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ISSN: 1040-8444 (Print) 1547-6898 (Online) Journal homepage: www.tandfonline.com/journals/itxc20

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To cite this article: Mohamed Kadry Taher, Franco Momoli, Jennifer Go, Shintaro Hagiwara, Siva Ramoju, Xuefeng Hu, Natalie Jensen, Rowan Terrell, Alex Hemmerich & Daniel Krewski (2024) Systematic review of epidemiological and toxicological evidence on health effects of fluoride in drinking water, Critical Reviews in Toxicology, 54:1, 2-34, DOI: 10.1080/10408444.2023.2295338

To link to this article: https://doi.org/10.1080/10408444.2023.2295338

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CRITICAL REVIEWS IN TOXICOLOGY
2024, VOL. 54, NO. 1, 2–34
https://doi.org/10.1080/10408444.2023.2295338

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REVIEW ARTICLE

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Systematic review of epidemiological and toxicological evidence on health effects of fluoride in drinking water

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ABSTRACT

Introduction: Fluoride is a naturally occurring substance that is also added to drinking water, dental hygiene products, and food supplements for preventing dental caries. Concerns have been raised about several other potential health risks of fluoride.

Objective: To conduct a robust synthesis of evidence regarding human health risks due to exposure to fluoride in drinking water, and to develop a point of departure (POD) for setting a health-based value (HBV) for fluoride in drinking water.

Methods: A systematic review of evidence published since recent reviews of human, animal, and *in vitro* data was carried out. Bradford Hill considerations were used to weigh the evidence for causality. Several key studies were considered for deriving PODs.

Results: The current review identified 89 human studies, 199 animal studies, and 10 major *in vitro* reviews. The weight of evidence on 39 health endpoints was presented. In addition to dental fluorosis, evidence was considered strong for reduction in IQ scores in children, moderate for thyroid dysfunction, weak for kidney dysfunction, and limited for sex hormone disruptions.

Conclusion: The current review identified moderate dental fluorosis and reduction in IQ scores in children as the most relevant endpoints for establishing an HBV for fluoride in drinking water. PODs were derived for these two endpoints, although there is still some uncertainty in the causal weight of evidence for causality for reducing IQ scores in children and considerable uncertainty in the derivation of its POD. Given our evaluation of the overall weight of evidence, moderate dental fluorosis is suggested as the key endpoint until more evidence is accumulated on possible reduction of IQ scores effects. A POD of 1.56 mg fluoride/L for moderate dental fluorosis may be preferred as a starting point for setting an HBV for fluoride in drinking water to protect against moderate and severe dental fluorosis. Although outside the scope of the current review, precautionary concerns for potential neurodevelopmental cognitive effects may warrant special consideration in the derivation of the HBV for fluoride in drinking water.

ARTICLE HISTORY
Received 9 March 2023
Revised 22 November 2023
Accepted 27 November 2023

KEYWORDS
Fluoride; drinking water; systematic review; dose-response; point of departure; health-based value; weight of evidence; dental fluorosis; childhood IQ; thyroid dysfunction; kidney dysfunction; sex hormone disruptions

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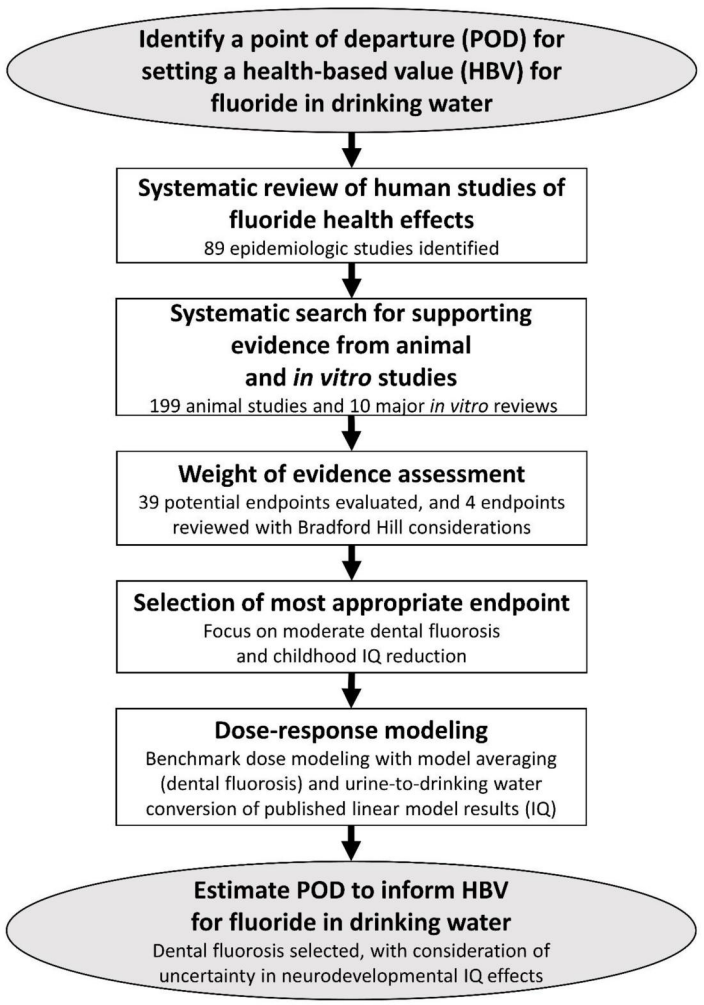
GRAPHICAL ABSTRACT



Abbreviations: 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; CKDu: chronic kidney disease of unknown 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; GLP: good lab practices; 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; OECD: office of economic collaboration and development; 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; WHO: world health organization; WQP: water quality program

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Introduction

Fluoride is a naturally occurring element that is present in various concentrations in fresh and sea water, soil, rocks and many foods (Botchey et al. 2016; Lima et al. 2019). Food represents the most common source of fluoride intake, except in areas with high consumption from ground water sources, where the fluoride concentration far exceeds that of community or municipal drinking water (World Health Organization (WHO) 2019).

Dental caries is a public health problem that, as reported by the World Health Organization (WHO), affects 60%–90% of children (USA), and most adults and elderly persons all over the world (Botchey et al. 2016; Petersen and Ogawa 2016; World Health Organization (WHO) 2016). In a 2019 assessment of the global burden of diseases, dental caries

reportedly impact an estimated 2 billion persons (permanent teeth) and 520 million children (primary teeth) (World Health Organization (WHO) 2020), with repercussions on quality of life (Kassebaum et al. 2017; Mariño and Zaror 2020). Such a global public health problem was attributed by the WHO to excessive intake of sugars and inadequate exposure to fluoride (World Health Organization (WHO) 2020).

At standard concentration of 1 part per million (PPM), fluoride was shown to prevent dental caries by improving resistance to the effect of acids, allowing the accumulation of minerals in teeth enamel (stimulating mineralization) (Medjedovic et al. 2015; World Health Organization (WHO) 2019; Mariño and Zaror 2020; Shyam et al. 2021). Additionally, fluoride disrupts the process of glycolysis, thus interfering with bacterial metabolism (World Health Organization (WHO) 2019). In the 1940s, many countries started adding fluoride to community drinking water (Petersen and Ogawa 2016; Mariño and Zaror 2020) at concentrations ranging between 0.6–1.0 mg/L (Mariño and Zaror 2020). As suggested by the WHO findings (Kassebaum et al. 2017; Mariño and Zaror 2020), such addition was successful in reducing the burden of dental caries, with some reported reductions of 26–35% in caries prevalence (Iheozor-Ejiofor et al. 2015). Due to its effectiveness in preventing dental caries, fluoride has also been added to foods, toothpaste and other dental hygiene products. Starting in 1969, WHO has officially endorsed the addition of fluoride to drinking water (Petersen and Ogawa 2016; Idowu et al. 2019). The uptake of such endorsement varied considerably across many developed and developing countries (Botchey et al. 2016).

However, this expansion of use was accompanied with an increased prevalence of dental and skeletal fluorosis, a state of hypomineralization of teeth enamel and bones, respectively (Medjedovic et al. 2015; Lima et al. 2019; Menya et al. 2019; Godebo et al. 2020). The resulting increase in tooth discoloration (with concerns for self-esteem and confidence in youth) and in teeth brittleness and reduced bone density show significant impacts on quality of life, including for instance an increase in prevalence of osteoporosis and bone fractures (Lima et al. 2019). Global concerns were heightened due to reports on possible associations of fluoride exposure with other health risks, such as cognitive, urogenital, endocrine, cardiovascular, and developmental/reproductive dysfunctions (NHMRC-National Health and Medical Research Council 2017; NTP-National Toxicology Program 2019; World Health Organization (WHO) 2019; Godebo et al. 2020). Evidence suggested that the prevalence of such outcomes was possibly related to genetics and the timing, degree, and duration of cumulative exposure to fluoride from all sources combined (World Health Organization (WHO) 2019; Godebo et al. 2020). Nevertheless, many studies affirmed fluoride in drinking water as a main contributor to the body's content of fluoride.

The objective of this review was to conduct a robust synthesis of current evidence on health risks due to exposure to fluoride in drinking water, and to recommend a point of departure that could be used for setting a health-based value (HBV)[1] for fluoride in drinking water designed to minimize potential health risks (Health Canada 2010; Minnesota Department of Health 2023).

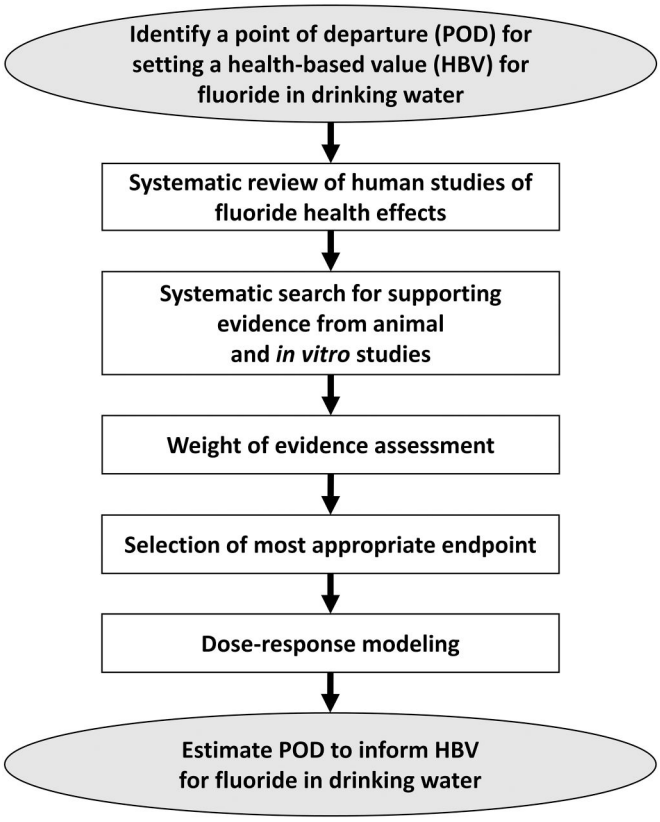

Figure 1. Risk assessment strategy for fluoride health effects.

The risk assessment strategy for deriving a POD for fluoride is summarized in Figure 1, outlining the review and weight of evidence approach, selection of appropriate health endpoints, and derivation of a point of departure. Additional details on the different sections of this manuscript are provided in supplementary material: a brief guide to the eight separate sections of this supplementary material can be found in the appendix to this manuscript.

Literature review strategy

Search methods

A rigorous, multi-step systematic review strategy was used to identify evidence from published or publicly available human, animal and *in vitro* streams, which examined the association of fluoride in drinking water with potential health risks. The separate streams of evidence were based on updating earlier comprehensive reviews in humans (CADTH 2019b, 2019a), animals (NTP-National Toxicology Program 2016), and *in vitro* studies (Health Canada 2010). The first review (CADTH 2019b) was an update of an earlier systematic review from Australia (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017). Given the extensive variety of outcomes under investigation, there was no requirement for a specific PECO (Population, Exposure, Comparison, Outcome) statement. The review strategy sought to include all outcomes reported across all population types and groups

(human, animal and *in vitro*), in conjunction with all eligible fluoride exposure scenarios.

The eligibility criteria for inclusion in the current review included all original studies published between 2016 and July 2021, which examined the association between exposure to fluoride in drinking water (fluoridation, or naturally occurring fluoride) and any health endpoints. The search was designed and implemented on July 21, 2021. An update of this search was conducted on February 2, 2023 for original studies reporting on two endpoints: dental fluorosis and effects on IQ scores. Studies from the earlier reviews were excluded from re-assessment, but weighed in as part of overall evidence assessment using the Bradford Hill considerations. Exclusion criteria included studies that 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

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 any health risks. The search included 10 bibliographic databases and 6 clinical trial registries. Eighteen major grey literature sources and web-based materials were also examined, including 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. The literature search used both controlled vocabulary and keywords, and 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 (Higgins and Green 2011). See Supplementary Material 1 for details on the searched sources, used terms, and search output.

Identified references from all sources were collated using EndNote (The EndNote Team 2018), and were subjected to automated then manual deduplication. A multi-level assessment including screening of titles and abstracts, and full-text examination was conducted by two independent reviewers at each step, using DistillerSR (2021). Any identified conflicts were resolved *via* consensus prior to moving to the next level.

The literature search considered original published reviews to assess whether they would suffice as updates to the earlier reviews (CADTH 2019b, 2019a), (NTP-National Toxicology Program 2016), and (Health Canada 2010). Published reviews were screened using the 3 following criteria, and were considered potentially eligible, if a review meets all criteria:

1. Does the review have sufficient description of its methodology (including searching 1 or more bibliographic databases)?
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?

Data abstraction spreadsheets were developed using Microsoft Office Excel (Microsoft Corporation 2020). Key characteristics of the included studies are summarized in Table 1 and detailed in Supplementary Material 2.

Animal evidence

Animal evidence on the association between fluoride in drinking water and a wide range of endpoints was conducted for identifying relevant original studies that were published after 2006 and not included in the 2010 report (Health Canada 2010). The search for animal evidence followed the same comprehensive, multi-step search strategy as human evidence, and in accordance with PRISMA guidelines, and guidance provided by the Cochrane Collaboration (Higgins and Green 2011). No filters other than "animals only" were applied to limit the search output. No restrictions on animal species or models were imposed. See Supplementary Material 1 for details on the searched sources, used terms and search output.

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 [lowest observed adverse effect level], 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 ($\sim$200), a tiered approach was employed to determine and select studies with "key" information relevant for the current objectives. This approach categorized studies into three tiers with tier-1 containing all "key" information for the review, and tier-2 containing supporting information. Furthermore, studies in tier-1 underwent full data abstraction and quality assessment. Tier-1 studies tended to be guideline studies (OECD [office of economic collaboration and development], GLP [good lab practices]) that assessed oral route of exposure at relevant concentrations ($\leq$ 20 ppm). 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 complete list of studies in Supplementary Material 4). In this approach, each study that passed level 2 screening was reviewed for the following "key" information and placed in the appropriate tier.

Table 1. Major characteristics of included human studies.

Study	Study design	Country	Participants	Health effect	Association	Quality of evidence
Mercado et al. (2023)	Cross-sectional	Peru	Children 12–15 years old	Dental fluorosis	Positive	2
Tang et al. (2023)	Cross-sectional	China	Children 7–14 years old	Dental fluorosis	Positive	2
Ahmad et al. (2022)	Cross-sectional	Peru	Children 9–11 years old	Intelligence quotient (IQ)	None	3
Feng et al. (2022)	Cross-sectional	Pakistan	Children 8–12 years old	Intelligence quotient (IQ)	Positive	2
García-Escobar et al. (2022)	Cross-sectional	India	Persons 10–60 years old	Dental fluorosis	Positive	2
Goodman et al. (2022)	Cohort	Mexico	Mother-child pairs	Intelligence quotient (IQ)	Positive	1
Gupta et al. (2022)	Case-control	India	All residents	Dental fluorosis	Positive	2
Ibarluzea et al. (2022)	Cohort	Spain	Mother-child pairs	Intelligence quotient (IQ)	None	1
Kaur et al. (2022)	Cross-sectional	India	Children 12–13 years old	Intelligence quotient (IQ)	Positive	2
Marques et al. (2022)	Cross-sectional	Brazil	Adolescents 17–20 years old	Dental fluorosis	Positive	1
McLaren et al. (2022)	Cross-sectional	Canada	Children 7 years old	Dental fluorosis	Positive	1
Rani et al. (2022)	Cross-sectional	India	Children 6–12 years old	Dental fluorosis	Positive	2
Saeed et al. (2022)	Cross-sectional	Pakistan	Children 5–16 years old	Dental fluorosis Intelligence quotient (IQ)	Positive Positive	2
Tawfik et al. (2022)	Cross-sectional	Egypt	Children 7–14 years old	Dental fluorosis	Positive	2
Thilakarathne and Ekanayake (2022)	Cross-sectional	Sri Lanka	Children 15 years old	Dental fluorosis	Positive	2
Al-Omoush et al. (2021)	Cross-sectional	Jordan	Children 13–18 years old	Dental fluorosis	Positive	2
Ayele et al. (2021)	Cross-sectional	Ethiopia	Residents 10–70 years old	Skeletal fluorosis Headache and paresthesia	Positive Possible	2[a]
Cao et al. (2021)	Cross-sectional	China	Children 8–13 years old	Dental fluorosis	Positive	2
Dong et al. (2021)	Cross-sectional	USA	Children 6–19 years old	Dental fluorosis	Positive	1
Du et al. (2021)	Cross-sectional	China	Children 7–12 years old	Thyroid hormone dysfunction	Positive	1
Farmus et al. (2021)	Cohort	Canada	Mother-child pairs	Intelligence quotient (IQ)	Positive	1
Fernandes et al. (2020)	Cross-sectional	Brazil	Children 6–12 years old	Dental fluorosis	Positive	2
Helte et al. (2021)	Cohort	Sweden	SMC residents <85 years old	Bone density	Positive	1
James et al. (2021)	Cross-sectional	Ireland	Children 7–12 years old	Dental fluorosis	Possible	1
Meghe et al. (2021)	Cross-sectional	India	Residents with no skeletal fluorosis	Skeletal fluorosis	Possible	2
Meng et al. (2021)	Cross-sectional	China	Adults >18 years old	Genotoxicity	Possible	2
Mohd Nor et al. (2021)	Cross-sectional	Malaysia	Residents 9 and 12 years old	Dental fluorosis	Positive	1
Rojanaworarit et al. (2021)	Cross-sectional	Thailand	Children 6–10 years old	Dental fluorosis	Positive	1
Sharma et al. (2021)	Cross-sectional	India	Children 6–19 years old	Dental fluorosis	Positive	2
Silva et al. (2021)						1
Tkachenko et al. (2021)	Cross-sectional	Ukraine	Children 7–10 years old with fluorosis	Biomarkers for myocardial infarction	Positive	2
Wang et al. (2021)	Cross-sectional	China	Children 6–13 years old	Dental fluorosis Intelligence quotient (IQ)	Positive Positive	1
Yani et al. (2021)	Cross-sectional	Indonesia	Children 6–12 years old	Dental fluorosis Intelligence quotient (IQ)	Positive Positive	2
Yu et al. (2021)	Cross-sectional	China	Children 7–13 years old	Intelligence quotient (IQ)	Positive	1
Zhao et al. (2021)	Cross-sectional	China	Children 6–11 years old	Intelligence quotient (IQ)	Positive	1
Bai et al. (2020)	Cross-sectional	USA	Children 6–19 years old	Sex hormone disruptions	Inverse	1
Cui et al. (2020)	Cross-sectional	China	School children 7–12 years old	Intelligence quotient (IQ) Thyroid dysfunction	Non-significant Possible	2
Das et al. (2020)	Cross-sectional	Saudi Arabia	Dental patients 9–50 years old	Dental Fluorosis	Positive	2
Fernandes et al. (2020)	Cross-sectional	Brazil	Children 6–12 years old	Dental fluorosis	Positive	2
Godebo et al. (2020)	Cross-sectional	Ethiopia	Adolescents and adult farmers	Skeletal fluorosis	Positive	1
Kim et al. (2020)	Case-control	USA	Adults less than 40 years old	Bone cancer (osteosarcoma)	None	1
Krishna et al. (2020)	Case-control	India	Adult patients 45–75 years old	Diabetes Mellitus	Positive	1
Lee et al. (2020)	Ecological	South Korea	All residents	Hip fracture Osteoporosis Bone cancer	None None None	1
Nanayakkara et al. (2020)	Cross-sectional	Sri Lanka	Adult non-dialysis CKDu[b] cases	CKDu	Possible	2
Russ et al. (2020)	Cohort	Scotland	Children 11 years old (mean age)	Dementia	Positive	1
Stangvaltaite-Mouhat et al. (2020)	Cross-sectional	Lithuania	Adults 35–74 years old	Dental fluorosis	Possible	2
Sun et al. (2020)	Cross-sectional	China	Female farmers 20–60 years old	Bone quality	Positive	1
Till et al. (2020)	Cohort	Canada	Females >17 years old, <14 weeks gestation	Intelligence quotient (IQ)	Positive	1
Wang et al. (2020)	Cross-sectional	China	Children 7–13 years old	Thyroid dysfunction Intelligence quotient (IQ)	Positive Positive	1
An et al. (2019)	Cross-sectional	China		Sex hormone disruptions	Positive	1

(*continued*)

Table 1. Continued.

Study	Study design	Country	Participants	Health effect	Association	Quality of evidence
			Male farmers 18–55 years old			
Crnosija et al. (2019)	Ecological	USA	Inpatients >18 years old with metastatic bone cancer	Bone cancer (secondary)	None	2
Fernando et al. (2019)	Case-control	Sri Lanka	Adults 19–76 years old, non-dialysis patients	CKDu	Possible	2
Jiménez-Córdova et al. (2019)	Cross-sectional	Mexico	Children 5–12 years old	Kidney dysfunction Cardiovascular diseases	Inconclusive Possible	1
Jiménez-Córdova et al. (2019)	Cross-sectional	Mexico	Adults	Arsenic methylation	Inverse	1
Khanoranga (2019)	Cross-sectional	Pakistan	Male brick kiln workers and controls (17–45 years old	Dental fluorosis	Positive	2
Liu et al. (2019)	Cross-sectional	China	Children 7–13 years old	Childhood obesity	Positive	1
Malin et al. (2019)	Cross-sectional	USA	Adolescents 12–19 years old	Liver dysfunction Kidney dysfunction	Possible Possible	1
Malin et al. (2019)	Cross-sectional	USA	Adolescents 16–19 years old	Sleep disturbance	Possible	1
Pei et al. (2019)	Cross-sectional	China	Residents ≥16 years old	Skeletal fluorosis	Possible	2
Riddell et al. (2019)	Cross-sectional	Canada	Children 6–17 years old	ADHD[c]	Positive	1
Shaik et al. (2019)	Cross-sectional	India	Children 9–13 years old with normal nutrition and iodine status	Thyroid dysfunction	None	2
Soto-Barreras et al. (2019)	Cross-sectional	Mexico	Children 9–10 years old	Intelligence quotient (IQ) Dental fluorosis	None Possible	2
Zhang et al. (2019)	Cross-sectional	USA	Women with a live birth (2009–2016)	Pre-term births	Positive	1
Zhou, Song, et al. (2019)	Cross-sectional	China	Adults ≥40 years old, with no congenital eye disease or ocular trauma	Eye diseases (selected)	Possible	1
Zhou, Yang, et al. (2019)	Cross-sectional	USA	Children 7–13 years old	Dental fluorosis Genotoxicity	Positive Positive	1
Bashash et al. (2018)	Cohort	Mexico	Mother-child pairs	ADHD	Positive	1
Cui et al. (2018)	Cross-sectional	China	Children 7–12 years old	Intelligence quotient (IQ)	Positive	1
Jiménez-Córdova et al. (2018)	Cross-sectional	Mexico	Adults 18–77 years old	Kidney dysfunction	Possible	1
Kumar et al. (2018)	Cross-sectional	India	Children 8–15 years old	Thyroid dysfunction	Positive	2
Kumar et al. (2018)	Cross-sectional	India	Adolescents 12–15 years old	Dental fluorosis	Positive	1
Malin et al. (2018)	Cross-sectional	Canada	Persons 3–79 years old	Thyroid dysfunction	Possible	1
Mohd Nor et al. (2018)	Cross-sectional	Malaysia	Children 9 and 12-years-old	Dental fluorosis	Positive	2
Mustafa et al. (2018)	Ecological	Sudan	Children 6–14 years	Intelligence quotient (IQ)	Possible	2
Oweis et al. (2018)	Cohort	USA	Adolescents 17 years old	Bone quality	None	2
Quadri et al. (2018)	Cross-sectional[d]	India	Children 4–12 years old with NS-MCD[e]	Renal tubule ultrastructural changes	Positive	2
Rathore et al. (2018)	Cross-sectional	India	Children 8–14 years old	Thyroid dysfunction	Positive	2
Shruthi and Anil (2018)	Cross-sectional	India	Adolescents and adults	Non-skeletal manifestations of fluoride toxicity	Possible	2
Yu et al. (2018)	Cross-sectional	China	Children 7–13 years old	Intelligence quotient (IQ)	Positive	1
Arulkumar et al. (2017)	Case-control	India	Cases with dental and skeletal fluorosis and matching controls	Cardiovascular diseases Liver dysfunction	Possible Possible	2
Bashash et al. (2017)	Cohort	Mexico	Mother-child pairs	Intelligence quotient (IQ)	Positive	1
Chauhan et al. (2017)	Abstract[f]	India	Adults with fluorosis	Sex hormone disruptions	Possible	N/A[g]
Stephenson et al. (2017)	Abstract	N/A	NR[h]	Suicide rates	Possible	N/A
Verma et al. (2017)	Cross-sectional	India	Adolescents 12–17 years old	Dental fluorosis	Positive	1
Cárdenas-González et al. (2016)	Cross-sectional	Mexico	Children 5–12 years old	Kidney dysfunction	None	1
de Moura et al. (2016)	Cross-sectional	Brazil	Children 11–14 years old	Dental fluorosis	Positive	2
Heck (2016)	Cross-sectional	USA	Children 14–15 years old and Adults 17–90 years old	Intelligence quotient (IQ) General health	None None	1
Kousik and Mondal (2016)	Ecological	India	Children 6–18 years old	Body mass index (BMI) Intelligence quotient (IQ)	Positive Positive	2
Sabokseir et al. (2016)	Cross-sectional	Iran	Children 9 years old	Dental fluorosis	Positive	1
Xiang et al. (2016)	Cross-sectional	China	Children 8–14 years old	Dental fluorosis	Positive	2

[a]Quality was assessed as level 1 (high quality) for the skeletal fluorosis outcome, and level 2 (moderate/acceptable quality) for the neurological outcomes. A conservative assessment of the study's overall quality was set to level 2. [b]CKDu: Chronic kidney disease of unknown origin. [c]ADHD: Attention deficit hyperactivity disorder. [d]Originally a case-control study. Only cross-sectional analysis results relevant to the current review are included. [e]NS-MCD: Minimal change nephrotic syndrome. [f]Study design not reported. [g]N/A: Not applicable due to lack of sufficient information on study quality in the retrieved abstract. [h]NR: Not reported.

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 2. Considerations for tiered approach for animal studies.

- 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 2.

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. See Supplementary Material 6 for more details.

Risk of bias assessment

The quality of included human, animal, and *in vitro* studies was assessed using the OHAT risk of bias tool (Rooney et al. 2014; National Institute of Environmental Health Sciences 2019). This tool enlists 11 questions across 7 domains against which each study is assessed for quality of provided evidence. In the current review, four questions associated with experimental evidence were excluded as they were considered irrelevant to the types of studies included in this review. For every question, 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 (––). Overall study risk of bias followed the rubric provided in the OHAT guidance.

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. Full details of the assessment of human and animal studies are provided in Supplementary Material 2 and 4, respectively.

Weight of evidence

Selection of candidates for the most appropriate endpoint

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 setting an HBV, based on identifying the most appropriate endpoint. All health endpoints studied in the literature were evaluated in the current review, but only select endpoints were considered further if the body of evidence was sufficiently concerning to warrant closer examination. In evaluating the body of evidence identified in the current 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 (Hill 1965) 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 20 ppm fluoride)
   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:

3. There was consistent evidence of an association in tier-1 animal studies
4. The association occurred in the studies at an exposure level (less than 20 ppm fluoride/L) relevant to current fluoride levels in North American drinking water context.

Throughout the review, evidence summaries and weight of evidence considerations were described in terms relevant to the North American drinking water context. Naturally occurring and fluoridated community water levels vary widely throughout the world; the shorthand used in the text is only meant to imply that focus was placed on health risks that may occur at fluoride levels in drinking water just above, close to, or below current maximum allowable concentrations–this was operationalized in the review at a level of approximately 2 ppm fluoride in drinking water.

Bradford Hill considerations

In weighing evidence of causality between fluoride and drinking water, the current review used the Bradford Hill considerations (Hill 1965) to assess the evidence drawn from human, tier-1 and tier-2 animal, and *in vitro* streams. The considerations included the following domains: strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy.

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?" (Hill 1965).

Evidence integration

For each endpoint, the updated evidence was classified into one of the following categories:

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

Literature search results

Identification of relevant studies

Human

The search strategy resulted in retrieval of 5,020 records, including 4,662 records from bibliographic databases and clinical trial registries, and 358 records from major grey literature sources. Deduplication resulted in removal of 2,307 records, leaving 2,713 studies for title and abstract screening (level 1). Upon excluding 2,202 irrelevant studies, there were 511 studies left for full-text examination (level 2). This examination led to the exclusion of an additional 422 references for not matching the inclusion/exclusion criteria.

Eighty-nine original studies including 2 abstracts were finally retained for further analysis. A detailed PRISMA flow diagram (Moher et al. 2009) showing the selection process for human studies is shown in Figure 3. Details on characteristics and assessment of quality of evidence for the included studies, and the list of excluded studies with rationale for exclusion are provided in Supplementary Material 2 and 3, respectively.

The retained studies included 70 (79%) cross-sectional in design, 9 (10%) cohort studies (Bashash et al. 2017; 2018; Oweis et al. 2018; Russ et al. 2020; Till et al. 2020; Farmus et al. 2021; Helte et al. 2021; Goodman et al. 2022; Ibarluzea et al. 2022), 4 (4%) case-control studies (Arulkumar et al. 2017; Fernando et al. 2019; Kim et al. 2020; Krishna et al. 2020) and 4 (4%) ecological studies (Kousik and Mondal 2016; Mustafa et al. 2018; Crnosija et al. 2019; Lee et al. 2020) and 2 (%) were only abstracts (Chauhan et al. 2017; Stephenson et al. 2017). All of the retrieved studies were published between 2016 and 2023. The sampling time-frame included variable time intervals between 1992 and 2019, with one third of studies that did not report a time-frame.

Eighteen studies (20%) were carried out in China followed by 17 (19%) in India, with USA, Mexico, Canada and Brazil involved in 9 (10%), 8 (9%), 7 (8%) and 5 (6%) of studies, respectively. Three studies were conducted in each of Pakistan and Sri Lanka, and two studies in each of Ethiopia and Malaysia. One study was conducted in each of Egypt, Indonesia, Iran, Ireland, Jordan, Lithuania, Peru, Saudi Arabia, Scotland, South Korea, Spain, Sudan, Sweden, Thailand, Ukraine. Sixty-one studies (69%) examined fluoride exposure in drinking water, 17 (19%) in ground water, 15 (17%) in urine, and 2 (2%) in serum, with many studies assessing exposure from more than one source.

The examined population were comprised of children and/or adolescents in 54 studies (61%), compared to 15 studies (17%) in adults, 13 (15%) in mixed populations, and finally 6 (7%) in mother/child pairs. The number of study participants ranged from 83 to 6,914,124 with 78 studies (88%) examined both men and women, 3 studies (3%) in men only, 2 studies (2%) in women only, and 6 studies (7%) that examined mother-child pairs.

A summary of major study characteristics and quality of evidence is shown in Table 1, where positive association refers to an increased health risk with increasing fluoride exposure, and negative association refers to a decreased

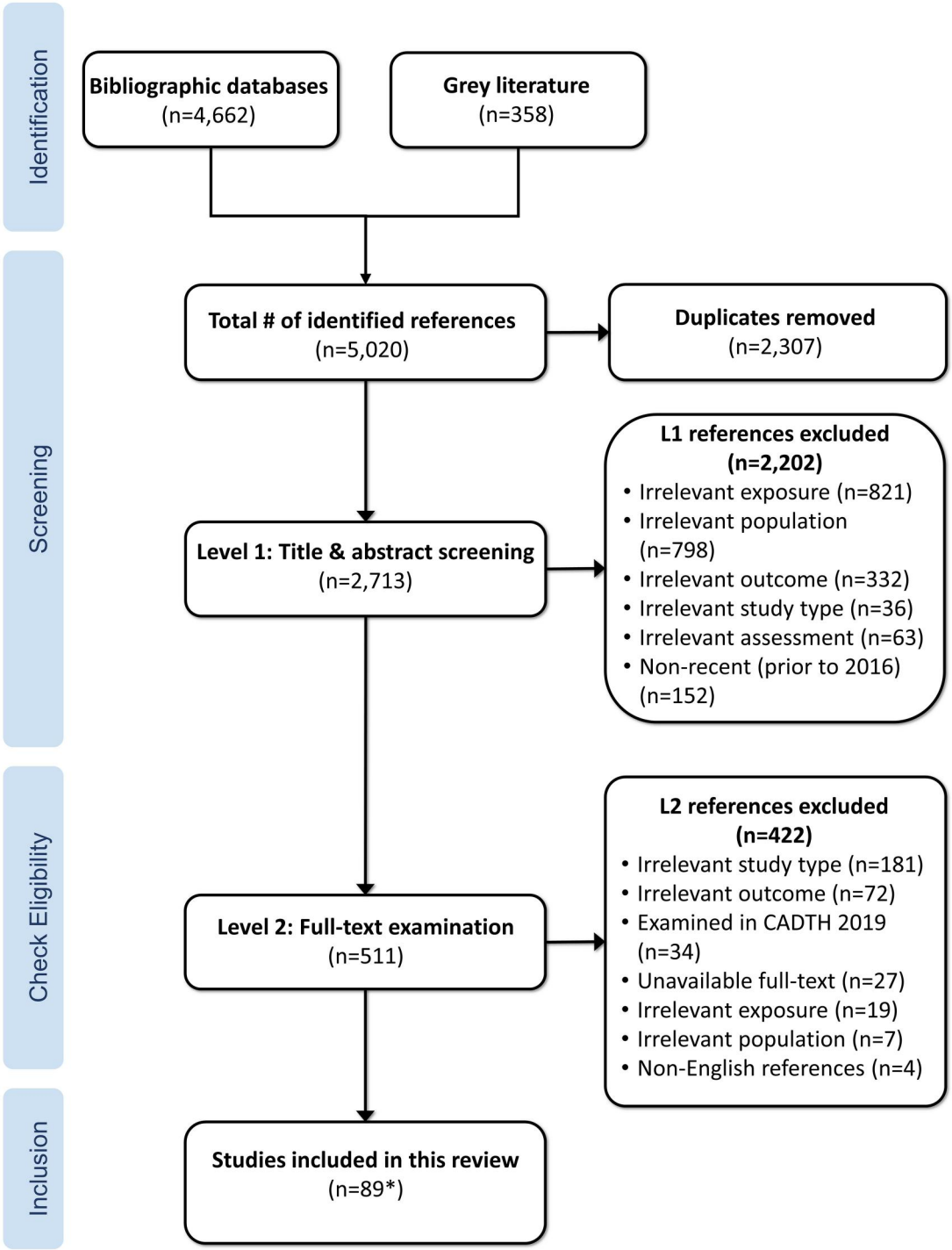

Figure 3. PRISMA flow diagram for human studies (* including 2 abstracts).

health risk with increasing fluoride exposure. A more detailed description is provided in Supplementary Material 2.

Animal

The search strategy resulted in retrieval of 2,119 non-duplicate records from bibliographic databases. Upon excluding 1,714 irrelevant studies during title and abstract screening, there were 405 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 (Moher et al. 2009) showing the selection process for animal studies is shown in Figure 4.

Most of the primary outcomes reported in the retained studies involve 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

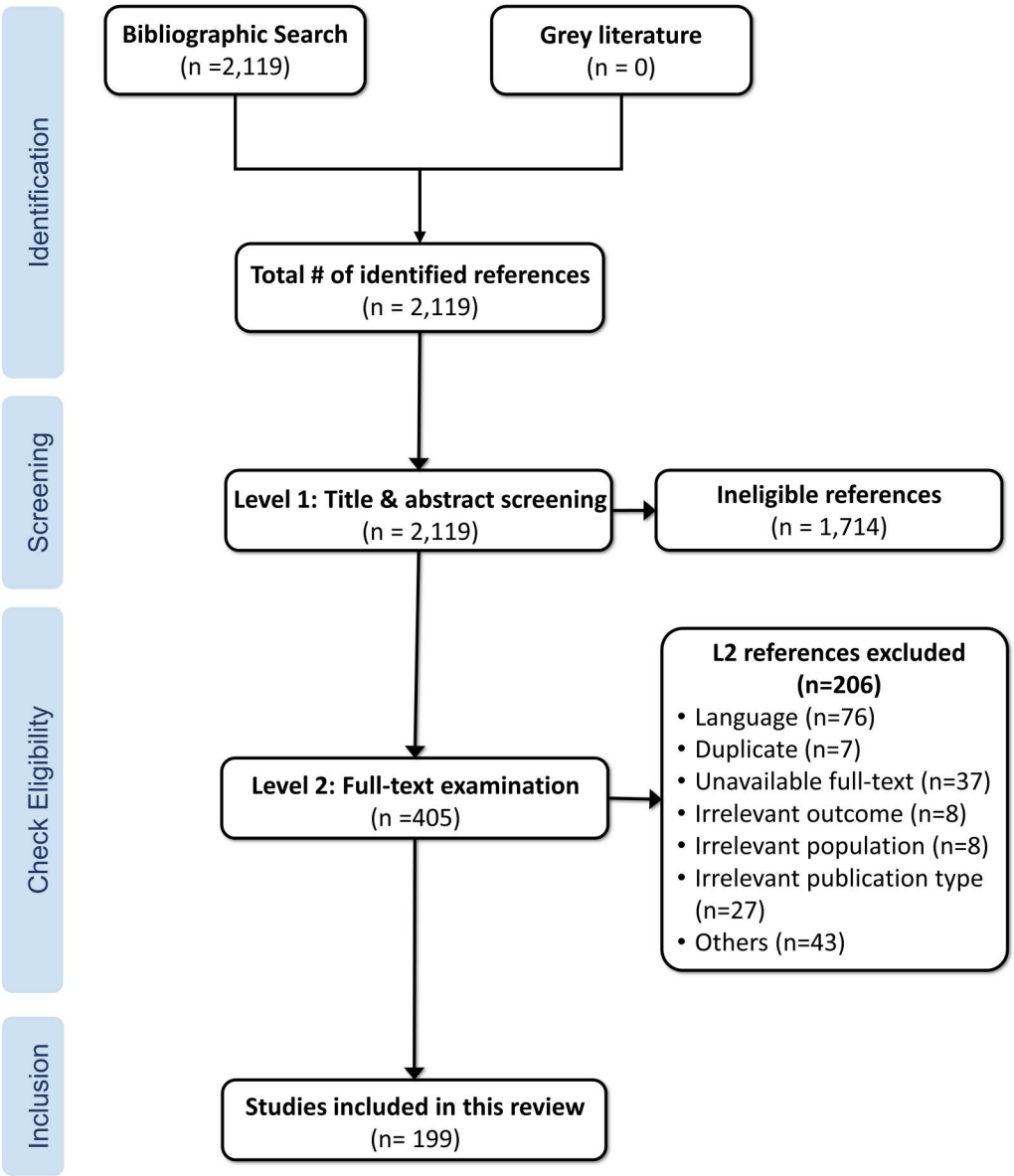

Figure 4. PRISMA flow diagram for animal studies.

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.

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 quality of evidence. Across all endpoints examined, and excluding neurological outcomes, the largest amount of data was related to reproductive outcomes.

Using the OHAT risk of bias tool (National Institute of Environmental Health Sciences 2019), an assessment of the quality of evidence for the included tier-1 studies showed that 56% of studies ($n = 20$) were of high quality ($Q = 1$), compared to 39% percent that were of acceptable quality ($n = 14$). A comprehensive summary of important study characteristics of all tier-1 and tier-2 studies and the quality of evidence assessment for tier-1 studies only, which are included in the current review, are shown in Supplementary Material 4. A listing of excluded animal studies in Supplementary Material 5.

In vitro

A focused search was conducted for identifying *in vitro* evidence reporting on the possible mechanism of action of fluoride at the cellular level. A thorough examination of the studies and reviews retrieved from 3 major bibliographic databases resulted in the selection of 10 major reviews based on the depth and quality of their reported *in vitro* evidence.

The strategy used for bibliographic database search, and a summary of the major characteristics of the included reviews are shown in Supplementary Material 6.

Overview of evidence

Human evidence

Out of a total of 38 endpoints reported in the current review, the current literature search identified new human evidence relating to 15 endpoints, which were not reported in earlier reports (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017; CADTH 2019b, 2019a). CADTH had initially reported on 23 endpoints, for which the current review updated the evidence on 14 of those endpoints and found no new evidence on the remaining 9 endpoints. This section describes the evidence reported in NHMRC 2016, CADTH 2019, and the current updated review of the literature. A summary of the human evidence for all endpoints is provided in Table 2 and detailed in Supplementary Material 2.

Where no earlier evidence was reported, the CADTH (2019b, 2019a) 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 North American context. Although these studies may involve fluoride water concentration higher than those in North American 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 Supplementary Material 2). Where available, fluoride levels in drinking water were listed in the following section for the purpose of comparison across studies. The 16 new endpoints identified in the current review include sex hormone disruptions, ADHD, dementia, liver dysfunction, memory loss, preterm births, genotoxicity, ultrastructural kidney changes, BMI, childhood obesity, selected eye diseases, general health, trouble working, suicide, and arsenic methylation.

The current review also updated the evidence on 14 additional endpoints that were identified earlier (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017; CADTH 2019b, 2019a). These endpoints include dental and skeletal fluorosis, IQ reduction, 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.

In the absence of new studies, the CADTH (2019b) summary of evidence remained unchanged as no association of drinking water fluoride exposure and each of cancer total incidence and mortality, and Down syndrome (limited). The CADTH (2019b) evidence remained insufficient for all-cause mortality, musculoskeletal pain, refractive errors, newborn's height & weight, hypertension, and abortion and female fertility. The CADTH (2019b) evidence for kidney stones remained limited for an inverse association with exposure to drinking water fluoride.

Dental fluorosis. Earlier evidence on the association of fluoride with dental fluorosis was reported by NHMRC (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017) (three systematic reviews) and CADTH (2019b) (21 studies: 1 acceptable, 19 low; $N = 35{,}374$), which reported consistent findings for an association between fluoride and dental fluorosis. The current literature search identified 33 cross-sectional studies, including 15 studies of high quality (Sabokseir et al. 2016; Verma et al. 2017; Kumar et al. 2018; Zhou et al. 2019; Cao et al. 2021; Dong et al. 2021; James et al. 2021; Mohd Nor et al. 2021; Rojanaworarit et al. 2021; Silva et al. 2021; Wang et al. 2021; Marques et al. 2022; McLaren et al. 2022; Saeed et al. 2022; Tawfik et al. 2022) and 18 studies of acceptable quality (de Moura et al. 2016; Xiang et al. 2016; Mohd Nor et al. 2018; Khanoranga 2019; Soto-Barreras et al. 2019; Das et al. 2020; Fernandes et al. 2020; Stangvaltaite-Mouhat et al. 2020; Al-Omoush et al. 2021; Sharma et al. 2021; Yani et al. 2021; García-Escobar et al. 2022; Gupta et al. 2022; Rani et al. 2022; Thilakarathne and Ekanayake 2022; Mercado et al. 2023; Tang et al. 2023) 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, 6 were conducted in China (Xiang et al. 2016; Zhou et al. 2019; Cao et al. 2021; Wang et al. 2021; Rani et al. 2022; Tang et al. 2023), 5 in India (Verma et al. 2017; Kumar et al. 2018; Sharma et al. 2021; García-Escobar et al. 2022; Gupta et al. 2022), 5 in Brazil (de Moura et al. 2016; Fernandes et al. 2020; Silva et al. 2021; Marques et al. 2022), 2 in Malaysia (Mohd Nor et al. 2018; 2021), 2 in Pakistan (Khanoranga 2019; Saeed et al. 2022), and 1 in each of Canada (McLaren et al. 2022), Egypt (Tawfik et al. 2022), Indonesia (Yani et al. 2021), Iran (Sabokseir et al. 2016), Jordan (Al-Omoush et al. 2021), Lithuania (Stangvaltaite-Mouhat et al. 2020), Mexico (Soto-Barreras et al. 2019), Peru (Mercado et al. 2023), Saudi Arabia (Das et al. 2020), Sri Lanka (Thilakarathne and Ekanayake 2022), Thailand (Rojanaworarit et al. 2021), and USA (Dong et al. 2021).

The study by Dong et al. (2021) 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. A study that was conducted on children (age 9 to 12 years) reported that compared to those exposed to non-fluoridated water, the odds of dental fluorosis (DFI $\geq$ 2) (95% CI) were 5.97 (95%CI: 3.32, 10.72) times greater among children with a lifetime exposure to 0.5 ppm fluoride, and 9.12 (95%CI: 5.15, 16.14) times greater among those exposed to 0.7 ppm fluoride during the first two years of life, followed by a level of 0.5 ppm (Mohd Nor et al. 2021).

Another study included children (age 7 to 13 years) from rural areas with low-to-moderate levels of fluoride and reported that each 1 mg/L increase of water fluoride was associated with increased odds of 1.47 (95%CI: 1.40, 1.55), 1.85 (95%CI: 1.63, 2.11), 1.68 (95%CI: 1.57, 1.79), and 3.85

Table 2. Summary of the evolving human evidence on all health effects

Outcome	NHMRC 2016 *Review/study (quality)*	CADTH 2019 *Study (quality)*	CADTH 2019 conclusion	Current review *Study (quality)*	Evolving evidence
All-cause mortality	1 study (acceptable)	No studies	Insufficient	No new studies	Insufficient
Musculoskeletal					
Bone cancer	2 SR[a] (2 NR[b]); 6 studies (3 acceptable, 3 low)	2 studies (2 acceptable)	No association	3 studies (1 high (Lee et al. 2020), 2 acceptable (Crnosija et al. 2019; Kim et al. 2020))	No association
Bone density and quality	1 SR (NR); 1 study (low)	No studies	Insufficient	5 studies (4 high (Godebo et al. 2020; Lee et al. 2020; Sun et al. 2020; Helte et al. 2021) and 1 acceptable (Oweis et al. 2018))	Inconsistent
Hip fracture	2 SRs (2 NR); 2 studies (acceptable)	1 study (acceptable)	No association	1 study (high (Lee et al. 2020))	No association
Musculoskeletal pain	2 studies (2 low)	No studies	Insufficient	No new studies	Insufficient
Cancer, total incidence and mortality	SRs (2 NR); 3 studies (acceptable)	1 study (acceptable)	No association	No new studies	No association
Cognitive dysfunction					
ADHD	No studies	No studies	N/A	2 studies (2 high (Bashash et al. 2018; Riddell et al. 2019))	Insufficient
Dementia	No studies	No studies	N/A	1 study (high (Russ et al. 2020))	Insufficient
Down syndrome	2 SRs (2 NR); 1 study (acceptable)	1 study (acceptable)	Limited (no association)	No new studies	Limited (no association)
IQ reduction	1 SR (NR); 11 studies (1 high, 2 acceptable, 8 low)	6 studies (1 acceptable, 5 low)	Limited (no association)	17 studies (12 high (Bashash et al. 2017; Cui et al. 2018; Yu et al. 2018; Till et al. 2020; Wang et al. 2020; Farmus et al. 2021; Wang et al. 2021; Yu et al. 2021; Zhao et al. 2021; Goodman et al. 2022; Ibarluzea et al. 2022; Saeed et al. 2022), 5 acceptable (Heck 2016; Kousik and Mondal 2016; Mustafa et al. 2018; Soto-Barreras et al. 2019; Cui et al. 2020))	Positive
Trouble working	No studies	No studies	N/A	1 study (acceptable (Heck 2016))	Insufficient
Cardiovascular					
Atherosclerosis	1 study (low)	No studies	Insufficient	3 studies (1 high (Jiménez-Córdova et al. 2019), 2 acceptable (Arulkumar et al. 2017; Tkachenko et al. 2021))	Limited
Hypertension	3 studies (low)	2 studies (2 low)	Insufficient	No new studies	Insufficient
Myocardial infarction	No studies	1 study (low)	Insufficient	1 study (acceptable (Tkachenko et al. 2021))	Insufficient
Diabetes Mellitus	No studies	2 studies (2 low)	Insufficient	1 study (high (Krishna et al. 2020))	Insufficient
Eye diseases					
Eye diseases[c]	No studies	No studies	N/A	1 study (high (Zhou, Song, et al. 2019))	Insufficient
Refractive errors	No studies	1 study (low)	Insufficient	No new studies	Insufficient
Fluorosis					
Dental	3 SRs (2 NR, 1 high)	21 studies (1 acceptable, 20 low)	Positive	32 studies (15 high quality (Sabokseir et al. 2016; Verma et al. 2017; Kumar et al. 2018; Zhou, Yang, et al. 2019; Cao et al. 2021; Dong et al. 2021; James et al. 2021; Mohd Nor et al. 2021; Rojanaworarit et al. 2021; Silva et al. 2021; Wang et al. 2021; Marques et al. 2022; McLaren et al. 2022b; Saeed et al. 2022; Tawfik et al. 2022) and 17 acceptable (de Moura et al. 2016; Xiang et al. 2016; Mohd Nor et al. 2018;	Positive

(*continued*)

Table 2. Continued.

Outcome	NHMRC 2016 *Review/study (quality)*	CADTH 2019 *Study (quality)*	CADTH 2019 conclusion	Current review *Study (quality)*	Evolving evidence
				Khanoranga 2019; Soto-Barreras et al. 2019; Das et al. 2020; Fernandes et al. 2020; 2020; Al-Omoush et al. 2021; Sharma et al. 2021; Yani et al. 2021; García-Escobar et al. 2022; Gupta et al. 2022; Rani et al. 2022; Thilakarathne and Ekanayake 2022; Mercado et al. 2023; Tang et al. 2023)	
Skeletal	1 SR (NR); 2 studies (2 low)	2 studies (2 low)	Insufficient	3 studies (1 high (Ayele et al. 2021), 2 acceptable (Pei et al. 2019; Meghe et al. 2021))	Limited
Genotoxicity	No studies	No studies	N/A	2 studies (1 high (Zhou, Yang, et al. 2019), 1 acceptable (Meng et al. 2021))	Insufficient
Growth & development					
BMI	No studies	No studies	N/A	1 study (acceptable (Kousik and Mondal 2016))	Insufficient
Childhood obesity	No studies	No studies	N/A	1 study (high (Liu L et al. 2019))	Insufficient
Newborn's height & weight	1 study (low)	1 study (low)	Insufficient	No new studies	Insufficient
Kidney					
Kidney dysfunction	No studies	1 study (low)	Insufficient	6 studies (4 high (Cárdenas-González et al. 2016; Jiménez-Córdova et al. 2018; Jiménez-Córdova et al. 2019; Malin et al. 2019), 2 acceptable (Fernando et al. 2019; Nanayakkara et al. 2020))	Limited
Kidney stones	No studies	1 study (acceptable)	Limited	No new studies	Limited
Ultrastructural	No studies	No studies	N/A	1 study (acceptable (Quadri et al. 2018))	Insufficient
Liver dysfunction	No studies	No studies	N/A	2 studies (1 high (Malin et al. 2019), 1 acceptable (Arulkumar et al. 2017))	Insufficient
Neurologic					
Headache	2 studies (2 low)	No studies	Insufficient	1 study (acceptable (Ayele et al. 2021))	Insufficient
Sleep-related outcomes	2 studies (2 low)	No studies	Insufficient	1 study (high (Malin et al. 2019))	Insufficient
Reproduction					
Abortion and fertility	No studies	2 studies (2 low)	Insufficient	No new studies	Insufficient
Preterm births	No studies	No studies	N/A	1 study (high (Zhang et al. 2019))	Insufficient
Sex hormone disruptions	No studies	No studies	N/A	2 studies (2 high(An et al. 2019; Bai et al. 2020)), 1 abstract (N/A (Chauhan et al. 2017))	Limited
Thyroid function	3 studies (3 low)	4 studies (1 acceptable, 3 low)	Insufficient	7 studies (3 high (Malin et al. 2018; Wang et al. 2020; Du et al. 2021), 4 acceptable (Kumar et al. 2018; Rathore et al. 2018; Shaik et al. 2019; Cui et al. 2020))	Limited
Other					
Arsenic methylation	No studies	No studies	N/A	1 study (high (Jiménez-Córdova et al. 2019))	Insufficient
General health	No studies	No studies	N/A	1 study (acceptable (Heck 2016))	Insufficient
Non-skeletal manifestations of fluoride toxicity	2 studies (2 low)	No studies	Insufficient	2 studies (acceptable (Shruthi and Anil 2018; Ayele et al. 2021))	Insufficient
Suicide	No studies	No studies	N/A	1 abstract (N/A (Stephenson et al. 2017))	Insufficient

[a]SR: Systematic review. [b]NR: Not reported. [c]Seven eye diseases were examined: pterygium, arteriosclerotic retinopathy, cataract, primary angle closure glaucoma, diabetic retinopathy, age-related macular degeneration, and strabismus.

(95%CI: 3.01, 4.92) of total, very mild, mild and moderate dental fluorosis, respectively (Zhou et al. 2019). The study by Kumar et al. (2018) 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) 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) 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 current literature search reported a wide range of fluoride concentrations ranging from 0.06 ppm in Brazil (Fernandes et al. 2020) to >4 ppm in Iran (Sabokseir et al. 2016).

Further to a study conducted in 2022 in Canada (McLaren et al. 2022) where the reported fluoride levels in tap water was $0.1-1.0$ ppm, other examples of fluoride concentrations relevant to the North American context were reported from Ireland: tap water, $0.6-1.0$ ppm (James et al. 2021), China: tap water, $0.89-0.91$ ppm (Xiang et al. 2016), Mexico: tap water, 1.22 ± 1.09 ppm (Soto-Barreras et al. 2019), and India: ground water, 0.67–0.83 (Rani et al. 2022), tap water, 1.1–2.92 (García-Escobar et al. 2022) and tap water, 1.27 ± 0.46 ppm (Kumar et al. 2018). Only 2 studies (Stangvaltaite-Mouhat et al. 2020; James et al. 2021) reported non-significant (possible) association between high drinking water fluoride (>6 ppm) and dental fluorosis.

Although no meta-analysis was conducted for the current review, an earlier review (Iheozor-Ejiofor et al. 2015) included a dose-response meta-analysis of 40 studies at high risk of bias (published up to that time). The results suggested that at 0.4 ppm fluoride, 10% of a population (95% CI: 6% to 15%) would be expected to have dental fluorosis of esthetic concern (defined as ≥ 3 TFI, ≥ 2 TSIF, or mild or worse DFI) [odds ratio= 2.90 (95% CI 2.05 to 4.10) for each 1 mg/L increase of fluoride exposure].

Current review 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.

Skeletal fluorosis. Earlier evidence on the association of fluoride with skeletal fluorosis was reported by NHMRC (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017) (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 (2019b) (two studies of low quality at Canadian CWF levels). Evidence was collectively reported by CADTH 2019 as insufficient to conclude an association. The current literature review search identified 3 cross-sectional studies with high/acceptable quality that were conducted in China (Pei et al. 2019), Ethiopia (Ayele et al. 2021), and India (Meghe et al. 2021) on individuals aged 10 years or older. Whereas only one study (Ayele et al. 2021) reported a positive association between fluoride exposure and skeletal fluorosis, the two other studies of acceptable quality reported a possible impact (Pei et al. 2019; Meghe et al. 2021). Reported ground water fluoride levels included a mean (SD) of 6.8 ppm (±4.3) in one study and a wide range of $\leq 1->4.0$ in another study. No water fluoride levels could be extracted, or extrapolated from the third study (Pei et al. 2019).

Current review evidence synthesis: Based on the available literature to date, there is limited evidence for an association of skeletal fluorosis with fluoride exposures relevant to North American drinking water.

Reduction in IQ score. Based on one systematic review and eleven studies (1 high, 2 acceptable, and 8 low quality), the NHMRC (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017) reported mixed findings regarding the association of fluoride exposure with lower IQ scores in children. A subsequent report by CADTH (2019a) identified a Canadian cohort study (Green et al. 2019) 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) 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 (2020) 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[2] (NTP-National Toxicology Program 2020) 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 (NTP-National Toxicology Program 2022) 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 current literature search identified 21 studies including 12 studies of high quality (Bashash et al. 2017; Cui et al. 2018; Mustafa et al. 2018; Yu et al. 2018; Till et al. 2020; Wang et al. 2020; Farmus et al. 2021; Wang et al. 2021; Yu et al. 2021; Zhao et al. 2021; Goodman et al. 2022; Saeed et al. 2022), and 5 studies of acceptable quality (Kousik and Mondal 2016; Mustafa et al. 2018; Yani et al. 2021; Feng et al. 2022; Kaur et al. 2022) that reported a positive/possible association between fluoride exposure and reduced IQ scores and school performance in children. Four studies of high (Heck 2016; Ibarluzea et al. 2022), moderate (Soto-Barreras et al. 2019), or low (Ahmad et al. 2022) quality reported the absence of an association with reducing IQ scores.

A number of cohort studies with high/acceptable quality examined the association between exposure to fluoride in drinking water and IQ score. A recent and high-quality analysis of critical time windows of exposure using the Canadian MIREC cohort (Farmus et al. 2021) 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. An earlier study that used the same cohort (Till et al. 2020) 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.

Results from a recent study (Goodman et al. 2022), which used data from the Mexican Cohort ELEMENT suggested 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. An earlier, high quality study (Bashash et al. 2017) that analyzed the same Mexican cohort reported a positive association of maternal exposure to fluoride during pregnancy with lower General Cognitive Index (GCI)/IQ scores in children at approximately 4 years old, and with lower Full-Scale IQ scores at 6–12 years old (Farmus et al. 2021). However, a fifth and recent study (Ibarluzea et al. 2022) examined prenatal fluoride exposure in a small mother-child birth cohort in Spain concluded that results in boys suggest improved scores in cognitive domains with maternal urinary concentrations.

Many cross-sectional studies provided evidence on an association between exposure to fluoride in drinking water and reduction in IQ score. A 2020 study (Wang et al. 2020) reported a significant IQ score reduction for each 1 mg/L increase in water fluoride concentration [β: −1.59 (−2.61, −0.57), $p = 0.002$]. An earlier study (Yu et al. 2018) 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). Another high quality study (Cui et al. 2018) reported an association with reduced IQ scores only in children carrying the dopamine receptor-2 (DRD2) Taq 1 A-TT genotype, with no similar association with the other DRD2 Taq 1 A genotypes. And finally, a cross-sectional study conducted by Kousik and Mondal (2016) reported a positive and significant correlation between exposure dose and IQ reduction ($r = -0.343$, $p < 0.01$).

These studies reported a reduction of IQ scores in association with water fluoride concentrations of 0.01–2.07 ppm (Mustafa et al. 2018), 0.58 ppm (Till et al. 2020), 0.1–1.6 ppm (Yani et al. 2021), 0.15–1.38 ppm (Bashash et al. 2017), 0.1–15.8 ppm (Saeed et al. 2022), 0.20–2.49 ppm (Cui et al. 2018), 0.20–3.90 ppm (Wang et al. 2021), >1.0 ppm (Feng et al. 2022),1.39 ppm (Wang et al. 2020), 1.53–2.84 ppm (Zhao et al. 2021), 2.0 ppm (Yu et al. 2018), 2.11 ppm (Kousik and Mondal 2016), 2–5 ppm (Kaur et al. 2022). Three studies with acceptable quality reported no effect of fluoride on children's IQ at fluoride exposures of 0.3–3.0 ppm (Heck 2016), 1.22 ppm ±1.09 (Soto-Barreras et al. 2019) and 2.04 ppm (Ahmad et al. 2022).

Current review evidence synthesis: Based on the available literature to date, the cumulative body of evidence suggests a positive association of reduced IQ scores for children and fluoride exposures relevant to current North American drinking water levels.

Thyroid dysfunction. Evidence on the association of fluoride with thyroid gland dysfunction was reported on by NHMRC (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017) (3 studies of low quality) and CADTH (2019b) (1 study of acceptable and three studies of low quality), which concluded mixed findings, flagging insufficient evidence for this association.

The current 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 (Kumar et al. 2018; Rathore et al. 2018; Shaik et al. 2019), 3 in China (Cui et al. 2020; Wang et al. 2020; Du et al. 2021), and 1 in Canada (Malin et al. 2018). Four studies of high (Wang et al. 2020; Du et al. 2021) or acceptable quality (Kumar et al. 2018; Rathore et al. 2018) reported a positive association with thyroid dysfunction, 1 study of high quality reported a possible association (Malin et al. 2018), and 1 study of acceptable quality that reported a non-significant association (Cui et al. 2020) with thyroid dysfunction. These studies identified disruption of thyroid hormones at water fluoride concentrations of 0.22 ppm (Malin et al. 2018), <1ppm (Rathore et al. 2018), 1.39 ppm (Wang et al. 2020), and 2.88 ppm (Kumar et al. 2018). A seventh study of acceptable quality reported no association between disruption of thyroid functions and drinking water fluoride levels (0.01–2.0 ppm) (Shaik et al. 2019).

Current review 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 North American drinking water levels.

Kidney dysfunction. There were no earlier studies identified in NHMRC (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017) that reported on the association of fluoride and kidney dysfunction. In 2019, the review by CADTH (2019b) identified a single study with low quality and concluded that there was insufficient evidence on the association between CWF and kidney dysfunction.

The current literature search identified 6 studies including 4 with high quality (Cárdenas-González et al. 2016; Jiménez-Córdova et al. 2018; 2019; Malin et al. 2019) and 2 with acceptable quality (Fernando et al. 2019; Nanayakkara et al. 2020), which examined the association of fluoride exposure with kidney dysfunction. Four out of these 6 studies reported results consistent with a possible association (Jiménez-Córdova et al. 2018; Fernando et al. 2019; Malin et al. 2019; Nanayakkara et al. 2020). The first study (Malin et al. 2019) 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 (Jiménez-Córdova et al. 2018) 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 (Nanayakkara et al. 2020) was conducted on men diagnosed with chronic kidney disease of unknown origin (CKDu), and concluded a possible association with serum fluoride. A fourth Sri Lanka-based study of case-control design with acceptable quality (Fernando et al. 2019) 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 (Malin et al. 2019), 1.33 ppm (Fernando et al. 2019), 1.5 ppm (Jiménez-Córdova et al. 2018) and 0.68 ppm (±0.48) (Nanayakkara et al. 2020).

One cross-sectional study with high quality was conducted on 5–12 years old Mexican school children (Jiménez-Córdova et al. 2019), and reported an inconclusive association with 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 (Cárdenas-González et al. 2016) at water fluoride concentration of 2.47 ppm.

Current review 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 North American drinking water levels.

Sex hormone disruptions. There were no earlier studies identified in NHMRC (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017) or CADTH (2019b) that reported on the association of fluoride exposure and disruption of male sex hormones. The current literature search identified 2 cross-sectional studies of high quality that examined US children and adolescents 6–19 years old (NHANES survey) (Bai et al. 2020), and male farmers from Henan Province in China (An et al. 2019). Results from the first study (Bai et al. 2020) indicated a gender- and age-specific inverse associations of fluoride in plasma and water with sex steroid hormones of total testosterone, estradiol and sex hormone binding globulin (SHBG) in U.S. children and adolescents, with a mean water fluoride level of 0.36 ppm (0.30 – 0.42). The second study (An et al. 2019) 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 0.37 ± 0.15 mg/L in the low exposure villages (LEG). The review also identified a relevant abstract (Chauhan et al. 2017) 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.

Current review 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 North American drinking water levels.

Other human endpoints. Based on the available literature to date, there is insufficient evidence to evaluate an association between fluoride exposures relevant to current North American drinking water levels and all-cause mortality, ADHD, dementia, memory loss, trouble working, headache, paresthesia, sleep-related outcomes, diabetes mellitus, liver dysfunction, ultrastructural kidney changes, abortion and female fertility, preterm births, genotoxicity, musculoskeletal pain, non-skeletal manifestations of fluoride toxicity, newborns' weight or newborns' height, childhood obesity, BMI, general health, errors of refraction or select eye diseases (pterygium, arteriosclerotic retinopathy, cataract, primary angle closure glaucoma, diabetic retinopathy, age-related macular degeneration, and strabismus), suicide or arsenic methylation.

A number of studies examining individual cardiovascular endpoints were reported in earlier reviews (Jack et al. 2016; CADTH 2019b) as well as by the current 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 investigation to properly assess the association of fluoride exposure with cardiovascular diseases. Based on the available literature to date, there is limited evidence for an association of fluoride exposures relevant to current North American drinking water levels with each of atherosclerosis and myocardial infarction. However, the evidence was deemed insufficient for hypertension.

Based on the available literature to date, there is consistent evidence of no association between fluoride exposures relevant to current North American drinking water levels and the overall incidence of cancer or cancer-related mortality, bone cancer or hip fracture. In the absence of new studies, the evidence remained limited for Down syndrome and kidney stones. The evidence was found to be inconsistent for

judging an association with bone quality. Detailed description of the evidence is provided in Supplementary Material 2.

Animal evidence

The full review of the animal evidence, with citations, is provided in Supplementary Material 4. An abbreviated summary is provided below on twelve primary endpoints, updating the evidence reported in two previous authoritative reviews of animal studies: Health Canada 2010 and the NTP 2020 draft report on neurocognitive outcomes. The emphasis of the review was on effects occurring at or below exposures (i.e. 20 ppm in animal studies) relevant to current fluoride levels in North American drinking water.

Neurological and cognitive outcomes. Summary based on the NTP 2020 draft report: NTP concluded that the evidence based was "inadequate" to assess whether exposure to fluoride could affect learning and memory. 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". 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". Current updated evidence synthesis: In the current review, a total of 3 low risk-of-bias studies with at least one test concentration $\leq$20 ppm (tier-1 study) and published since 2019 were identified. Although 1 study 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). Two other low risk of bias tier-1 studies found no significant effects below 20 ppm.

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. Current updated evidence synthesis: Two tier-1 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 did not find a significant association with thyroid hormone levels (TSH, T3, or T4); the other study reported statistically significant changes, though inconsistent across time points. Overall, the studies included in the current review suggest no or inconsistent evidence of thyroid dysfunction in animals exposed to fluoride in 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. Current updated evidence synthesis: Six low to medium risk of bias animal studies were identified that evaluated fluoride effects on kidney function at test concentrations 20 ppm or below. These studies investigated different exposure durations (chronic or sub-chronic) over a range of concentrations (from 0.05–150 mg/L). 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.

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. High quality multigeneration guideline studies did not find effects on reproductive function. Current updated evidence synthesis: Twelve low to medium risk-of-bias tier-1 studies were identified that evaluated adverse effects on reproductive system. 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. Overall, there was evidence of effects on male fertility, primarily decrease in sperm quality and increased testicular damage.

Cancer. Summary based on Health Canada 2010 report: 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. Current 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 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. Current updated evidence synthesis: Low risk-of-bias tier-1 studies were identified. One study reported no significant increase in any bone indexes in a male and female nephrotic mice model. Another study reported that fluoride in drinking water for 8 weeks did not induce any significant changes in bone mineral density or bone modeling. Another study reported an 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. Another study 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).

Diabetes or glucose or lipid metabolism related outcomes. Summary based on Health Canada 2010 report: No relevant animal evidence on diabetes, or any metabolism-related outcomes. Current updated evidence synthesis: Three lower risk-of-bias tier-1 studies were identified. One study reported that intake of fluoridated water from water supply (up to 15 ppm for 60 days) modified plasma insulin levels without affecting plasma glycemia in Sprague-Dawley rats. No change in glycemia, insulinemia, KITT, and HOMA2-IR were found in Wistar rats exposed to 10 ppm NaF for 22 days. In another study, 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 $\geq$ 22.5 mg/L) accompanied by increase in markers of oxidative stress such as superoxide dismutase, GSH peroxidase, and catalase. Current updated evidence synthesis: A single tier-1 study 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.

Respiratory outcomes. Summary based on Health Canada 2010 report: No animal evidence on respiratory outcomes were identified. Current 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. Current updated evidence synthesis: Two lower risk-of-bias tier-1 studies were identified. One study 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 15 ppm NaF exposure for 30 to 90 days. Another study 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.

Immune system related outcomes. Summary based on Health Canada 2010 report: No animal evidence on immunotoxicity was identified. Current updated evidence synthesis: Two low risk-of-bias tier-1 studies were identified. One study 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). Another study 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).

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." Current updated evidence synthesis: One lower risk-of-bias tier-1 study was identified. The study 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.

Intestinal outcomes. Summary based on Health Canada 2010 report: No animal evidence on intestinal outcomes was identified. Current updated evidence synthesis: No tier-1 or tier-2 studies were identified.

In vitro evidence

The full review of the *in vitro* evidence, with citations, is provided in Supplementary Material 6. An abbreviated summary is provided below. The goal of this review was a better understanding of the mechanisms of action of fluoride in exposed animals or humans.

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" (Barbier et al. 2010). Reactive Oxygen species (ROS) can be generated from a variety of exogenous and endogenous sources. 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 the cellular level, fluoride appears to induce oxidative stress, cell cycle arrest, and apoptosis through various pathways such as inhibition of metalloproteins, organelle disruption, altered pH, and electrolyte imbalance.

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.

Mitochondrial dysfunction. Mitochondrial dysfunction has been shown to contribute to the occurrence of apoptosis and it is central to the apoptotic pathway. Evidence shows that fluoride exposure induces apoptosis by regulating the mitochondrial pathway (decreased MMP and increased ROS) in H9C2 cardiomyocytes, human thyroid cells, and umbilical vein endothelial cells. Fluoride exposure can trigger apoptosis *via* increasing mRNA or protein levels of specific cell types.

Endoplasmic reticulum dysfunction. The endoplasmic reticulum is the main site for the folding and maturation of transmembrane, secretory, and ER-resident proteins. 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, and human thyroid follicular epithelial cells. 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 induce ER stress and unfolded protein response (UPR).

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. Studies using mice splenic lymphocytes show that fluoride exposure cause ER stress and UPR, 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.

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. Fluoride exposure inhibits the activity of Na, K-ATPase through multiple pathways. 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.

Inflammatory response. Inflammation is the immune system's response to an irritant, e.g. infection or tissue damage. Chronic inflammation plays an important role in the development of chronic conditions, such as diabetes, atherosclerosis, cardiovascular disease, allergies, and COPD. Studies have shown that fluoride exposure can promote inflammatory response *via* increasing oxidative stress and ROS in human umbilical vein endothelial cells, human monocytic line THP-1, and RAW 264.7 murine macrophage line. Fluoride-related phosphorylation of c-Jun NH (2)-terminal kinase (JNK) is involved in the pro-inflammatory response in the MDPC-23 odontoblast-like cells and human ameloblast lineage cells.

Weight of evidence for causality

Selection of critical endpoints

After consideration of all evidence identified in the current review, consolidated with earlier reviews from (Health Canada 2010), (CADTH 2019b, 2019a), (NTP-National Toxicology Program 2016), 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 relevant to the North American context. The four endpoints identified as candidates for most appropriate endpoint (other than dental fluorosis, for which causality is not in question) were examined using the Bradford Hill considerations (Hill 1965):

- Cognitive dysfunction (specifically, reduction in IQ scores in children)
- Thyroid dysfunction
- Kidney dysfunction
- Sex hormone alterations

Bradford Hill considerations

In weighing evidence of causality for each of the selected candidate endpoints, relevant human, animal (tier-1 and tier-2), and *in vitro* evidence was organized and evaluated along the nine Bradford Hill considerations (Hill 1965). Only evidence from original studies of high or acceptable quality was included in the evaluation of each endpoint.

To support each of the considerations, evidence was cited where available from the earlier comprehensive reviews (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017; CADTH 2019b, 2019a), and the current review. 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. Details of this weighing of evidence are detailed in full in Supplementary Material 7.

Reduction in IQ scores

NHMRC (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017) identified a study of acceptable quality (Rocha-Amador et al. 2007), which reported a statistically significant negative correlation between drinking water fluoride and IQ. The review also assessed a prospective study in New Zealand (Broadbent et al. 2015) that was mostly consistent with little to no effect of water fluoride on childhood IQ. CADTH (2019a) identified one study of acceptable quality (no association), and 5 studies of low quality (mixed findings). Seventeen studies of high or acceptable quality were identified, which reported a positive/possible association between reduced IQ scores and water fluoride (Kousik and Mondal 2016; Mustafa et al. 2018; Yu et al. 2018; Till et al. 2020; Wang et al. 2020; 2021; Yani et al. 2021; Yu et al. 2021; Zhao et al. 2021; Kaur et al. 2022; Saeed et al. 2022), or urinary fluoride levels (Bashash et al. 2017; Cui et al. 2018; Farmus et al. 2021; Feng et al. 2022; Goodman et al. 2022; Ibarluzea et al. 2022). Based on the available literature to date, the cumulative body of evidence suggests a positive association of reduced IQ scores for children and fluoride

exposures relevant to current North American 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 childhood IQ at fluoride exposures relevant to current North American drinking water levels. Focusing on high quality cohort studies, most of the evidence suggests a reduction in childhood IQ scores associated with fluoride levels, though results from one 2023 study in Spain (Ibarluzea et al. 2022) documented an improvement in specific cognitive domain scores in boys. Results from a 2015 study in New Zealand (Broadbent et al. 2015) that compared children living in fluoridated and non-fluoridated areas, was mostly consistent with little to no effect of fluoride on childhood IQ.

Fluoride appears to play a role in the induction of a range of health risks and is **not specific**. Although **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. 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 provided in Supplementary Material 7.

Thyroid dysfunction

CADTH (2019b) identified a study of acceptable quality (Barberio et al. 2017), which reported no association between drinking water fluoride and thyroid function. The current literature review eligible studies identified 5 studies of high or acceptable quality that reported a positive/possible association of thyroid dysfunction with water fluoride (Kumar et al. 2018; Malin et al. 2018; Rathore et al. 2018; Wang et al. 2020) or urinary fluoride levels (Du et al. 2021). (Cui et al. 2020) reported a non-significant association between thyroid dysfunction and urinary fluoride levels. A seventh study reported no association between thyroid dysfunction and water (Shaik et al. 2019). 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 North American 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 North American drinking water levels. Fluoride appears to play a role in the induction of a range of health risks 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 provided in Supplementary Material 7.

Kidney dysfunction

No studies from NHMRC 2016 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 current review identified four new studies (Jiménez-Córdova et al. 2018; Fernando et al. 2019; Malin et al. 2019; Nanayakkara et al. 2020) of high or acceptable quality that reported a possible association between water fluoride and kidney dysfunction. Two other studies reported either inconclusive (Jiménez-Córdova et al. 2019) or no association (Cárdenas-González et al. 2016). 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 North American 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 North American drinking water levels. The effects of fluoride appear to be **not specific** to one health effect. **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 provided in Supplementary Material 7.

Sex hormone disruptions

The current review identified 2 cross-sectional human studies (An et al. 2019; Bai et al. 2020) that reported a positive association and one abstract with insufficient data (Chauhan et al. 2017) 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 North American 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 (Hill

1965) was not strongly supportive of a causal association with fluoride in drinking water. Results from this assessment of the included studies are provided in Supplementary Material 7.

Dose-response assessment for critical endpoints

Point of departure for dental fluorosis

Identification of the key study

In its 2010 report (USEPA 2010) 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 using data from Dean (1942). 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. Only studies published after 2008 were further considered (earlier studies were reviewed by the US EPA). Studies included in two earlier reviews (Iheozor-Ejiofor et al. 2015; CADTH 2019b) and in the current review were found to be of variable risk of bias, particularly based on concerns for exposure assessment and potential confounding. 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 modeling of the effects of fluoride in drinking water. Based on the above considerations, including adequacy of dose-response data for modeling moderate dental fluorosis in a child or adolescent study population, no other candidate key studies were identified.

Dataset

The study by Dean (1942) was a cross-sectional study of 5,824 children, in 22 cities across 10 states of the U.S. The children were 9–14 years old or in grades 2–12, depending on the township where they resided (USEPA 2010)[3]. 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 method reported by Elvove (1933), which was derived from the mean of twelve-monthly samples.

Bayesian dose-response modeling

Although the US EPA selected severe dental fluorosis as the adverse endpoint of concern for deriving a POD, in the current work moderate dental fluorosis was selected as an important fluorosis category (defined as all tooth surfaces affected; marked wear on biting surfaces; and brown stain may be present), possibly leading to esthetic concerns and impacts on psychological wellbeing. Accordingly, the benchmark dose modeling of data from Dean was constructed by combining rates of moderate (DFI = 3) and severe (DFI = 4) dental fluorosis. A Bayesian framework was employed for the benchmark dose estimation using Benchmark BMD software (BBMD) developed by Shao and Shapiro (2018).

All 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 analysis. For the prior distributions for all parameters, the uniform distribution with the default lower and upper bounds were used. These default values were chosen based on the biological considerations (Shao and Shapiro 2018). Section 2 of the supplementary material from Shao and Shapiro (2018) provides more details on the remaining models.

Benchmark-dose modeling of added and extra risks

The objective of the dose-response analysis was to derive a 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 BMRs of 1%, 5%, and 10%. This 1–10% BMR range corresponds to the lower limit of risks that can typically be reliably estimated in exposure-response studies, and corresponds to values that have been considered by regulatory authorities (Benchmark dose technical guidance 2012; Hardy et al. 2017). BMRs are based on extra-risk rather than added-risk, as the BMD defined in terms of extra risk is always less than or equal to the BMD based on added risk. A more detailed description of the methods and results is provided in Supplementary Material 8.

Based on individual model results, estimated model weights, and fit statistics, the Hill model may be the single most plausible model to describe the dose-response relationship using the data from Dean (1942). However, considering results from sensitivity analyses, model averaging over log-logistic, log-Probit, and Hill models was nevertheless considered preferable. Table 3 provides estimated BMD and BMDL based on model averaging.

The model average benchmark dose for 1% extra-risk and the corresponding BMDL were estimated as 1.66 mg/L, and 1.56 mg/L, respectively. The BMDL estimates for the 1% BMR are between the NOAEL of 1.3 mg/L and the LOAEL of

Table 3. Estimated BMD and BMDL values by model averaging.

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

The extra-risk based BMRs are used.

1.8 mg/L for moderate dental fluorosis in the Dean study (the latter having a positivity rate of 1.2% in the study population). The model averaging estimates are similar to those derived under the Hill model alone, which had a model weight of 99.95%. Sensitivity analyses are described in Supplementary Material 8.

Point of departure for other candidate endpoints

Current health-based values for fluoride in drinking water are predominantly based on protecting against dental fluorosis. In considering updates to maximal allowable concentrations of fluoride, the current evidence synthesis also considered the merit of using points of departure for a selection of candidate endpoints for which there is sufficient evidence to be concerned about a causal effect due to fluoride ingestion. A systematic review of *in vitro*, animal, and epidemiologic evidence suggests a possible role of fluoride on epigenetic processes; although implications are not clear and the evidence is preliminary, Balasubramanian and Perumal (2022) identified studies that suggest correlation of fluoride exposure and various methylation, histone modification, non-coding RNA alterations affecting numerous genes.

Cognition, IQ

The body of evidence considered in the current review suggests a positive association of reduced IQ scores for children and fluoride exposures relevant to current North American drinking water levels. Using the 2022 NTP dose-response mean-effects meta-analysis (NTP-National Toxicology Program 2022) 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 draft NTP-National Toxicology Program (2022) also includes a mean effects meta-analysis, 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 draft NTP-National Toxicology Program (2022) 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 ($\beta = -4.77$; 95% CI: −9.09, −0.45) and −1.81 (−2.80, −0.81) for urinary fluoride.

Benchmark dose (BMD) modeling results have been recently published, based on high-quality birth cohort data. Grandjean et al. (2022) 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 (Green et al. 2019; Till et al. 2020) 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).

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 (Bashash et al. 2017; Green et al. 2019; Till et al. 2020) 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. (2022), 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 et al. (2022) 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 modeling by Grandjean et al. (2022) included 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, secondhand 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 modeling, 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 et al. (2022). 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 et al. (2022) 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 et al. (2022), 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. (2022) 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 (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,[4] 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:

$$\begin{aligned} F_{ing} &= [BMCL_{MUF} \text{x } 24-\text{hour urine volume}]/FUFE \\ &= [0.192 \text{ mg/L x } 1.07 \text{ L/d}]/0.75 \\ &= 0.274 \text{ mg/day} \end{aligned}$$

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

$$\begin{aligned} BMCL_{DW} &= F_{ing}/\text{water intake} \\ &= (0.274 \text{ mg/day})/(1.53 \text{ L/day}) \\ &= 0.179 \text{ mg F/L} \end{aligned}$$

(Villa et al. 2004; Grandjean et al. 2022)[5]

Grandjean et al. (2022) 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 et al. (2022), 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 current 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 North American drinking water contexts and effects on thyroid dysfunction. No epidemiologic study was considered adequate to derive a point of departure. In considering the animal stream of evidence, only two low risk of bias studies with dose-response information were considered relevant (Liu et al. 2016; McPherson et al. 2018). Out of these two rat chronic studies, one study did not find a change in thyroid 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 North American 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 subchronic), 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 disruptions

In the human stream of evidence, two low-risk of bias cross-sectional studies were identified. While considered low risk of bias in the OHAT scoring (Rooney et al. 2014; National Institute of Environmental Health Sciences 2019), cross-sectional studies were not considered adequate for consideration in deriving a point of departure. Recent animal studies identified in the current 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.

Discussion

Summary of results

The current review retained 89 original human studies that were conducted between 2016 and 2021, which were predominantly cross-sectional studies, with few cohorts and case-control studies. Examination of these studies identified evidence relating to 15 new endpoints, in addition to updating the earlier evidence on 14 endpoints that were reported in the earlier reviews (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017; CADTH 2019b, 2019a). The current search did not find any new evidence on 9 additional endpoints that were reported earlier. In addition to dental and skeletal fluorosis, four endpoints were flagged for a detailed evidence synthesis: reduction in intelligence quotient (IQ) scores in children, thyroid and kidney dysfunction, and sex hormone disruptions.

Using a tiered approach considering relevance and quality, 199 original animal studies were identified, examined and analyzed, leading to retention of 35 tier-1 studies in the current synthesis. These studies examined the effects 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 the cellular level 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 nonspecific to support particular biological mechanisms leading to specific health outcomes. Recent reviews also discuss the context of blood-brain barrier permeability to hydrogen fluoride, the role of altered pH, and the implications for neurotoxicity (Johnston and Strobel 2020).

NTP published updated draft reports in 2020 (NTP-National Toxicology Program 2020) and 2022 (NTP-National Toxicology Program 2022) following an independent review by the National Academy of Sciences, Engineering, and Medicine (NASEM) (NASEM-National Academies of Sciences 2021). 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.

Four high-quality studies utilized data from two significant cohorts: the Mexican ELEMENT cohort (Bashash et al. 2017; Goodman et al. 2022) (Cohort 2 A from 1997 to 1999 and Cohort 3 from 2001 to 2003) and the Canadian MIREC cohort (Till et al. 2020; Farmus et al. 2021) (from 2008 to 2011). These investigations involved 348 mother-child pairs (Goodman et al. 2022), 299 mother-child pairs (Bashash et al. 2017), 596 mother-child pairs (Farmus et al. 2021), and 398 mother-child pairs (Till et al. 2020). After accounting for major confounding factors, all these studies consistently reported a significant and measurable association between children's performance IQ and fluoride exposure during both the perinatal period and early childhood.

Both formula-fed and breastfed infants were affected by fluoride exposure, but the impact was more pronounced in formula-fed infants, even upon adjusting for fetal fluoride exposure (Till et al. 2020). Additionally, the timing of exposure appeared to differ by gender, with prenatal exposure potentially being more critical for boys, while infancy (beyond the first year) might be the more critical window of exposure for girls (Farmus et al. 2021). On the other hand, a recent study in Spain (Ibarluzea et al. 2022), involving a small mother-child birth cohort, found no association between prenatal fluoride exposure and cognitive functions and reported improved scores in boys for some cognitive domains; and a study in New Zealand (Broadbent et al. 2015), comparing children living in fluoridated and non-fluoridated areas, reported results mostly consistent with little to no effect of fluoride on childhood IQ.

There are several methodological aspects that differ among these cohort studies. Analyses did not provide adjustment for the same candidate confounders (for example, arsenic and lead in drinking water); studies did not assess IQ in children at the same ages; measurement of fluoride and water intake was undertaken with different methods or not at all; and different tools were used to assess cognitive IQ (including use of sub-domain measures). Although the preponderance of results supports the view that fluoride exposure at low levels is concerning, given some conflicting results in at least two follow-up studies and unclear results about the relevant period of exposure in boys and girls, there remains some uncertainty in the weight of evidence for causality.

Strengths, limitations, and data gaps

This review reflects a systematic search of evidence across multiple evidence streams that examined the possible association of fluoride in drinking water spanning a wide range of potential health risks. This evidence was acquired from 10 bibliographic databases, 6 clinical trial registries, and 18 major grey literature and web-based sources including national and international authoritative and technical health agencies, academic dissertations, major scientific hubs, and international conference proceedings.

The majority of studies across all evidence streams were of high or acceptable quality, as assessed by design-specific questions on the OHAT risk of bias tool. Characterization of exposure and ascertainment of outcomes were predominantly completed using reliable tools and qualified examiners, respectively. Studies were conducted in 20 countries, across all age groups, in both sexes, and among different races and ethnicities.

Evidence summaries for the examined human endpoints were based on the cumulative evidence identified in the current review, as well as in earlier reviews of human studies (Jack et al. 2016; NHMRC-National Health and Medical Research Council 2017; CADTH 2019b, 2019a). A weight-of-evidence synthesis combined human, animal, and *in vitro* streams, including summaries from earlier reviews of non-human evidence (Health Canada 2010; NTP-National Toxicology Program 2016), several authoritative reviews, and numerous published peer-reviewed systematic reviews. This synthesis was used to flag four endpoints of major concern based on considerations of the overall evidence and whether these effects were plausibly occurring at relevant exposure levels.

A major limitation of this review is that 79% of the retained studies were cross-sectional, which, despite showing high to acceptable methodological quality, present numerous challenges in interpretation concerning causality. Concerns of bias due to potential confounding were raised due to differences in reporting on covariates, the possibility of exposure to other sources of fluoride such as use of dental hygiene and care products, or exposure to burning coal, and whether studies accounted for other potential neurotoxicants in drinking water (such as arsenic).

Considerable diversity was identified across studies as to sources for exposure assessment, ranging from drinking water (households, community tap, city records), water intake, and fluoride content in serum and urine for children, adults and pregnant women. Furthermore, a wide range of fluoride concentration with different cutoff points among retained studies was also noticeable. Randomized controlled trials and large-scale cohort studies will help close the gap in our knowledge base for the effects of exposure to fluoride in drinking water and on health outcomes such as cognitive impairment, thyroid and kidney dysfunction, and sex hormones disruption for which uncertainty remains.

Derivation of exposure guidelines for fluoride in drinking water

The current evidence synthesis encompassed a thorough, multi-stream examination of the effects of exposure to fluoride in drinking water on human health risks. The ultimate goal of this review was to identify evidence with which current allowable concentrations of fluoride in drinking water may be revisited. To identify the most sensitive and most appropriate endpoint for setting human exposure guidelines, the review included considerations for deriving an appropriate POD, notably the availability of good quality data demonstrating a well-defined exposure-response relationship.

This examination involved the identification and assessment of quality of evidence from human, animal, and *in vitro* studies that had been published after the release of CADTH (2019b, 2019a), (NTP-National Toxicology Program 2016), and (Health Canada 2010). 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 evidence streams across the current review and the previous reviews was then examined using the Bradford Hill considerations (Hill 1965) for identification of credible causal adverse effects due to fluoride exposure.

In reconsidering allowable concentrations of fluoride in drinking water, a new POD was derived using benchmark dose modeling of moderate dental fluorosis in the Dean (1942) data. Furthermore, based on weight of evidence, four new endpoints were considered as credible candidates for most appropriate endpoint. While effects on sex hormones, thyroid dysfunction, and kidney dysfunction are potential human health risks of fluoride exposure through drinking water, with sufficient supporting evidence to warrant concern, no points of departure were derived because of inadequate data for dose-response modeling.

The totality of evidence identified to date supports the likelihood that there are causal effects on cognitive outcomes in children, at levels close to those currently seen in North American drinking water. However, among high quality cohort studies, some uncertainty remains in this interpretation. For the current assessment, the selection of the most appropriate endpoint nevertheless requires a comparison of the POD for moderate dental fluorosis and the POD for IQ effects. For both endpoints, the vulnerable population is young, school-aged children, though critical periods of exposure possibly differ (prenatal vs. early life).

First, the POD for moderate 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%. Second, based on the evidence to date, concern is warranted for fluoride having a possible effect on childhood IQ. There remains, however, some uncertainty in the causal weight of evidence for causality and significant uncertainty in the POD. The draft NTP-National Toxicology Program (2022) concluded that effects below 1.5 mg/L are unclear; however, benchmark dose analyses conducted by Grandjean et al. (2022) lead to lower POD values.

Different linear and non-linear models fit by Grandjean et al. (2022) 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 (National Research Council 2006), 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 guided by a better understanding of the mechanism of action of fluoride neurotoxicity.

Consideration should also be given to the severity of the two end points–moderate dental fluorosis and IQ reduction. 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 (Grosse et al. 2002), though more recent work has not necessarily supported these relationships (Aggeborn and Öhman 2021).

Although outside of the scope of the present work, the establishment of an HBV for fluoride in drinking water will require consideration of possible adjustment factors to be applied to any derived POD. Since the POD of 1.56 mg fluoride/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, the possibility of cognitive IQ effects in children–arguably a more severe adverse health outcome than moderate dental fluorosis–may be borne in mind when 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 fluoride/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 fluoride/L, an additional overall database uncertainty factor could be applied to this POD.

One of the challenges in evaluating the potential human health risks of fluoride is estimating risks at low levels of exposure. 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 most human epidemiological studies to date–demonstrated a shallower exposure-response relationship, with less evidence of the threshold-like behavior seen for dental fluorosis.

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 nonspecific 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, coupled with challenges in modeling low exposure epidemiologic data, the risk of possible effect on reducing IQ scores across relevant exposure ranges 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 (Adkins and Brunst 2021).

Future large epidemiologic studies should include follow-up from conception, standardized urine collection over several days, as well as consideration of all relevant confounders and effect modifiers pertinent to clarifying possible neurodevelopmental and cognitive effects. Greater understanding of the mode of action of how fluoride might induce neurodevelopmental effects would be of value in understanding the exposure-response curve at low concentrations.

Conclusion

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 for causality, in addition to dental fluorosis. These endpoints included reduction of IQ levels in children, thyroid dysfunction, kidney dysfunction, and sex hormone disruptions. The evidence supports a conclusion that fluoride exposure reduces IQ levels in children at concentrations close to those seen in North American drinking water, although there is some uncertainty in the weight of evidence for causality and considerable uncertainty in the point of departure. 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. Using moderate dental fluorosis as the most appropriate endpoint, a point of departure of 1.56 mg fluoride/L may be preferred as a starting point for setting a health-based guidance value for fluoride in drinking water.

Notes

1. An HBV is "the level of a contaminant that can be present in water and pose little or no health risk to a person drinking that water; these values do not take into account cost or technological challenges, and may be lower than drinking water guidelines, which take into account these and other practical considerations in risk management.
2. 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.

3. The US EPA (2010) 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.
4. Urine 24-hour volume Information | Mount Sinai–New York.
5. The derivation of the drinking water BMCL was based on Grandjean et al. 2022. Results from Villa et al. 2004, assume that all fluoride ingested is via drinking water. They reported for their participants, about 75% of fluoride could be attributed to drinking fluids (but food, drinks, and toothpaste were all controlled in the study, and the study was conducted in Chile, which may be less applicable to a North American population).

Acknowledgments

This manuscript is based on a report prepared by Risk Sciences International under a contract with Health Canada to review current evidence on the potential human health effects of fluoride to identify a point of departure for establishing a health-based value for fluoride in drinking water. The authors would like to acknowledge the comments that were provided by Health Canada on the original report, as well as technical comments by an independent third-party contractor (Tetratech). The evidence presented in this review was discussed by a six-member expert panel convened by Health Canada in June 2023, to provide recommendations on deriving an HBV for fluoride in drinking water. The authors would like to thank the journal Editor and three peer reviewers for helpful comments on the manuscript. Although all comments were carefully considered, the views and conclusions expressed are those of the authors alone.

Declaration of interest

All authors who contributed to the current systematic review report no conflict of interest existed at any stage of planning or preparation for this review, as well as the drafting, critical review, and approval of the aforementioned manuscript.

This work was requested by Health Canada under a competitive master standing offer agreement, which includes RSI as a provider of health risk assessment services. The contract report on which this manuscript is based was completed during the period from January 2020 to March 2023.

Supplemental material

Supplemental data for this article can be accessed online at https://doi.org/10.1080/10408444.2023.2295338.

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Appendix.

Appendix–Guide to supplementary material

In order to provide full documentation on the methods used in the conduct of this comprehensive review of the health effects of fluoride, additional details on the literature search, quality assessment, weight of evidence evaluation, and dose-response modeling is provided in supplementary material. The supplementary material is divided into eight sections, described briefly below. This guide to the detailed supplementary material is intended to direct readers to where they may find additional information on specific aspects of this review, to supplement that presented in the main manuscript.

Supplementary Material 1. Literature search for human and animal studies

This supplement describes the detailed search strategies across multiple bibliographic databases for human and animal studies published since Health Canada's 2010 monograph (Health Canada 2010) and CADTH's 2019 review (CADTH 2019b, 2019a), including grey literature search strategies.

Supplementary Material 2. Included human studies

This supplement expands on the summary tables of epidemiologic studies included in the main manuscript, providing comprehensive study details and quality of evidence summaries. Lists of all included studies, according to health endpoint, are provided.

Supplementary Material 3. Excluded human studies

This supplement lists all epidemiologic studies identified, but considered ineligible for the systematic review, with reasons provided for exclusion.

Supplementary Material 4. Included animal studies

This supplement lists all animal studies eligible for inclusion in the systematic review, categorized by endpoint and tier of relevance. Comprehensive study characteristics are provided, along with risk of bias assessments. To supplement the description of animal evidence in the main manuscript, a longer summary of currently available evidence is included.

Supplementary Material 5. Excluded animal studies

This supplement provides a list of animal studies identified but considered ineligible for the systematic review, with reasons provided for exclusion.

Supplementary Material 6. *In vitro* evidence

This supplement provides details of the search strategies for multiple bibliographic databases, with the resulting eligible reviews of *in vitro*

data on the health effects of fluoride. To supplement the description of *in vitro* evidence in the main manuscript, a longer summary is included.

Supplementary Material 7. Weight of evidence using Bradford Hill considerations for causality

This supplement details the discussion of Bradford Hill considerations pertaining to the four endpoints considered candidates for developing a point of departure in addition to dental fluorosis (specifically, reduction in IQ scores in children, thyroid dysfunction, kidney dysfunction, and sex hormone alterations). Additional details involved in assessing the weight of evidence for these effects is provided. Tables are included showing cited supporting studies considered in weight of evidence evaluations.

Supplementary Material 8. Point of departure derivation

This supplement provides further technical detail on the statistical derivation of the POD for dental fluorosis, the conversion of urinary benchmark concentrations to drinking water concentrations, and issues for consideration when selecting the most appropriate endpoint for setting a health-based value for fluoride in drinking water. A list of current international health-based values for fluoride in drinking water is also provided.