI examination identified four new studies [36, 44, 49, 60] of high or acceptable quality that reported a possible association between water fluoride and kidney dysfunction. Two other studies reported either inconclusive [45] or no association [73]. Based on the available literature to date, there is limited evidence for an association of kidney dysfunction (mainly CKDu) and fluoride exposures relevant to current Canadian drinking water levels.

The available human evidence demonstrated a **moderate magnitude (strength) of association, with weak consistency** between fluoride and multiple kidney injury biomarkers at fluoride exposures relevant to current Canadian drinking water levels. The effects of fluoride appear to be **not specific** to one adverse health outcome. **Temporality** cannot be evaluated as the available evidence is entirely based on cross-sectional studies. **Exposure-response relationships** between fluoride exposure and kidney dysfunction were reported in four studies with variable levels of statistical significance. Although fluoride has been reported to impact the level of multiple kidney biomarkers, **no specific mechanisms** could confirm the impact of fluoride on the kidney functions. **Experimental evidence** showed some significant histological kidney alterations in association with fluoride exposure. Results from the included studies are summarized in Section 6 of the Supplementary Material.

Table 20: Hill's consideration of causality for fluoride and kidney dysfunction

Criterion	Summary of Evidence
Strength of association	• Four studies [36, 44, 49, 60] of high/acceptable quality reported results demonstrating a possible association between kidney dysfunction and fluoride exposure. • One study [60] reported a significantly positive association for each unit increase in water fluoride (mg/L) with ALB (β=1.20 µg/mL), Cys-C (β=0.03 mg/mL), OPN (β=0.10 mg/mL), TFF-3 (β=2.88 ng/mL). Change in CLU, KIM-1, and eGFR was non-significant. • Another study reported an inverse association per each 1mg/L increase in water fluoride with BUN concentration (β=−0.93 mg/dL, [−1.44, −0.42], p=0.007), whereas change in eGFR and ACR were non-significant.

	Additionally, a positive association was observed with SUA (non-significant) [49]. • A third study [44] reported a significant increase in serum fluoride of 1.43 (0.47–9.58) in CKDu patients compared to 1.07 (0.51–1.92) in controls. Similarly, a significant increase was reported for urinary fluoride as 1.53 ([0.45–6.92) in CKDu patients compared to 1.26 (0.36–3.80) in controls. • The fourth study [36] also reported that CKDu patients showed significantly higher serum fluoride concentrations than the healthy controls (p-value: <0.05).
Consistency	• Four studies [36, 44, 49, 60] suggested a possible association between kidney dysfunction and fluoride exposure. • Within studies showing a possible association, 3 studies were cross-sectional [36, 49, 60] and 1 was a case-control [44], geographic location included Mexico, Sri Lanka, and United States, and the studied population involved children/adolescents [49, 60] and adults [36, 44]. • The directionality of the association (positive vs. inverse association) varied depending on the indicator of kidney dysfunction assessed. • Two additional studies reported inconclusive [45] or no association [73] between water fluoride and kidney dysfunction.
Specificity	Fluoride appears to play a role in the induction of a range of adverse health outcomes, and kidney dysfunction can be caused by a number of risk factors including exposure to toxic factors other than fluoride.
Temporality	Of the 4 studies that suggested a possible association, 3 were of cross-sectional design [36, 49, 60], whereby an inference of causality should not be inferred
Biological gradient (exposure-response)	Of the two studies [49, 60] that reported results supporting a possible association between water fluoride levels and kidney dysfunction, both examined the exposure on a continuous scale. • One study reported a positive association for each unit increase in water fluoride (mg/L) with ALB (albumin), Cys-C (cystatin-C), OPN (osteopontin), TFF-3 (trefoil factor 3, significant), CLU (clusterin), KIM-1 (kidney injury molecule 1), and eGFR (estimated glomerular filtration rate, non-significant) [60]. • The other study reported an inverse association for each 1mg/L increase in water fluoride with BUN (blood urea nitrogen, significant), eGFR, and ACR (urinary albumin to creatinine ratio, non-significant). Additionally, a positive association was observed with SUA (serum uric acid, non-significant) [49].

Biological plausibility	• Histopathological changes in the kidney due to high fluoride exposure [44]. • Increased apoptosis and tubular epithelial damage, including necrosis, have also been observed among children with high fluoride exposures [49]. • Studies with adult rats have shown that chronic low-level fluoride exposure can lead to glomerular hypercellularity and mesangial cell proliferation, reduced kidney enzyme activity, interstitial nephritis, and renal tubule hypertrophy and hyperplasia [49]. • In experimental studies, exposure to fluoride has been associated with enzymatic inhibition, mitochondrial dysfunction (by the sirtuin 3 (SIRT3) pathway), oxidative stress generation and apoptosis induction (via activation of Bax expression and Bcl-2), especially in the S3 segment of the proximal tubules, which is considered the section most susceptible to fluoride toxicity [60] • In animal models, these kidney toxicants have been shown to target predominantly the proximal tubule causing reactive oxygen species generation followed by endoplasmic reticulum stress and mitochondrial damage, culminating in cellular necrosis/apoptosis [73]. • In humans, chronic exposure to heavy metals and fluoride has been associated with kidney disease. However, most of these studies have been conducted in adults that are occupationally exposed and therefore, the effects of environmental exposure in children largely remains uninvestigated [73]. • Primary route of elimination is fluoride through kidneys and large number of experimental studies provide evidence that tubular area of kidneys is the most vulnerable to excess amounts of fluoride [112, 377]. • One of the plausible mechanisms of fluoride induced kidney damage particularly in renal tubules is by increasing lipid peroxidation and decreasing activities of antioxidant enzymes (oxidative stress) at cellular level or by activating apoptotic pathways leading to cell death and renal injury.
Coherence	Coherence with previous evidence cannot be assessed based on the findings: • No specific mechanisms were directly linked to fluoride and kidney dysfunction • Animal evidence was inconclusive/inadequate
Experimental evidence	• There has been no experimental evidence generated from human studies. • Six experimental studies [112, 116, 149, 174, 232, 282] were identified that evaluated fluoride effects on kidney function at test concentrations required to achieve comparable blood fluoride levels in humans exposed to drinking water in Canada and are of lower risk of bias • These studies investigated the impact of fluoride exposure in rodents (mice or rats) at different exposure durations (chronic or sub-chronic) given a range of

	drinking water fluoride concentrations (ranged from 0.05 – 150 mg/L); specific outcomes measured include kidney dysfunction markers such as blood urea nitrogen (BUN) or creatinine (CRE) levels or histological analysis • Three out of six studies found some histopathological changes in kidneys (such as proximal tubule injury) but none reported any significant changes in kidney dysfunction markers such as BUN or CRE at or above test concentrations relevant to humans (except one study found slight but significant increase in CRE levels after long term exposure at 20 ppm fluoride concentrations)
Analogy	No suitable analogies identified

Sex Hormones

The RSI search for the human evidence stream identified 2 cross-sectional studies [28, 42] that reported a positive association and one abstract with insufficient data [15] that reported a possible association between fluoride exposure and sex hormone alterations. Based on the available literature on humans to date, there is limited evidence for an association of levels of sex hormones and fluoride exposures relevant to current Canadian drinking water levels.

The search also identified multiple animal studies that reported a possible association between fluoride exposure and some proxy measures for male infertility, such as sperm quality and testicular damage; however, older multi-generational guideline rodent studies on reproductive toxicity indicated no association with number of pups delivered or with a fertility index. Moreover, the overall assessment of evidence from all streams using the Bradford Hill considerations was not strongly supportive of a causal association with fluoride in drinking water. Results from this assessment of the included studies are summarized in Section 6 of the Supplementary Material.

Table 21: Hill's consideration of causality for fluoride and male sex hormones

Criterion	Summary of Evidence
Strength of association	• One human study [28] of high-quality reported inverse association of fluoride in plasma and water with sex steroid hormones of total testosterone, where a possible biological gradient could be identified among all study groups except for male children. Similar disruptions of estradiol and sex hormone binding globulin (SHBG) could not be observed in U.S. children and adolescents. • Another human study [42] reported chronic fluoride exposure from drinking water is associated with significant differences of serum SHBG concentration among local male farmers in the high-exposure gp. (30.07 ± 28.32), compared to the low-exposure gp. (35.90 ± 28.58). The effect of fluoride exposure on androgen binding protein (ABP) levels was non-significant, and varied depending on estrogen receptor α gene (ESRα) gene polymorphisms. • All 11 experimental animal studies [109, 114, 115, 182, 183, 262, 263, 277, 284, 288, 313] identified in this review reported statistically significant changes in one or more outcomes related to male reproductive system dysfunction (male infertility) such as change in sperm quality or testosterone levels or histology of testis • These changes are observed at test concentrations relevant to humans i.e., concentrations required to achieve comparable serum fluoride levels in humans exposed to Canadian CWF levels. • These studies [109, 114, 115, 182, 183, 262, 263, 277, 284, 288, 313] included multiples species (rats and mice), dose ranges that relevant to current CWF levels, exposure pattern (i.e., continuous exposure through drinking water) and sufficient group size (i.e., 10 or more animals per treatment group).
Consistency	• The two identified human studies [28, 42] reported significant inverse associations with serum levels of different sex hormones in male and female adults, children and adolescents.

	• Drinking water fluoride studies in mice and rats over a range of study durations (chronic and sub-chronic) and doses (5 to >20 ppm) that are relevant to current drinking water levels in Canada demonstrate a consistent association on the male reproductive outcomes such as sperm quality, histology of testis and testosterone levels (see experimental evidence table for male fertility in supplementary material 5).
Specificity	Fluoride appears to play a role in the induction of a range of adverse health outcomes, and dysregulating male reproductive system/functions can be caused by a number of risk factors including exposure to toxic factors other than fluoride.
Temporality	• The 2 human studies [28, 42] were cross-sectional design, and an inference of causality cannot be inferred. • In all experimental animal studies, the exposure (i.e., fluoride treatment) preceded the observation of outcomes (i.e., change in male reproductive outcomes)
Biological gradient (exposure-response)	• One human study [28] of high-quality reported inverse association of fluoride in plasma and water with sex steroid hormones of total testosterone, where a possible biological gradient could be identified among all study groups except for male children. Similar disruptions of estradiol and sex hormone binding globulin (SHBG) could not be observed in U.S. children and adolescents. • A second study by An and Colleagues [42] did not provide enough comparison groups to allow for a proper assessment of a biological gradient (high exposure vs. low exposure only). • All the included experimental animal studies reported significant changes in sperm quality with changes in fluoride levels in DW • Four studies found a linear dose response relationship between fluoride concentration in DW and one or more outcomes related to sperm quality (e.g., reduction in sperm motility, count or hyperactivity or increase in sperm abnormalities)

Biological plausibility	• Animal experiments reported chronic fluoride exposure to damage Sertoli cells in, whether such damage can further alter serum ABP concentrations remains uncertain [42]. • In addition to ABP regulation by SHBG, ABP regulation in vivo has been reported to be regulated by androgen and FSH [42]. • Mounting evidence has indicated that both gene-gene and gene-environment interactions play important roles in regulating hormone levels. Males who carried different ESRα genotypes with the same fluoride exposure group had different serum ABP concentrations, suggesting that genetic polymorphisms also significantly affect serum ABP levels [42]. • One of the plausible mechanisms proposed for fluoride induced male reproductive toxicity is reducing gene/protein expression of NGF and other genes in MAPK pathways; nerve growth factor (NGF) plays a critical role in male reproductive system such as in sperm motility, inducing proliferation and differentiation of stem Leydig cells and production of testosterone [307]. • Another proposed mechanism is by induction of reactive oxygen species mediated endoplasmic reticulum (ER) stress pathway followed by apoptosis of Sertoli cells which play an important role in spermatogenesis [378]. Several studies linked toxicant induced ER stress pathways to impairment of male reproductive function such as changes in spermatogenesis, sperm function/ hyperactivation etc. [378].
Coherence	Evidence is predominantly based on animal stream
Experimental evidence	• There has been no experimental evidence generated from human studies. • Evidence is predominantly based on animal experimental studies
Analogy	No suitable analogies identified

IX. Derivation of a point of departure for moderate dental fluorosis

Methodology

Summary of the US EPA (2010) dose-response analysis

In its 2010 report [379] entitled *Fluoride: Dose-Response Analysis For Non-cancer Effects*, the US EPA performed a dose-response analysis on severe dental fluorosis as a function of fluoride in drinking water. The dose-response analysis was conducted using the dataset from Dean (1942) [380]. Five models were fit to the data (log-Probit model, Probit model, log logistic model, logistic model, and dichotomous Hill model) with the dichotomous Hill model as the preferred model based on its AIC value (see Table 4-2 from EPA (2010) [379]). The resulting benchmark dose (BMD) for 1% severe dental fluorosis was 2.43 mg/L, while the corresponding lower bound of the benchmark dose (BMDL) was found to be 2.18 mg/L. It should also be noted that USEPA [379] attempted to fit these models to data on moderate dental fluorosis; however, none of the models provided an acceptable model fit to derive benchmark dose using the selected analytic strategy.

Identification of the key study

As described above in the systematic review methods and results sections, a bibliographic search was conducted for all epidemiologic studies on fluoride in drinking water and dental fluorosis. The review was an update of the CADTH (2019) systematic review. However, in considering whether there existed a preferable study to Dean (1942), candidate key studies were considered from all studies published after 1942. The selection of a preferable key study involved several considerations: the study would have a low risk of bias based on its design and analytic methodology, there would be data adequate for a dose-response analysis of moderate dental fluorosis, the design of the study would not be entirely ecologic (that is, aggregate statistics) to allow for proper inference, other sources of fluoride would ideally be considered, the study would be based on a child or adolescent study population, ideally drinking water fluoride levels would be reflected in a range that includes levels below 0.7 ppm, the study size would be sufficiently large for dose-response modelling, and the study setting would offer natural parallels to a Canadian context. The team did not further consider studies

published before 2008 (as the US EPA had already reviewed these in detail). Risk of bias of studies published after 2008 were based on assessments conducted by Iheozor-Ejiofor et al [395], and CADTH (2019), and within the current RSI review.

Epidemiologic studies published to date were of variable risk of bias levels, particularly based on concerns for exposure assessment and potential confounding, among other issues. A major consideration was that other sources of fluoride (such as dental cleaning products and rinses) are common in more recent eras. This poses considerable uncertainty in dose-response modelling of the effects of fluoride in drinking water, as was also noted by the US EPA (2008). At the time of its review, the US EPA also preferred Dean (1942) because it used a standardized protocol to assess dental fluorosis, had a relatively large study size, and had a requirement for continuous residency of the children participating in the study.

As no candidate key studies were identified, for the reasons described above, Dean (1942) was still preferred for statistical modeling purposes.

Dataset

Dean (1942) [380] was a cross-sectional study for 5824 children, in 22 cities across 10 states of the U.S. The children were 9-19 years old (grades 2-12). The design was comprised of a comparison of regions with varying water fluoride levels. Drinking water was the only route of exposure considered in the study. Dental fluorosis was measured using Dean's Index. Community fluoride concentrations were based on the Elvove (1933) method, derived from the mean of twelve-monthly samples.

The data used for the dose-response analysis is summarized in Table 33, which is generated by aggregating Table 33 from Dean (1942) [380] and sorting by the fluoride concentration levels in the drinking water supplies. The number of moderate (DFI=3) or severe (DFI=4) dental fluorosis cases for each community is calculated by combining proportions of moderate and severe dental fluorosis and multiplying by the number of study participants in each town.

The US EPA (2010) [379] argued that data from the town of Bauxite, AR, was an outlier, with a confounding factor of the excessive amounts of alumina in the environment due to the aluminum mine and smelter in the region. Therefore, data for Bauxite (26 children at an exposure level of 14.1mg/L) is excluded from the present analysis.

Table 22: Fluoride concentration in drinking water supplies and number of cases of moderate/ severe dental fluorosis (modified from Dean, 1942)

Town	State	Sample size	Age (Years)	F (mg/L)	Cases (Moderate + Severe)
Waukegan	IL	423	12-14	0	0
Michigan City	IN	236	12-14	0.1	0
Zanesville	OH	459	12-14	0.2	0
Lima	OH	454	12-14	0.3	0
Marion	OH	263	12-14	0.4	0
Elgin	IL	403	12-14	0.5	0
Pueblo	CO	614	12-14	0.6	0
Kewanee	IL	123	12-14	0.9	0
Aurora	IL	633	12-14	1.2	0
Joliet	IL	447	12-14	1.3	0
Elmhurst	IL	170	12-14	1.8	2
Galesburg	IL	273	12-14	1.9	3
Clovis	NM	138	9-11	2.2	16
Colorado Springs	CO	404	12-14	2.6	42
Plainview	TX	97	9-12	2.9	26
Amarillo	TX	289	9-13	3.9[1]	136
Conway	SC	59	9-14	4	21
Lubbock	TX	189	9-15	4.4	121
Post	TX	38	~8-11[3]	5.7[2]	34
Chetopa	KS	65	~7-17[4]	7.6[2]	45
Ankeny	IA	21	~6-17[5]	8.0[2]	19
Bauxite	AR	26	14-19	14.1[2]	24

[1] *"Subject to possible correction to 4.2mg/L during susceptible period of age group examined." (Dean, 1942)*

[2] *Those observations are based on a single determination. Others are calculated as the average of samples across 12-month timeline.*

[3] *Corresponds to children in grades 4 to 6*

[4] *Corresponds to children in grades 3 to 12*

[5] *Corresponds to children in grades 2 to 12*

Bayesian vs. frequentist dose-response modelling

As mentioned in the US EPA (2010) report [379], none of the models used in the dose-response modelling on the moderate and severe dental fluorosis provided acceptable fit to the data. For the current project, a Bayesian framework was employed for the benchmark dose estimation using the more recently available Benchmark BMD software (BBMD) developed by Shao and Shapiro (2018) (https://benchmarkdose.com)[381]. This approach would in principle provide improved model fit to a given dataset. Briefly, the Bayesian analysis calculates the posterior probability for parameter set $\boldsymbol{\theta}$ given data (i.e., $p(\boldsymbol{\theta}|\text{Data})$) as

$$p(\boldsymbol{\theta}|\text{Data}) \propto \pi(\boldsymbol{\theta})L(\text{Data}|\boldsymbol{\theta}) \,,$$

where $\pi(\boldsymbol{\theta})$ denotes the prior distribution for $\boldsymbol{\theta}$, $L(\text{Data}|\boldsymbol{\theta})$ represents the likelihood function. In particular, since the data is dichotomous (i.e., "success" if a study participant exhibits moderate or severe dental fluorosis, or "failure" otherwise), the likelihood function can be described using binomial distribution.

$$L(\text{Data}|\boldsymbol{\theta}) = \prod_{i=1}^{I} \binom{n_i}{y_i} \, p(d_i|\boldsymbol{\theta})^{y_i}[1 - p(d_i|\boldsymbol{\theta})]^{n_i - y_i} \,,$$

where n_i represents the number of participants in each exposure group, and y_i corresponds to number of participants developing moderate or severe dental fluorosis in ith exposure group, and $p(d_i|\boldsymbol{\theta})$ is the probability of developing moderate or severe dental fluorosis given exposure concentration at ith group. For all analyses, to derive a BMD and POD to protect against moderate dental fluorosis, a DFI cutoff of 3+ was used for modelling (i.e., a combination of moderate (DFI=3) and severe (DFI=4) categories, as described in Dean (1942).

There are a number of plausible dose-response model that can be used for the analysis. In particular, the BBMD software provides eight models available for the dose-response analysis to fit dichotomous data:

- Quantal linear model
- Probit model
- Logistic model
- Weibull model
- Multistage (2nd order) model

- Log Logistic model
- Log Probit model
- Dichotomous Hill model

All eight models provided by the BBMD software were used for the dose-response analysis; however, only log-logistic, log-Probit, and dichotomous Hill models provided convergence and adequate fit for the main analysis. These three models, as well as the prior distributions for corresponding model parameters are shown below.

Log-logistic model

$$f(d) = a + \frac{1-a}{1+e^{-(c+b\times\log(d))}},$$

where the prior distributions for the parameters are given by: $a \sim Uniform(0,1)$; $b \sim Uniform(1,15)$; and $c \sim Uniform(-5,15)$.

Log-Probit model

$$f(d) = a + (1-a) \times \Phi(c + b \times \log(d)),$$

where the prior distributions for the parameters are given by: $a \sim Uniform(0,1)$; $b \sim Uniform(1,15)$; and $c \sim Uniform(-5,15)$.

Dichotomous Hill model

$$f(d) = a \times g + \frac{a(1-g)}{a+e^{-(c+d\times\log(d))}},$$

where the prior distributions for the parameters are given by: $a \sim Uniform(0,1)$; $b \sim Uniform(1,15)$; $c \sim Uniform(-5,15)$; and $g \sim Uniform(0,1)$.

For the prior distributions for all parameters, the uniform distribution with the default lower and upper bounds are used. These default values were chosen based on the biological

considerations (Shao and Shapiro, 2018) [381]. See section 2 of the supplemental material from Shao and Shapiro (2018) [381] for the details of the remaining models.

Benchmark-dose modelling of added and extra risks

The objective of the dose-response analysis conducted in this section is to derive a point-of-departure (POD) using the BMD and the BMDL. The added-risk and extra-risk-based BMDs, for a prespecified benchmark response (BMR), can be defined as

$$\mathrm{BMD}_{\mathrm{ad}} = \{d: f(d) - f(0) = \mathrm{BMR}\},$$

and

$$\mathrm{BMD}_{\mathrm{ex}} = \left\{d: \frac{f(d) - f(0)}{1 - f(0)} = \mathrm{BMR}\right\},$$

where $f(d)$ and $f(0)$correspond to the risk of developing moderate or severe dental fluorosis at exposure levels d and 0, respectively.

Choice of benchmark response

Derivation of BMD and BMDL estimates were based on the extra-risk BMR of 1%, 5%, and 10%, given that extra-risk based BMR would always produce an estimate less than or equal to the BMD based on added risk.

Adequacy of model fit

As in classical (i.e., frequentist) statistics, the model fit to the data can be checked for adequacy in the Bayesian analysis. One such measure is called the posterior predictive p-value (PPP). The PPP indicates the discrepancy between the observed data and the plausibility of generating the observed data based on the posterior predictive distribution. If the model's PPP value is between 0.05 and 0.95, the model's fit it thought to be adequate. For a more detailed explanation of the PPP, see Gelman (2013) [382].

Model selection and model averaging

When there is more than one plausible model to describe the dose-response relationship, there would be more than one BMD estimate derived. There are two ways to determine a single "best" BMD estimate from a set of BMDs. One way is to choose the BMD from the most plausible model (model selection), and another way is to calculate the weighted average of the BMD estimates (model averaging). Either way, such determination requires the use of posterior model weights.

First, assume that equal prior model weights are assigned to each of candidate model, as there is no reason to believe one model is more plausible than others. Consequently, the model weight [383] for the j[th] model can be calculated as

$$\widehat{m}_j = \exp\left(\hat{l}_j - \frac{d_j}{2} log(n)\right),$$

where $\hat{l}_j$ denotes the estimated loglikelihood value, d_j represents the number of parameters used in j[th] model, with sample size represented by n. Therefore, using equal prior model weights, the posterior model weight for each of the model included in model selection/averaging can be calculated as

$$\widehat{pm}_j = \frac{\widehat{m}_j}{\sum_{t=1}^{T} \widehat{m}_t},$$

and therefore, the BMD estimate for model selection is given by

$$\widehat{\mathrm{BMD}}_{MS} = \underset{\widehat{pm}_j}{\operatorname{argmax}}\, \widehat{\mathrm{BMD}}_j .$$

Similarly, the BMD estimate for the model averaging is given by

$$\widehat{\mathrm{BMD}}_{MA} = \sum_{t=1}^{T} \widehat{pm}_t \times \widehat{\mathrm{BMD}}_j .$$

Results

NOAEL and LOAEL

Although the purpose of this section is to determine the POD based on BMD, it is worthwhile noting that the no observed adverse effect level (NOAEL) and lowest observed adverse effect

level (LOAEL) for the moderate or severe dental fluorosis are 1.3 mg/L and 1.8 mg/L, respectively. The LOAEL corresponds to a 1.2% positivity rate in the study participants from Elmhurst, IL (when all communities in Dean (1942) are sorted by fluoride concentration, the lowest concentration at which moderate dental fluorosis manifests is within the Elmhurst community, which had 1.8 ppm F concentration).

BMD estimates based on individual models

Dose-response analysis using log logistic model

Figure 4 shows the estimated dose-response curve using log logistic model. With the PPP value of 0.453, there is no reason to believe the inadequacy of the model fit. The estimated BMD and BMDL based on extra-risk based BMR of 1%, 5%, and 10% are presented in Table 23. When the BMR is set to 1%, the BMD and BMDL estimates are 1.45 mg/L and 1.35 mg/L, respectively. The estimated model weight for log logistic model is less than 0.001%, indicating that, of the three models used in this analysis, log logistic model has the lowest loglikelihood.

It should also be noted that, as the estimated background risk of developing moderate or severe dental fluorosis is 2.4×10^{-4} (Table 24), the added-risk based BMD and BMDL estimates would be the same as those shown in Table 23. The parameter estimates given in Table 24 uses the normalized exposure levels. In short, exposure levels in all data sets are normalized to the scale between 0 to 1 by dividing the exposure level d by the maximum exposure level ($8.0 mg/L$) from the study.

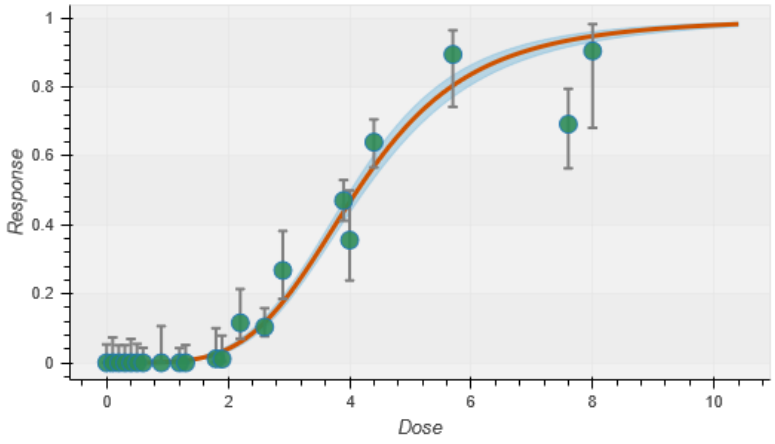

Figure 4: Estimated dose-response curve using log logistic model (orange line), with 90% confidence interval shown in light blue shade.

(Green dots represent the observed data while the vertical bars denote the 90% confidence interval about the observation.)

Table 23: Estimated BMD and BMDL values based on log logistic model. The extra-risk based BMR are used.

BMR	Log Logistic Model	
	BMD (mg/L)	BMDL (mg/L)
1%	1.45	1.35
5%	2.11	2.01
10%	2.50	2.41

Table 24: Estimated parameters for log logistic model.

Parameter	Log Logistic model		
	Mean	SE(Mean)	Standard Deviation
$\boldsymbol{a}$	2.4×10^{-4}	2.0×10^{-6}	2.4×10^{-4}
$\boldsymbol{b}$	4.38	2.1×10^{-3}	0.2
$\boldsymbol{c}$	2.9	1.9×10^{-3}	0.18
$\boldsymbol{lp}$	-737.6	0.01	1.27

Dose-response analysis using log Probit model

Figure 5 shows the estimated dose-response curve using log logistic model. With the PPP value of 0.396, there is no reason to believe the inadequacy of the model fit. Table 23 summarizes the estimated BMD and BMDL based on extra-risk based BMR of 1%, 5%, and 10%. When the BMR is set to 1%, the BMD and BMDL estimates are 1.58 mg/L and 1.49 mg/L, respectively. The estimated model weight for log logistic model is 0.046%, implying that log Probit model is a more plausible underlying model than the log logistic model for

describing dose-response relationship between fluoride concentration in drinking water and development of moderate or severe dental fluorosis.

Similar to the log logistic model, as the estimated background risk of developing moderate or severe dental fluorosis is 2.4×10^{-4} (Table 25), the added-risk based BMD and BMDL estimates would be the same as those shown in

Table *26*. Note also that the parameter estimates given in Table 25 uses the normalized exposure levels.

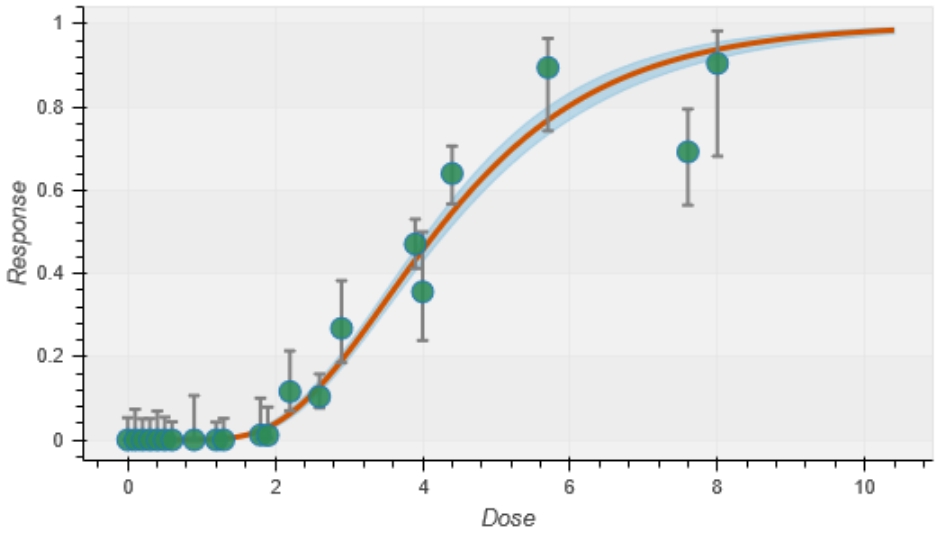

Figure 5: Estimated dose-response curve using log Probit model (orange line), with 90% confidence interval shown in light blue shade.

(Green dots represent the observed data while the vertical bars denote the 90% confidence interval about the observation.)

Table 25: Estimated BMD and BMDL values based on log Probit model. The extra-risk based BMR are used.

BMR	Log Probit Model	
	BMD (mg/L)	BMDL (mg/L)
1%	1.58	1.49
5%	2.10	2.02
10%	2.45	2.37

Table 26: Estimated parameters for log Probit model.

Parameter	Log Probit Model		
	Mean	SE(Mean)	Standard Deviation
$\boldsymbol{a}$	2.4×10^{-4}	2.0×10^{-6}	2.4×10^{-4}
$\boldsymbol{b}$	2.38	1.1×10^{-3}	0.10
$\boldsymbol{c}$	1.5	1.0×10^{-3}	0.10
$\boldsymbol{lp}$	-732.5	0.01	1.29

Dose-response analysis using dichotomous Hill model

Figure 6 shows the estimated dose-response curve using log logistic model. With the PPP value of 0.452, there is no reason to believe the inadequacy of the model fit. As shown in Figure 4, the dichotomous Hill model plateaus at 78% response rate, meaning that Hill model assumes that even as fluoride concentration increase infinitely, only about 78% of population would develop either moderate or severe dental fluorosis. The estimated BMD and BMDL based on extra-risk based BMR of 1%, 5%, and 10% are presented in Table 27. When the BMR is set to 1%, the BMD and BMDL estimates are 1.66 mg/L and 1.56 mg/L, respectively, which are slightly larger than those based on the log logistic and the log Probit model. When considering the model averaging results, the estimates were heavily weighted toward those of the Hill model (estimated model weight of 99.95%).

The background rate for the Hill model is given by $(a \times g)$. From Table 28, we obtain that $a \times g = 2.5 \times 10^{-4}$, and therefore the added-risk based BMD and BMDL estimates for the Hill model are identical to those presented in Table 27. Note also that the parameter estimates given in Table 28 uses the normalized exposure levels.

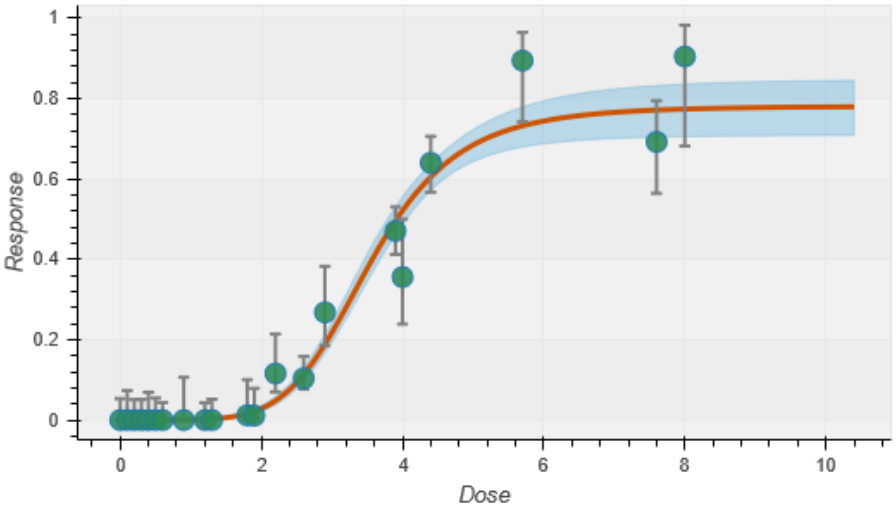

Figure 6: Estimated dose-response curve using dichotomous Hill model (orange line), with 90% confidence interval shown in light blue shade.

(Green dots represent the observed data while the vertical bars denote the 90% confidence interval about the observation.)

Table 27: Estimated BMD and BMDL values based on dichotomous Hill model. The extra-risk based BMR are used.

BMR	Dichotomous Hill Model	
	BMD (mg/L)	BMDL (mg/L)
1%	1.66	1.56
5%	2.22	2.13
10%	2.53	2.46

Table 28: Estimated parameters for dichotomous Hill model.

	Dichotomous Hill Model		
Parameter	**Mean**	**SE(Mean)**	**Standard Deviation**
a	0.78	4.3×10^{-4}	0.04
b	5.77	4.3×10^{-3}	0.38
c	4.71	5.3×10^{-3}	0.46
g	3.2×10^{-4}	2.5×10^{-6}	3.2×10^{-4}
lp	-732.5	0.01	1.29

BMD estimates using model averaging

In the previous section, based on an estimated model weights and fit statistics, the Hill model may be the single most plausible model to describe the dose-response relationship based on the data from Dean (1942) [380]. Since there always is an uncertainty about which model is the "true" model, it may be beneficial to adjust the BMD and BMDL estimates by taking the weighted average of the BMD estimates from different models. As shown in Table *29*, BMD and BMDL estimates based on model averaging is identical to those of Hill model. This is because the model weight for the Hill model is 99.95%. Therefore, whether model averaging or model selection is used, the BMD and BMDL estimates corresponding to 1% BMR would be 1.66 mg/L and 1.56 mg/L, respectively.

Table 29: Estimated BMD and BMDL values by model averaging. The extra-risk based BMR are used.

BMR	**Model Averaging**	
	BMD (mg/L)	**BMDL (mg/L)**
1%	1.66	1.56
5%	2.22	2.13
10%	2.53	2.46

Sensitivity analysis

Effect of correction in exposure

As stated in the footnote on Table 22, Dean (1942) [380] indicated the exposure level in Amarillo, TX, may be subject to a possible correction to 4.2 mg/L (instead of 3.9 mg/L) “during susceptible period of age group examined”. Although the age group of study participants from Amarillo does not seem to differ greatly from children from other communities, a sensitivity analysis was based on the dose-response analysis with a modified fluoride concentration for the Amarillo subjects.

The BMD and BMDL estimates under log logistic, log Probit, and Hill models, as well as from model averaging are provided in Table 30. As expected, these estimates are very similar to those from the original analysis (Table 23, Table 25, Table 27, and Table 29). In particular, BMD and BMDL estimates for 1% extra risk based on model averaging are 1.63 mg/L and 1.52 mg/L, respectively.

Table 30: BMD and BMDL estimates under various models. The exposure level for Amarillo, TX, has been modified to 4.2 mg/L for possible susceptible period of age group examined, as noted by Dean (1942).

BMR	Log logistic Model		Log Probit Model		Dichotomous Hill Model		Model Average	
	BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL
1%	1.63	1.52	1.44	1.34	1.58	1.49	1.63	1.52
5%	2.21	2.12	2.13	2.04	2.12	2.03	2.22	2.12
10%	2.55	2.46	2.54	2.45	2.48	2.39	2.55	2.46

Effect of higher concentration groups

The Hill model provided the best fit in the main analysis. This may be due to the extra parameter in the Hill model that allows for the model to plateau before reaching a 100% incidence rate. To investigate the effect of the plateauing effect on the estimation of BMD, two additional analyses were considered, where the highest concentration group (i.e., Ankeny, IA at 8.0 mg/L) and two highest concentration groups (i.e., Ankeny, IA, and Chetopa, KS at 7.6

mg/L) are removed. The BMD estimates based on these additional analyses are presented in Table 31 and Table 32. When only the highest concentration group is excluded, the resulting model average BMD and BMDL estimates are nearly identical to those found in the original analysis (1.68 mg/L vs. 1.66 mg/L for BMD, and 1.57 mg/L vs. 1.56 mg/L for BMDL for 1% BMR). When the two highest exposure groups are excluded, the model average BMD estimates are slightly increased at 1.72mg/L for 1% BMR. Although the BMD estimates are mostly unaffected by the plateauing effect, when both Ankeny and Chetopa subjects were excluded from the analysis, the log Probit model became the dominant dose-response model (with the model weight of 91.2%). This indicates that the model fit may be heavily influenced by the plateauing effect (i.e., whether incidence rate will become 100% as exposure increase or not), and therefore it would be preferable to use the BMD and BMDL estimates from model averaging, rather than using only the most plausible model.

Table 31: BMD and BMDL estimates under various models. The highest exposure group (Ankeny, IA) is excluded from this dose-response analysis.

BMR	Log logistic Model		Log Probit Model		Dichotomous Hill Model		Model Average	
	BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL
1%	1.45	1.35	1.58	1.49	1.68	1.57	1.68	1.57
5%	2.11	2.02	2.1	2.02	2.22	2.13	2.22	2.13
10%	2.50	2.42	2.45	2.37	2.53	2.45	2.53	2.45

Table 32: BMD and BMDL estimates under various models. The two highest exposure groups (Ankeny, IA, and Chetopa, KS) are excluded from this dose-response analysis.

BMR	Log logistic Model		Log Probit Model		Dichotomous Hill Model		Model Average	
	BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL
1%	1.60	1.50	1.72	1.63	1.63	1.52	1.72	1.59
5%	2.21	2.12	2.2	2.11	2.22	2.13	2.2	2.11
10%	2.57	2.48	2.5	2.42	2.55	2.47	2.51	2.43

Conclusion

The dose-response analysis using data from Dean (1942) [380] was performed to investigate the relationship between the fluoride concentration in drinking water and the development of moderate or severe dental fluorosis. The model average benchmark dose for 1% extra-risk and corresponding BMDL were determined to be 1.66 mg/L, and 1.56 mg/L, respectively. These values are slightly less than the LOAEL (1.8 mg/L), which had the positivity rate of 1.2% in the study population. Although three models were used to derive the model average BMD, the Hill model was the dominant model with a 99.95% model weight.

There were some uncertainties in some of the collected data. For example, Dean (1942) suggested that a potential correction for the exposure level for the town of Amarillo may be needed. A sensitivity analysis with modified data suggested that the 1% BMD and BMDL are slightly decreased at 1.62mg/L and 1.53mg/L, respectively. Another sensitivity analysis was performed to investigate the impact of plateauing effect on the BMD estimates. When two of the highest exposure groups were excluded, the BMD and BDML estimates increased slightly, to 1.72 mg/L and 1.59 mg/L. Although the BMD estimates were stable, removal of highest concentration groups led to change in the dominant model (log Probit model had model weight of 91.2%), indicating that BMD estimation should be based on model averaging rather than model selection.

X. Derivation of points of departure for other candidate endpoints

The following Table 33 presents a selection of international HBV and MAC for fluoride in drinking water. These guidelines were based on considerations of the beneficial prevention of dental caries and the protection against dental fluorosis (as the most sensitive endpoint). The selection of candidate endpoints in the current RSI report and the derivation of points of departure in a subsequent section were based on a review of all other health endpoints, excluding dental fluorosis.

Table 33: HBV and MAC values reported by different authoritative agencies

Country / Organization	Reference Values	Source
WHO	"The guideline value for fluoride in drinking-water is 1.5 mg/L, based on increasing risk of dental fluorosis at higher concentrations and that progressively higher levels lead to increasing risks of skeletal fluorosis. This value is higher than that recommended for artificial fluoridation of water supplies for prevention of dental caries, which is usually 0.5–1.0 mg/L." (p. 3)	**WHO 2019** [384]
Canada	"… the optimal concentration of fluoride in drinking water for dental health has been determined to be 0.7 mg/L for communities who wish to fluoridate." (p. 2) "The maximum acceptable concentration (MAC) for fluoride in drinking water is 1.5 mg/L … Mild and very mild dental fluorosis are not considered to be adverse effects, whereas moderate dental fluorosis is found to be an adverse effect, based on its potential cosmetic concern, and is used as the endpoint of concern in the risk assessment used to establish the Maximum Acceptable Concentration." (p. 1) "Health Canada has calculated a health-based value of 0.9 mg/L for fluoride in drinking water, which is deemed protective against any potential adverse health effect from fluoride." (p. 64)	**Health Canada 2010** [1].
USA	"For community water systems that add fluoride to their water, PHS recommends a fluoride concentration of 0.7 mg/L (parts per million [ppm]) to maintain caries prevention benefits and reduce the risk of dental fluorosis." (p. 319)	**U.S. Department of Health and Human Services 2015** [385]
	"The current enforceable drinking water standard for fluoride is 4.0 mg/L. This is the maximum amount that is allowed in water from public water systems. It is set to meet the current public health goal for protection against increased risk of crippling skeletal fluorosis, a condition characterized by pain and tenderness of the major joints." (p. 2) "EPA also has a non-enforceable secondary standard for fluoride of 2.0 mg/L, which is recommended to protect children against the tooth discoloration and/or pitting that can be caused by excess fluoride exposures during the formative period prior to eruption of the teeth. Although water systems are not required to comply with secondary standards, for fluoride, EPA does require that systems notify customers if the average water levels exceed the secondary standard." (p. 2 – 3)	**US Environmental Protection Agency 2011** [386]

Country / Organization	Reference Values	Source
Australia	"Based on health considerations, the concentration of fluoride in drinking water should not exceed 1.5 mg/L." (p. 668) "The guideline value of 1.5 mg/L has been set to protect children from the risk of dental fluorosis." (p. 669)	**National Health and Medical Research Council, Australia 2021** [10]
	"NHMRC supports Australian states and territories fluoridating their drinking water supplies within the range of 0.6 to 1.1 mg/L. This range is aimed at reducing tooth decay, while avoiding any occurrence of dental fluorosis of aesthetic concern. In each Australian state or territory, the government health authority determines the appropriate operational levels within the range of 0.6 to 1.1 mg/L." (p. 1)	**National Health and Medical Research Council, Australia 2017** [10]
Ireland	"Community water fluoridation at a level of 1 ppm began in Ireland in 1964 as a measure to prevent dental caries. A major review of Ireland's water fluoridation policy in 2002 showed an increasing occurrence of dental fluorosis. As a result, in 2007, the fluoride level in drinking water in Ireland was lowered to a range of 0.6 to 0.8 ppm, with a target of 0.7 ppm. This remains the target and range applied in Ireland today." (p. 6)	**Sutton et al. 2015** [387]
	"In Europe, the maximum level of fluoride currently allowed in drinking water is 1.5 parts per million (ppm) (2). However, in Ireland, the 1960 Health (Fluoridation of Water Supplies) Act restricts the maximum level of fluoride to only 1ppm and this supersedes the European maximum limit." (p. 18)	**Food Safety Authority of Ireland 2006** [388]
New Zealand	"The NZMoH [New Zealand Ministry of Health] recommends that, for oral health reasons, the level of fluoride in drinking water in New Zealand should be between 0.7 and 1.0 mg/L. Based on WHO advice, the maximum acceptable value for fluoride in drinking water is 1.5 mg/L to prevent any known adverse health effects (dental or skeletal fluorosis)." (p. 22)	**Royal Society of New Zealand 2014** [389]

Cognition, IQ

The body of evidence considered in the current RSI review suggests a positive association of reduced IQ scores for children and fluoride exposures relevant to current Canadian drinking

water levels. Using the 2022 NTP dose-response mean-effects meta-analysis [421] of 29 human epidemiologic studies with aggregate-level exposure measurement, the linear dose-response model resulted in a change (a reduction) in IQ of -0.15 (standardized mean difference (SMD), 95% CL: −0.20, −0.11) between the drinking water fluoride exposed group and the reference group within each study. Restricting the dose-response meta-analysis to those studies that included an exposed (non-reference) group with mean fluoride concentrations below 1.5 mg/L (7 studies contributed 7 observations to the dose-response estimate) resulted in an estimate of the change in IQ of 0.05 (standardized mean difference, 95% CL: -0.36, 0.45) between the exposed group and the reference group using a linear model. This latter result could be used as evidence to reconsider the HBV for fluoride in drinking water in Canada; however, the estimate was based on largely cross-sectional studies with high risk of bias, including lack of adjustment for effects of other contaminants, such as arsenic and lead. The 2022 NTP draft also includes a mean effects meta-analysis [421], with studies that reported sex-stratified results (14 studies of boys, 13 studies of girls) with these subgroup analyses resulting in IQ changes of (SMD) −0.62 (95% CI: −0.81, −0.42) in boys and −0.53 (95% CI: −0.72, −0.34) in girls. The 2022 NTP draft includes a regression slopes meta-analysis of epidemiologic studies with individual-level fluoride exposure measures (including several cohort studies) with an estimated -4.77 IQ point change for a 1-mg/L increase in water fluoride (β = −4.77; 95% CI: −9.09, −0.45) and -1.81 (-2.80, -0.81) for urinary fluoride.

Benchmark dose (BMD) modelling results have been recently published, based on high-quality birth cohort data. Grandjean and Colleagues [390] conducted a BMD analysis using the pooled MIREC and ELEMENT cohorts, with assessment of maternal urinary fluoride levels. The MIREC Canadian cohort (Maternal–Infant Research on Environmental Chemicals) was the basis of previous assessments of prenatal fluoride exposure and childhood IQ (Till et al. (2020) [40] and Green et al. (2019) [326]) and the ELEMENT longitudinal birth cohort (Early Life Exposures in Mexico to Environmental Toxicants) was used to assess maternal and fetal fluoride exposure and childhood IQ in a Mexican population (Bashash et al. (2017)) [71]. The combined cohort represents high quality evidence partly based on a Canadian population, conducted within a context relevant to Canadian drinking water fluoride exposure levels. Both studies included prospective data collection, with prenatal exposure assessment (maternal urine collection over successive trimesters) and follow-up during the early life of the infants

and children. In risk of bias assessments conducted by NTP, the earlier publications by Green et al., Till et al., and Bashash et al. were assessed at low risk of bias due to unlikely concerns from measurement error on cognition and urine F concentration, selection of study samples, and confounding adjustment from known factors [These assessments are relevant to the publication by Grandjean et al., which used the same data sources]. Exposure coverage in the cohort reflects (urinary) fluoride levels below the current health-based value of 0.9 mg/L for fluoride in drinking water (with Grandjean reporting the mean urinary fluoride concentration [creatinine-adjusted] among pregnant women was 0.89 mg/L in Mexico City and 0.84 mg/L in Canada). Regression modelling by Grandjean et al. (2022) includes adjustment for critical confounders, including other chemical neurotoxicants in drinking water and socioeconomic impacts that would affect cognitive and mental health development. Adjustment included arsenic and lead exposures, as well as non-chemical determinants (gestational age, age at measurement, maternal education, race/ethnicity, child sex, parity, second-hand smoke, city, and quality of home environment [emotional support; cognitive stimulation]). Stratified and models with interaction terms were include to the relationship between sex and urinary-fluoride exposure. In the BMD modelling, various regression models (linear, quadratic, segmented) were used to estimate the benchmark concentration for a benchmark response of a 1-point reduction in IQ. Model fits were similar but resulted in widely varying estimated benchmark concentrations, with some models for girls not converging. At present, mode and mechanism of action information is insufficient to establish a preference for the linear or nonlinear models considered by Grandjean and Colleagues [390]. Based on a benchmark response (BMR) of 1 IQ point and using the linear model results, the benchmark concentration (BMC) for maternal urinary fluoride (MUF) was 0.312 mg MUF/L, and the one-sided lower limit of the BMC (the BMCL) was 0.192 mg MUF/L) when pooling General Cognitive Index (GCI) scores for the youngest children of both sexes in both cohorts. In sex-stratified results, estimated benchmark concentrations were lower in boys than in girls. Results varied in the two cohorts and by age at measurement – but when pooled for the youngest aged children, the derived BMCL from the linear model for boys was 0.125 MUF/L and for girls was 0.315 MUF/L.

To derive a potential *BMCL for fluoride in drinking water* based on the maternal urinary results from the pooled analysis of the MIREC and ELEMENT cohorts conducted by Grandjean and Colleagues [390] requires a conversion based on the following assumptions:

- Because of the uncertainty as to the shape of the dose-response curve at low concentrations of drinking water, the more stringent linear model, rather than the squared or break-point models considered by Grandjean and Colleagues [390], was selected in order that the BMCL not be overestimated.
- For a BMR of 1 IQ point, the $BMCL_{MUF}$ was 0.192 mg MUF/L, based on the linear model results from Grandjean et al. for the pooled cohorts at younger ages
- Daily drinking water intake is 1.53 L/day (Health Canada default value).
- 24-hour fraction of fluoride excretion in adults is 0.75 (from Villa et al., 2004). This fractional urinary fluoride excretion (FUFE) is the ratio of fluoride excreted and fluoride ingested, FUFE = F_{excr}/F_{ing},
- F_{excr} is a product of urinary volume (over 24h) and the urinary fluoride concentration. A normal range of 24-hour urine volume is 800 to 1,200 mL,[34] with 2 L of fluid intake per day. Given the mid-value of 1.4 L of urine volume per 2 L of fluid intake, and assuming linearity, the 24-hour urine volume for Canadians (with 1.53 L intake) would be 1.07 L.
- The susceptible population was young school-aged children, with the critical window of exposure being during prenatal periods and thus based on maternal intake.

Under these assumptions, the amount of fluoride ingested per day corresponding to the $BMCL_{MUF}$ is:

F_{ing} = [$BMCL_{MUF}$ x 24-hour urine volume] / FUFE

= [0.192 mg/L x 1.07 L/d] / 0.75

= 0.274 mg/day

And the BMCL for fluoride in drinking water is then calculated as:

$BMCL_{DW}$ = F_{ing} / water intake

= (0.274 mg/day) / (1.53 L/day)

= 0.179 mg F/L[35]

[34] Urine 24-hour volume Information | Mount Sinai - New York

[35] The derivation of the drinking water BMCL, based on Grandjean and Colleagues 390. Grandjean, P., et al., *A Benchmark Dose Analysis for Maternal Pregnancy Urine-Fluoride and IQ in Children.* Risk Anal, 2022. **42**(3): p. 439-449. results, assumes that all fluoride ingested is via drinking water. Villa et al. (2004) reported for their participants, about 75% of Fing could be attributed to drinking fluids (but food, drinks, and toothpaste were all

Grandjean and Colleagues [390] fit different linear and non-linear models, which resulted in lower bounds of benchmark concentrations which differed by more than 9-fold (when converted to drinking water concentration, with the method described above, the variously derived BMCLs ranged from 0.077 mg F/L to 0.753 mg F/L drinking water).

The point of departure of 0.179 mg F/L from the combined high-quality cohorts stands in contrast to the 2022 draft NTP report conclusions that evidence for fluoride effects on cognitive function in children is less consistent below 1.5 mg F/L. In choosing between the BMCL of 0.179 mg F/L based on the more stringent model fit to the MIREC and ELEMENT cohorts by Grandjean and Colleagues [390], and a weight of evidence conclusion that evidence for neurological effects of fluoride in children below concentrations of 1.5 mg F/L was less consistent, consideration was also given to the quality of evidence. While the BMCL derived from the cohort data suggests a much lower POD than 1.5 mg/L, the overall body of evidence suggests significant uncertainty in any low exposure-range derivation with current evidence. At this point in time, 1.5 mg/L may be considered as a provisional point of departure for establishing an HBV for fluoride in Canadian drinking water based on protection against neurocognitive effects in children. This POD should be reviewed as additional data accumulates on the biological mechanisms by which fluoride impacts cognitive function, providing additional insights into the shape of the exposure-response curve at lower concentrations.

Thyroid dysfunction

The RSI review and weighing of evidence under Bradford Hill considerations provided reasonable credibility from generally low to acceptable risk of bias – albeit cross-sectional – human epidemiologic studies to suggest a possible association of fluoride exposure in Canadian drinking water contexts and effects on thyroid dysfunction. No study was considered adequate to derive a point of departure. In considering the animal stream of evidence, only 2 low risk of bias studies with dose-response information were considered relevant [187, 208]. Out of these 2 rat chronic studies, one study did not find a change in thyroid

controlled in the study, and the study was conducted in Chile, which may be less applicable to a Canadian population).

hormone levels (T3, T4, or TSH) at the highest test concentrations (20 ppm), and the other study did not consistently demonstrate significant change across time points. Overall, these studies were considered insufficient for derivation of a point of departure for thyroid-related effects in humans.

No point of departure was derived.

Kidney dysfunction

Epidemiologic human studies were broadly consistent on supporting a possible association of fluoride exposure in Canadian contexts and effects on kidney dysfunction, with weighing of evidence under Bradford-Hill considerations supportive of the association being possibly causal. However, all human studies were cross-sectional in design and were not considered adequate for a derivation of a point of departure. Although a few low risk-of-bias animal studies demonstrated selective histopathological changes in the kidney (such as proximal tubule injury, but without any significant changes in kidney dysfunction markers such as BUN or CRE), the studies were of insufficient duration (mostly sub-chronic), or small group size (less than 10 per sex per group), or considered inadequate to derive a point of departure for kidney dysfunction in humans. Overall, these studies were considered insufficient for derivation of a point of departure for kidney dysfunction in humans.

No point of departure was derived.

Sex hormone dysfunction

In the human stream of evidence, 2 low risk of bias cross-sectional studies were identified. While considered low risk of bias in the OHAT scoring, cross-sectional studies were not considered adequate for consideration in deriving a point of departure. Recent animal studies identified in the RSI review suggested an association with proxy measures of male infertility such as sperm quality and testicular damage; however, older multi-generational guideline rodent studies on reproductive toxicity indicated no association with number of pups delivered or with a fertility index. Weighing of evidence under Bradford Hill considerations was not strongly supportive of a causal association with fluoride in drinking water. Overall, these studies were considered insufficient for derivation of a point of departure for sex hormone derangement effects in humans.

No point of departure was derived.

XI. Considerations for selection of most appropriate endpoint and a point of departure

The current RSI review encompassed a thorough, multi-pronged examination of the effects of exposure to fluoride in drinking water on adverse health outcomes. The ultimate goal of this review was to provide evidence with which Health Canada could consider updating the current MAC for fluoride in drinking water. To identify the most sensitive and most appropriate endpoint of concern, the review included considerations for deriving an appropriate POD, for which there is good quality data demonstrating a well-defined exposure-response relationship. This examination involved the identification and assessment of quality of all new evidence from human, animal, and in vitro studies that had been published after the release of two CADTH reports in 2019 [2, 3], the NTP report in 2016 [4], and the Health Canada report in 2010 [1]. In synthesizing this evidence, the quality and potential risk of bias of individual studies was taken into consideration. The combined evidence generated from these different streams was then examined via the Bradford Hill considerations [8] for identification of credible causal adverse effects due to fluoride exposure.

In reconsidering an update of the MAC for fluoride in drinking water for Canadians, the HBV based on dental fluorosis was revisited using the newly published scientific evidence and results from new modelling of the Dean (1942) data on dental fluorosis. Moderate dental fluorosis was selected as the level of fluorosis of concern by Health Canada. Furthermore, based on weight of evidence, four new endpoints were considered as credible candidates for most sensitive endpoint. While effects on sex hormones, thyroid dysfunction, and kidney dysfunction are potential adverse effects of fluoride exposure through drinking water, with enough supporting evidence to warrant concern, no points of departure were derived because of inadequate data sources to conduct dose-response modelling.

The overall evidence identified to date strongly suggests that fluoride can affect cognitive outcomes in children (specifically, reduction in IQ scores), at levels close to those currently seen in Canadian drinking water.

Hence, the selection of the most sensitive endpoint requires a comparison of the point of departure for moderate dental fluorosis and the point of departure for IQ effects. For both endpoints, the vulnerable population is young, school-aged children, though critical periods of exposure likely differ (prenatal vs. early life).

First, the POD for moderate/ severe dental fluorosis was derived in the current report as:

- 1.56 mg/L for a BMR of 1%, 2.13 mg/L for a BMR of 5%, and 2.46 mg/L for a BMR of 10%

  While data on moderate dental fluorosis could not be fit at the time of the US EPA report (2010) using a classical approach, the current values were estimated using Bayesian model averaging to derive the extra risk-based BMDL across three alternative dose-response models using Bayesian BMD software, which only recently became available. The BMDL of 1.56 mg/L lies between the NOAEL of 1.3 mg/L and the LOAEL of 1.8 mg/L for moderate dental fluorosis in the Dean study.

Second, based on the weight of evidence to date, fluoride can credibly be considered to have an effect on childhood IQ. There is, however, significant uncertainty as to the POD. The draft NTP 2020 and 2022 reports concluded that evidence for effects below 1.5 mg/L was less consistent than that above 1.5 mg/L. Based on high quality MIREC and ELEMENT cohorts with individual-level measures, Grandjean and Colleagues (2022) [390] estimated the benchmark concentration for maternal urinary fluoride associated with a 1-point reduction in IQ. Different linear and non-linear models fit by Grandjean and Colleagues [390] resulted in benchmark concentrations differing by more than 9-fold. Although the NRC concluded that fluoride is an endocrine disruptor, leading to thyroid dysfunction at very low exposure levels among individuals with iodine deficiency [393], the mechanism of action of fluoride for neurotoxicity is still poorly understood. Uncertainties in the shape of the dose-response curve at low levels of exposure to fluoride based on epidemiologic data will likely require extrapolation with a better understanding of the mechanism of action. For these reasons, a POD for IQ effects was provisionally selected as:

- 1.5 mg F/L, but acknowledging that credible support exists that the POD may be lower than this concentration (based on cohort data) and that the majority of studies to date

are cross-sectional studies with significant concerns regarding exposure assessment and potential confounding.

Consideration should also be given to the severity of the two end points - moderate dental fluorosis and IQ reduction – for which PODs have been derived. The choice of a BMR of 1 IQ point (corresponding to a 1% reduction from a mean IQ of 100) has been adopted as an appropriate benchmark on this endpoint by several regulatory bodies, including the US EPA and EFSA. This level of cognitive effect (in the context of assessing the exposure to lead) has been shown to be associated with reduced educational attainment, employment status, productivity, and earned wages, reflecting substantial public health concerns [391].

Although outside of the scope of the present report, the establishment of an HBV for fluoride in drinking water will require consideration of possible adjustment factors to be applied to either of the two PODs derived above. Since the POD of 1.56 mg F/L for moderate dental fluorosis is based on high-quality population-based data in the target population (children), with only minor concern about other sources of ingested fluoride, a minimal adjustment factor could be entertained in deriving an HBV based on fluorosis. However, with currently available evidence suggesting that fluoride leads to reductions in children's IQ – arguably of more concern than moderate dental fluorosis – the possibility of cognitive effects in children should be taken into account in setting an HBV for fluoride in drinking water. As the POD for IQ reduction is not yet well defined, the POD of 1.56 mg F/L for moderate dental fluorosis may be preferred as a starting point for deriving the HBV. To allow for protection against potential cognitive effects in children at levels below the POD of 1.56 mg F/L, an additional overall database uncertainty factor could be applied to this POD.

As additional information on the association between fluoride in drinking water and reduction in children's IQ becomes available, the choice of the most appropriate endpoint on which to base the POD to serve as the starting point to deriving an HBV for fluoride can be revisited.

A better understanding of low concentration fluoride risks

One of the challenges in evaluating the potential human health risks of fluoride is estimating risks at low levels of exposure. Following a comprehensive review of the scientific literature on health effects of fluoride in drinking water, neurological effects in children and dental

fluorosis emerged as the two key endpoints with exposure-response data suitable for determination of a point of departure for risk assessment purposes. Dental fluorosis demonstrates a very steep exposure-response curve, with risk increasing markedly between 1 ppm F in drinking water, at which there is a low risk of mild dental fluorosis, and 4 ppm, where there is a high risk of severe dental fluorosis. Reductions in children's IQ – the key indicator of neurological impairment noted in human epidemiological studies – demonstrated a shallower exposure-response relationship, with less evidence of the threshold-like behaviour seen for dental fluorosis. Considering the currently available evidence, possible fluoride effects on childhood IQ should be taken into consideration as an area of public health concern, although less consistent evidence at low exposure levels remains a source of significant uncertainty. Benchmark dose modeling of high-quality epidemiologic data by Grandjean et al. (2022) predicted increased risks at levels lower than 1 mg/L F in drinking water; however, different models – including linear, quadratic, and segmented models – predicted notably different levels of risk from fluoride at these low concentrations.

At this point in time, mechanistic explanation and key mode of action events are insufficiently understood to guide the choice of the most appropriate model to use for predicting risks at low exposure levels.

Given the challenges of using available epidemiological data to characterize potential fluoride health effects at low levels of exposure with a high degree of precision, an evaluation of the biological mode and mechanisms of action underlying fluoride toxicity was included in the current review to provide some guidance on extrapolation at low concentrations. Although fluoride was found to cause a number of biological changes through various toxicity pathways (including oxidative stress, changes in gene expression, mitochondrial dysfunction, ER stress, perturbation of the Na/K+ ATPase pathway, apoptosis, inflammation, or death receptor-mediated pathways), the evidence from in vitro studies was considered to be too non-specific for health endpoints to, in particular, explain the occurrence of neurological effects in children following fluoride exposure. Absent a clear understanding of the underlying biological mechanisms and mode of action by which exposure to fluoride may act to reduce children's IQ, evidence for such effects below 1.5 mg/L remains uncertain. Experimental studies are needed to better understand the key mode of action events and their timing with respect to neurodevelopmental effects following maternal and early life exposure to fluoride [394]. Future

epidemiologic studies incorporating molecular and genetic components may also be of value in clarifying the shape of the exposure-response curve at low concentrations.

XII. References

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