National Toxicology Program
U.S. Department of Health and Human Services

DRAFT NTP MONOGRAPH ON THE

SYSTEMATIC REVIEW OF FLUORIDE EXPOSURE AND NEURODEVELOPMENTAL AND COGNITIVE HEALTH EFFECTS

September 6, 2019

Office of Health Assessment and Translation
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

TABLES:

FIGURES:

DATA FIGURES:

RISK-OF-BIAS FIGURES:

ABSTRACT

Background: Previous reviews of epidemiological studies, including a 2006 evaluation by the National Research Council (NRC), found support for an association between consumption of high levels of naturally occurring fluoride in drinking water and neurological effects in humans and recommended further investigation (NRC 2006). Most of the evidence is from dental and skeletal fluorosis-endemic regions that have higher levels of naturally occurring fluoride than the fluoride concentrations historically added to water in community water fluoridation programs (0.7–1.2 ppm). NTP previously published a systematic review of the evidence from experimental animal studies of the effects of fluoride on learning and memory (NTP 2016). The systematic review found a low-to-moderate level of evidence that learning and memory deficits occur in non-human mammals exposed to fluoride. Studies in animals generally used fluoride drinking water concentrations that far exceeded the levels used in water fluoridation, and the lack of studies at lower fluoride concentrations was identified as a data gap. The evidence for effects on learning and memory was strongest (moderate) in animals exposed as adults, and evidence was weaker (low) in animals exposed during development. Since the publication of the NTP (2016) systematic review of the animal evidence, additional animal studies have been published. This systematic review extends the scope of the 2016 review by including human epidemiological studies, along with updated animal evidence and selected mechanistic information in order to reach hazard identification conclusions for fluoride and neurodevelopmental and cognitive effects.

Objective: To conduct a systematic review of the human, experimental animal, and mechanistic literature to evaluate the evidence and develop hazard conclusions about whether fluoride exposure is associated with neurodevelopmental and cognitive effects.

Method: A systematic review protocol was developed and utilized following the Office of Health Assessment and Translation (OHAT) approach for conducting literature-based health assessments.

Results: The literature search and screening process identified 149 published human studies, 339 published experimental animal studies, and 60 in vitro/mechanistic studies relevant to the objective. Eighty-two of the 149 human studies evaluated the association between fluoride exposure and neurodevelopmental or cognitive effects, and the remaining human studies evaluated thyroid effects or other potential mechanistic data. The majority of the experimental animal studies were mechanistic studies, which were not assessed in the NTP (2016) report. Thirty-five new experimental animal[1] studies evaluating effects on learning and memory and/or motor activity and sensory effects of fluoride were identified since the NTP (2016) systematic review.

The human body of evidence provides a consistent pattern of findings that high fluoride exposure is associated with decreased intelligence quotient (IQ) in children. There is a moderate level of evidence from cognitive neurodevelopmental studies in children based on four prospective cohort studies and nine cross-sectional studies that are considered functionally prospective in nature. Because the majority of available studies evaluate cognitive neurodevelopmental effects in children, the focus of the hazard conclusions is on cognitive neurodevelopmental effects. The evidence for cognitive effects in adults is limited, coming from two cross-sectional studies, and is inadequate to evaluate whether fluoride exposure in adults is associated with cognitive effects. The assessment of the new animal data focuses

[1] The term “animal” in this document refers to non-human mammals.

on evaluating a deficiency identified during the prior NTP (2016) review concerning the difficulty in distinguishing potential effects of fluoride on motor and sensory functions from effects specifically on learning and memory functions. Further examination of the animal data, including studies carried out at the NTP, have further highlighted this deficiency in the animal studies. For this reason, the animal body of evidence is now considered inadequate to inform conclusions on whether fluoride exposure is associated with cognitive effects (including cognitive neurodevelopmental effects) in humans primarily due to the inability to separate these effects from the other effects on the nervous system, including motor activity or motor coordination. While the animal data provide evidence of effects of fluoride on neurodevelopment, the human evidence base is primarily focused on cognitive neurodevelopmental effects and is the focus of conclusions.

Conclusions: NTP concludes that fluoride is *presumed to be a cognitive neurodevelopmental hazard to humans*. This conclusion is based on a consistent pattern of findings in human studies across several different populations showing that higher fluoride exposure is associated with decreased IQ or other cognitive impairments in children. However, the consistency is based primarily on higher levels of fluoride exposure (i.e., >1.5 ppm in drinking water). When focusing on findings from studies with exposures in ranges typically found in the United States (i.e., approximately 0.03 to 1.5 ppm in drinking water, NHANES (Jain 2017)) that can be evaluated for dose response, effects on cognitive neurodevelopment are inconsistent, and therefore unclear. There is inadequate evidence to determine whether fluoride exposure lowers IQ or impairs cognitive function in adults. There are few human studies available that provide data to evaluate whether fluoride exposure is associated with other neurodevelopmental effects, beyond IQ or other cognitive measures. Although conclusions were reached by integrating evidence from human and animal studies with consideration of relevant mechanistic data, the conclusions are based primarily on the human evidence. The evidence from animal studies is inadequate to inform conclusions on cognitive effects, and the mechanisms underlying fluoride-associated cognitive neurodevelopmental effects are not well characterized.

INTRODUCTION

The NTP's Office of Health Assessment and Translation (OHAT) conducted a systematic review to evaluate the evidence that exposure to fluoride is associated with neurodevelopmental or cognitive effects. This review was initiated in response to a nomination from the Fluoride Action Network. There are numerous human and animal studies reporting neurodevelopmental and cognitive health effects of exposure to excess fluoride. As noted by the National Research Council (NRC) in their 2006 report, although the studies lacked sufficient detail to fully assess their quality and relevance to the U.S. populations, the consistency of the results suggesting that fluoride may be neurotoxic warrants additional research (NRC 2006).

Fluoride salts are added to community water systems and dental products in the United States (e.g., toothpaste, mouth rinses, and supplements) for the prevention of dental caries. Approximately 67% of the U.S. population receives fluoridated water through a community drinking water system (CDC 2013). In other countries fluoride supplementation has been achieved by fluoridating food products such as salt, or milk. Fluoride supplementation has been recommended to prevent bone fractures (Jones *et al.* 2005). Fluoride also can occur naturally in drinking water. Other sources of human exposure include other foods and beverages, industrial emissions, pharmaceuticals, and pesticides (e.g., cryolite, sulfuryl fluoride). Soil ingestion is another source of fluoride exposure in young children (US EPA 2010).

The U.S. Public Health Service (PHS) first recommended communities add fluoride to drinking water in 1962. PHS guidance is advisory, not regulatory, which means that while PHS recommends community water fluoridation as a public health intervention, the decision to fluoridate water systems is made by state and local governments.[2] For community water systems that add fluoride, PHS now recommends a fluoride concentration of 0.7 milligrams/liter (mg/L). Under the Safe Drinking Water Act, the U.S. Environmental Protection Agency (EPA) sets maximum exposure level standards for drinking water quality. The current enforceable drinking water standard for fluoride, or the maximum contaminant level goal (MCLG, a concentration at which no adverse health effects are expected), is 4.0 mg/L. This is the maximum amount of fluoride contamination (naturally occurring not from water fluoridation) that is allowed in water from public water systems; it is set to protect against increased risk of skeletal fluorosis, a condition characterized by pain and tenderness of the major joints. EPA also has a non-enforceable secondary drinking water standard of 2.0 mg/L, which is recommended to protect children against the tooth discoloration and/or pitting that can be caused by severe dental fluorosis during the formative period prior to eruption of the teeth. Although the secondary standard is not enforceable, EPA does require that public water systems notify the public if the average levels exceed it (NRC 2006).

Controversy over community water fluoridation stems from concerns about the potential harmful effects of fluoride and the ethics of water fluoridation. Commonly cited health concerns related to fluoride are bone fractures and skeletal fluorosis, decreased intelligence quotient (IQ) and other neurological effects, cancer, and endocrine disruption. Effects on neurological function, endocrine function (e.g., thyroid, parathyroid, pineal), metabolic function (e.g., glucose metabolism), and carcinogenicity were assessed in the 2006 NRC report Fluoride in Drinking Water: A Scientific Review of EPA's Standards (NRC 2006). The NRC review considered adverse effects of water fluoride, focusing on a range of concentrations (2–4 mg/L) above the current 0.7-mg/L recommendation for community water

[2] For many years, most fluoridated community water systems used fluoride concentrations ranging from 0.8 to 1.2 mg/L (US DHHS 2015)

fluoridation (NRC 2006). The NRC report concluded that the current MCLG should be lowered to protect against severe enamel fluorosis and to reduce the risk of bone fractures associated with skeletal fluorosis (NRC 2006). Other than severe fluorosis, the NRC did not find sufficient evidence of negative health effects at fluoride levels below 4.0 mg/L; however, the NRC concluded that the consistency of the results of IQ deficits in children exposed to fluoride at 2.5 to 4 mg/L in drinking water from a few epidemiological studies of Chinese populations appeared significant enough to warrant additional research on the effects of fluoride on intelligence. The conclusions from the NRC review were the primary source of information for the potential hazard summary in a 2015 report by the U.S. Department of Health and Human Services (DHHS), Federal Panel on Community Water Fluoridation. The NRC report noted several challenges to evaluating the literature, citing deficiencies in reporting quality, lack of consideration of all sources of fluoride exposure, incomplete consideration of potential confounding, selection of inappropriate control subject populations in epidemiological studies, absence of demonstrated clinical significance of reported endocrine effects, and incomplete understanding of the biological relationship between histological, biochemical, and molecular alterations with behavioral effects (NRC 2006).

In 2016, NTP conducted a systematic review of the evidence from experimental animal studies on the potential effects of fluoride exposure on learning and memory (NTP 2016). The NTP (2016) systematic review found a low-to-moderate level of evidence that learning and memory deficits occur in experimental animals exposed to fluoride. Based on the findings in NTP (2016), NTP decided to conduct additional animal studies before carrying out a full systematic review to incorporate human, animal, and potentially relevant mechanistic evidence in order to reach hazard identification conclusions for fluoride and learning and memory effects. As the NTP (2016) report focused on learning and memory and developed confidence ratings for bodies of evidence by life stage of exposure (i.e., exposure during development or adulthood), this report also evaluates two different age groups (i.e., children and adults) with a focus on cognitive neurodevelopmental effects in children and cognitive effects in adults in order to address potential differences in the health impact based on timeframe of exposure (i.e., during development or during adulthood). This evaluation has been conducted separately from the 2016 assessment, but like the 2016 assessment, it has assessed mainly learning and memory effects in experimental animal studies to support the assessment of cognitive neurodevelopmental effects in children and cognitive effects in adults.

OBJECTIVE AND SPECIFIC AIMS

Objective

The overall objective of this evaluation is to undertake a systematic review to develop NTP hazard identification conclusions on the association between exposure to fluoride and neurodevelopmental and cognitive effects based on integrating levels of evidence from human and non-human animal studies with consideration of the degree of support from mechanistic data.

Specific Aims

- Identify literature that assessed neurodevelopmental and cognitive health effects, especially outcomes related to learning, memory, and intelligence, following exposure to fluoride in human, animal, and relevant in vitro/mechanistic studies.
- Extract data on potential neurodevelopmental and cognitive health effects from relevant studies.

- Assess the internal validity (risk of bias) of individual studies using pre-defined criteria.
- Assess effects on thyroid function to help evaluate potential mechanisms of impaired neurological function.
- Summarize the extent and types of health effects evidence available.
- Describe limitations of the systematic review, limitations of the evidence base, identify areas of uncertainty, as well as data gaps and research needs for neurodevelopmental and cognitive health effects of fluoride.

Dependent on the extent and nature of the available evidence:

- Synthesize the evidence using a narrative approach or meta-analysis (if appropriate) considering limitations on data integration such as study design heterogeneity.
- Rate confidence in the body of evidence for human and animal studies separately according to one of four statements: High, Moderate, Low, or Very Low/No Evidence Available.
- Translate confidence ratings into level of evidence of health effects for human and animal studies separately according to one of four statements: High, Moderate, Low, or Inadequate.
- Combine the level of evidence ratings for human and animal data to reach one of five possible hazard identification conclusions: Known, Presumed, Suspected, Not classifiable, or Not identified to be a hazard to humans.

METHODS

Problem Formulation and Protocol Development

The research question and specific aims stated above were developed and refined through a series of problem formulation steps including: (1) nomination received from the public in June 2015 to conduct analyses of fluoride and developmental neurobehavioral toxicity; (2) analysis of the amount of evidence available and the merit of pursuing systematic reviews, given factors such as the extent of new research published since previous evaluations and whether these new reports address or correct the deficiencies noted in the literature (NRC 2006, OEHHA 2011, SCHER 2011); (3) comments on the draft PECO statement from the NTP Board of Scientific Counselors during its December 1–2, 2015 meeting; and (4) review of the draft protocol by technical advisors. NTP published a systematic review of the animal evidence on the effects of fluoride on learning and memory (NTP 2016). NTP has conducted additional studies in animals to assess the effect of fluoride exposure on learning and memory. The results from this experimental animal work were published (McPherson *et al.* 2018) and are incorporated into the current review, which considers the epidemiological, animal, and mechanistic evidence in its conclusions. The protocol for the systematic review was posted in June 2017 (https://ntp.niehs.nih.gov/go/785076) and used to conduct this review. A brief summary of the methods is presented below.

PECO Statements

PECO (**P**opulation, **E**xposure, **C**omparators and **O**utcomes) statements were developed as an aid to identify search terms and inclusion/exclusion criteria as appropriate for addressing the overall research question (effects on neurodevelopmental or cognitive function and thyroid associated with fluoride exposure) for the systematic review (Higgins and Green 2011).The PECO statements are listed below for human, animal, and in vitro/mechanistic studies (see **Table 1**, **Table 2**, and **Table 3**).

Using the PECO statements, the evaluation searched for evidence of neurodevelopmental or cognitive function, and thyroid effects associated with fluoride exposure from human studies, controlled exposure animal studies, and mechanistic/in vitro studies. Mechanistic data can come from a wide variety of studies that are not intended to identify a disease phenotype. This source of experimental data includes in vitro and in vivo laboratory studies directed at cellular, biochemical, and molecular mechanisms that attempt to explain how a substance produces particular adverse health effects. The mechanistic data were first organized by general categories (e.g., biochemical effects in the brain and neurons, neurotransmitters, oxidative stress, etc.) to evaluate the information available. Categories focused on were those with more robust data at levels of fluoride more relevant to human exposure. The intent was not to develop a mechanism for fluoride induction of learning and memory effects, but to evaluate if there is biological plausibility for the effects observed in the low-dose region (below approximate drinking water equivalent concentrations of 20 ppm) that may support the hazard conclusion.

Table 1. Human PECO (Population, Exposure, Comparator and Outcome) Statement

PECO Element	Evidence
Population	Humans without restriction as to age or sex, geographic location, or life stage at exposure or outcome assessment
Exposure	Exposure to fluoride based on administered dose or concentration, biomonitoring data (e.g., urine, blood, other specimens), environmental measures (e.g., air, water levels), or job title or residence. Relevant forms are those used as additives for water fluoridation: • Fluorosilicic acid (also called hydrofluorosilicate; CASRN 16961-83-4) • Sodium hexafluorosilicate (also called disodium hexafluorosilicate or sodium fluorosilicate; CASRN 16893-85-9) • Sodium fluoride (CASRN 7681-49-4) • Other forms of fluoride that readily dissociate into free fluoride ions (e.g., potassium fluoride, calcium fluoride, ammonium fluoride)
Comparators	Comparable populations not exposed to fluoride or exposed to lower levels of fluoride (e.g., exposure below detection levels)
Outcomes	Neurodevelopmental outcomes including learning, memory, intelligence, other forms of cognitive behavior, other neurological outcomes (e.g., anxiety, aggression, motor activity), and biochemical changes in the brain or nervous system tissue; or measures of thyroid function, biochemical changes, or thyroid tissue

Table 2. Animal PECO Statement	
PECO Element	**Evidence**
Population	Non-human mammalian animal species (whole organism)
Exposure	Exposure to fluoride based on administered dose or concentration, and biomonitoring data (e.g., urine, blood, other specimens). Relevant forms are those used as additives for water fluoridation: • Fluorosilicic acid (also called hydrofluorosilicate; CASRN 16961-83-4) • Sodium hexafluorosilicate (also called disodium hexafluorosilicate or sodium fluorosilicate; CASRN 16893-85-9) • Sodium fluoride (CASRN 7681-49-4) • Other forms of fluoride that readily dissociate into free fluoride ions (e.g., potassium fluoride, calcium fluoride, ammonium fluoride)
Comparators	Comparable animals that were untreated or exposed to vehicle-only treatment
Outcomes	Neurodevelopmental outcomes including learning, memory, intelligence, other forms of cognitive behavior, other neurological outcomes (e.g., anxiety, aggression, motor activity), and biochemical changes in the brain or nervous system tissue; or measures of thyroid function, biochemical changes, or thyroid tissue

Table 3. In Vitro/Mechanistic PECO Statement	
PECO Element	**Evidence**
Population	Human or animal cells, tissues or biochemical reactions (e.g., ligand binding assays)
Exposure	Exposure to fluoride based on administered dose or concentration. Relevant forms are those used as additives for water fluoridation: • Fluorosilicic acid (also called hydrofluorosilicate; CASRN 16961-83-4) • Sodium hexafluorosilicate (also called disodium hexafluorosilicate or sodium fluorosilicate; CASRN 16893-85-9) • Sodium fluoride (CASRN 7681-49-4) • Other forms of fluoride that readily dissociate into free fluoride ions (e.g., potassium fluoride, calcium fluoride, ammonium fluoride)
Comparators	Comparable cells or tissues that were untreated or exposed to vehicle-only treatment
Outcomes	Endpoints related to neurological and thyroid function, including neuronal electrophysiology; mRNA, gene, or protein expression; cell proliferation or death in brain or thyroid tissue/cells; neuronal signaling; synaptogenesis, etc.

Literature Search

Search terms were developed to identify all relevant published evidence on developmental neurobehavioral toxicity or thyroid-related health effects potentially associated with exposure to fluoride by reviewing Medical Subject Headings for relevant and appropriate neurobehavioral and thyroid-related terms, and by extracting key neurological and thyroid-related health effects and developmental neurobehavioral terminology from reviews and a sample of relevant primary data

studies. A combination of relevant subject headings and keywords were subsequently identified. A test set of relevant studies was used to ensure the search terms retrieve 100% of the test set. The following six electronic databases were searched using a search strategy tailored for each database (specific search terms used for the PubMed search are presented in **Appendix 1**; the search strategy for other databases are available in the protocol (https://ntp.niehs.nih.gov/go/785076). No language restrictions or publication year limits were imposed, and databases were searched in December 2016. The search was updated during the review process through April 1, 2019, and these publications are categorized as "references identified through database searches" in **Figure 4**. To complete evaluations, there must be a cutoff date on new studies while balancing consideration of new information. Therefore, databases were searched and screened manually up to release dates of draft documents (August 20, 2019 for this document). If studies were identified that might impact conclusions, they were considered under "references identified through other sources" in **Figure 4**. Literature searches for this systematic review were conducted independent of the literature search conducted for the NTP (2016) report using a similar strategy. As relevant animal studies published prior to 2015 were identified in the NTP (2016) assessment, the focus of the literature searches for this systematic review was to identify relevant animal studies that were published since completion of the literature searches for the NTP (2016) assessment.

Databases Searched

- BIOSIS (Thomson Reuters)
- EMBASE
- PsycINFO (APA PsycNet)
- PubMed (NLM)
- Scopus (Elsevier)
- Web of Science (Thomson Reuters, Web of Science indexes the journal Fluoride)

Searching Other Resources

The reference lists of all included studies; relevant reviews, editorials and commentaries; and the Fluoride Action Network website (http://fluoridealert.org/) were manually searched for additional relevant publications.

Unpublished Data

Unpublished data were eligible for inclusion provided the owner of the data was willing to have the data made public and peer reviewed (see protocol for more details https://ntp.niehs.nih.gov/go/785076). No unpublished data were identified during the literature search.

Study Selection

Evidence Selection Criteria

In order to be eligible for inclusion, studies had to satisfy eligibility criteria that reflect the PECO statement in **Table 1**, **Table 2**, and **Table 3**. The following additional exclusion criteria were applied:

(1) Case studies and case reports.

(2) Articles without original data (e.g., reviews, editorials, or commentaries). Reference lists from these materials, however, were reviewed to identify potentially relevant studies not identified from the database searches. These studies were assessed for eligibility for inclusion.

(3) Conference abstracts or reports.

Screening Process

References retrieved from the literature search were screened for relevance and eligibility against the evidence selection criteria using a structured form in SWIFT-Active Screener, a machine-learning software program used to priority-rank studies for screening. SWIFT-Active Screener employs active learning to incorporate user feedback during the screening process to refine a statistical model that continually ranks the remaining studies according to their likelihood for inclusion. In addition, the software includes a statistical algorithm to estimate predicted recall (percent of truly relevant studies identified) while users work, thus providing a statistical basis for a decision about when to stop screening (Miller *et al.* 2016). The title and abstract screen was stopped once the statistical algorithm in SWIFT-Active Screener estimated ≥98% predicted recall. References were independently screened by two trained screeners at the title and abstract level to determine whether a reference met the evidence selection criteria. For citations with no abstract or non-English abstracts, articles were screened based on title relevance (title would need to indicate clear relevance); number of pages (articles ≤2 pages long were assumed to be conference reports, editorials, or letters unlikely to contain original data); and/or PubMed Medical Subject Headings (MeSH).

Studies that were not excluded during the title and abstract screening were further screened for inclusion with a full-text review by two independent reviewers using DistillerSR® by Evidence Partners, a web-based, systematic-review software program with structured forms and procedures to ensure standardization of the process. Screening conflicts were resolved through discussion and consultation with technical advisor(s), if necessary. During full-text review, studies that were considered relevant were tagged to the appropriate evidence streams (i.e., human, animal, and/or in vitro). Studies tagged to human or animal evidence streams were also categorized by outcome as primary neurodevelopmental or cognitive outcomes (learning, memory, and intelligence); secondary neurobehavioral outcomes (anxiety, aggression, motor activity, or biochemical); or related to thyroid effects. In vitro data were tagged as being related to neurological effects or thyroid effects. Translation assistance was sought to assess the relevance of non-English studies. Following full-text review, the remaining studies were "included" and used for the evaluation.

Data Extraction

Data were collected (i.e., extracted) from included studies by one member of the evaluation team and checked by a second member of the team for completeness and accuracy. Any discrepancies in data extraction were resolved by discussion or consultation with a third member of the evaluation team.

Data extraction was completed using the Health Assessment Workspace Collaborative (HAWC), an open source and freely available web-based interface application.[3] Data extraction elements are listed separately for human, animal, and in vitro studies in the protocol (https://ntp.niehs.nih.gov/go/785076). Data for primary and secondary outcomes as well as thyroid hormone level data were extracted from human studies. Studies evaluating only goiters or thyroid size were not extracted. All primary outcomes and functional neurological secondary outcomes (e.g., motor activity) were extracted from animal studies identified since the NTP (2016) report. For animal mechanistic data, studies were tiered based on exposure dose (with preference given to fluoride drinking water equivalent exposures, which were calculated using the method described in the NTP (2016) report) of 20 ppm or less as deemed most

[3] HAWC (Health Assessment Workspace Collaborative): A Modular Web-based Interface to Facilitate Development of Human Health Assessments of Chemicals (https://hawcproject.org/portal/).

relevant to exposures in humans), exposure duration or relevant time window (i.e., developmental), exposure route (with preference given to oral exposures over injection exposures), and commonality of mechanism (e.g., inflammation, oxidative stress, changes in neurotransmitters, and histopathological changes were considered pockets of mechanistic data). Data were not extracted from in vitro studies; however, these studies were evaluated and summarized for biological plausibility of the human and animal results. Thyroid data were also reviewed but not extracted. The data extraction results for included studies are publicly available and can be downloaded in Excel format through HAWC (https://hawcproject.org/assessment/405/). Methods for transforming and standardizing dose levels and results from behavioral tests in experimental animals are detailed in the protocol (https://ntp.niehs.nih.gov/go/785076).

Quality Assessment of Individual Studies

Risk of bias was assessed for individual studies using a tool developed by OHAT that outlines a parallel approach to evaluating risk of bias from human, animal, and mechanistic studies to facilitate consideration of risk of bias across evidence streams with common terms and categories. The risk-of-bias tool is comprised of a common set of 11 questions that are answered based on the specific details of individual studies to develop risk-of-bias ratings for each question. Study design determines the subset of questions used to assess risk of bias for an individual study (see **Table 4**).

Assessors were trained with an initial pilot phase undertaken to improve clarity of rating criteria and to improve consistency among assessors. Studies were independently evaluated by two trained assessors who answered all applicable risk-of-bias questions with one of four options in **Table 5** following pre-specified criteria detailed in the protocol (https://ntp.niehs.nih.gov/go/785076). The criteria describe aspects of study design, conduct, and reporting required to reach risk-of-bias ratings for each question and specify factors that can distinguish among ratings (e.g., what separates “definitely low” from “probably low” risk of bias).

Key Risk-of-bias Questions

In the OHAT approach, some risk-of-bias questions or elements are considered potentially more important when assessing studies because there is more empirical evidence that these areas of bias have a greater impact on estimates of the effect size or because these issues are generally considered to have a greater effect on the credibility of study results in environmental health studies (Rooney *et al.* 2016). There were three Key Questions for observational human studies: confounding, exposure characterization, and outcome assessment. There were also three Key Questions for experimental animal studies: randomization, exposure characterization, and outcome assessment. In addition, for animal developmental studies, failure to consider the litter as the unit of analysis was also a key risk-of-bias concern. When there was not enough information to assess the potential bias for a risk-of-bias question and authors did not respond to an inquiry for further information, a conservative approach was followed, and the studies were rated probably high risk of bias for that question.

Table 4. OHAT Risk-of-bias Questions and Applicability by Study Design						
Risk-of-bias Questions	**Experimental Animal***	**Human Controlled Trials****	**Cohort**	**Case-Control**	**Cross-Sectional*****	**Case Series**
1. Was administered dose or exposure level adequately randomized?	X	X				
2. Was allocation to study groups adequately concealed?	X	X				
3. Did selection of study participants result in the appropriate comparison groups?			X	X	X	
4. Did study design or analysis account for important confounding and modifying variables?			X	X	X	X
5. Were experimental conditions identical across study groups?	X					
6. Were research personnel blinded to the study group during the study?	X	X				
7. Were outcome data complete without attrition or exclusion from analysis?	X	X	X	X	X	
8. Can we be confident in the exposure characterization?	X	X	X	X	X	X
9. Can we be confident in the outcome assessment (including blinding of outcome assessors)?	X	X	X	X	X	X
10. Were all measured outcomes reported?	X	X	X	X	X	X
11. Were there no other potential threats to internal validity?	X	X	X	X	X	X

*Experimental animal studies are controlled exposure studies. Non-human animal observational studies can be evaluated using the design features of observational human studies such as cross-sectional study design.

**Human Controlled Trials are studies in humans with controlled exposure (e.g., Randomized Controlled Trials, non-randomized experimental studies)

***Cross-sectional studies include population surveys with individual data (e.g., NHANES) and surveys with aggregate data (i.e., ecological studies).

Any discrepancies in ratings between assessors were resolved by a senior technical specialist and through discussion when necessary to reach the final recorded risk-of-bias rating for each question along with a statement of the basis for that rating. Members of the evaluation team were consulted for assistance if additional expertise was necessary to reach final risk-of-bias ratings based on specific aspects of study design or performance reported for individual studies. Study procedures that were not reported were assumed not to have been conducted, resulting in an assessment of "probably high" risk of bias. Authors were queried by email to obtain missing information and responses received were used to update risk-of-bias ratings.

Table 5. The Four Risk-of-bias Rating Options

Answers to the risk-of-bias questions result in one of the following four risk-of-bias ratings	
++	***Definitely Low*** *risk of bias*: There is direct evidence of low risk-of-bias practices
+	***Probably Low*** *risk of bias*: There is indirect evidence of low risk-of-bias practices **OR** it is deemed that deviations from low risk-of-bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias
– NR	***Probably High*** *risk of bias*: There is indirect evidence of high risk-of-bias practices (indicated with "-") **OR** there is insufficient information provided about relevant risk-of-bias practices (indicated with "NR" for not reported). Both symbols indicate probably high risk of bias.
––	***Definitely High*** *risk of bias*: There is direct evidence of high risk-of-bias practices

Organizing and Rating Confidence in Bodies of Evidence

Health Outcome Categories for Neurodevelopmental and Cognitive Effects

After data were extracted from all studies, the health effects results within the category of neurodevelopmental or cognitive effects were grouped across studies to develop bodies of evidence or collections of studies with data on the same or related outcomes. The vast majority of the human studies evaluated intelligence quotient (IQ) in children as the single outcome; therefore, the discussion of cognitive neurodevelopmental effects in children focuses on IQ studies with supporting information from data on other endpoints. Cognitive function in adults was evaluated separately. Consistent with the NTP (2016) assessment, the primary focus within the animal study body of evidence was on animal studies with endpoints related to learning and memory.

Considerations for Pursuing a Narrative or Quantitative Evidence Synthesis

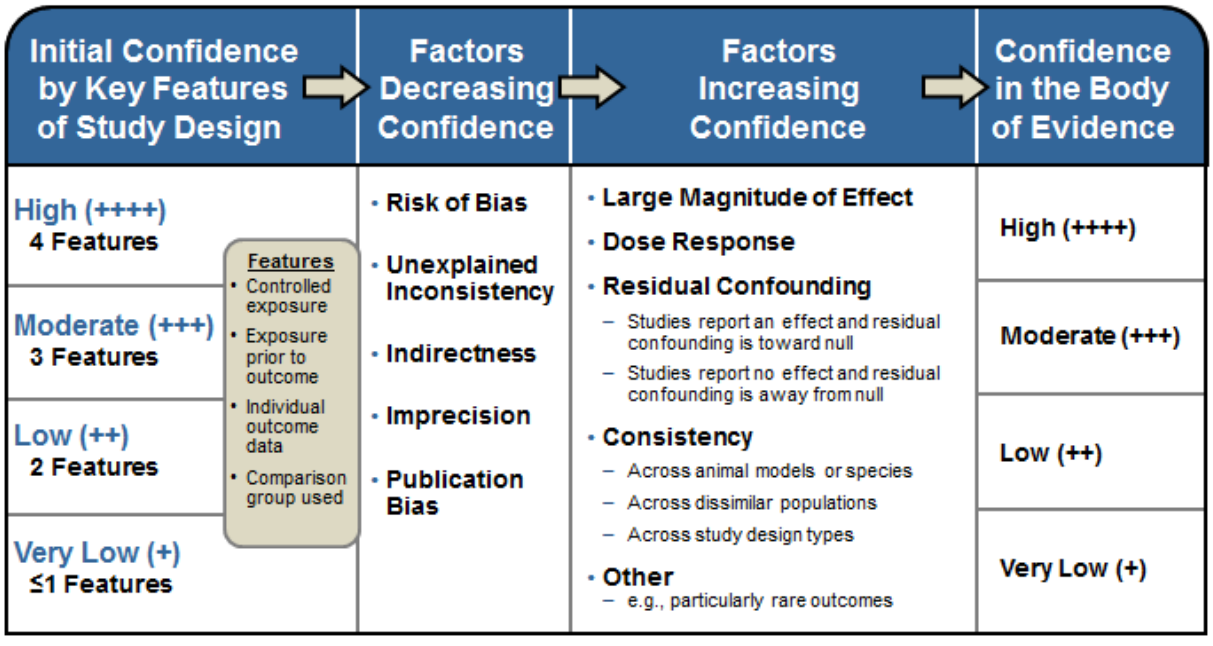
Heterogeneity within the available evidence was used to determine which type of evidence integration was appropriate—a quantitative synthesis (meta-analysis) or narrative approach for evidence integration. Choi *et al.* (2012) conducted a meta-analysis and found that high fluoride exposure was associated with a decrease in IQ. Choi *et al.* (2012) was able to determine a risk ratio for living in an endemic fluorosis area but was unable to develop a dose-response relationship. The study authors suggested that future research should include more precise individual-level exposures, including prenatal exposures, and should better address potential confounders. Although there have been a number of studies published since the Choi *et al.* (2012) meta-analysis, few studies have addressed the

issues identified by Choi *et al.* (2012). The majority of the available studies compare populations with high fluoride exposure to those with lower fluoride exposure (many times in the range of drinking water fluoridation in the United States). After evaluating the available data, NTP determined that a narrative review—not a meta-analysis or other quantitative assessment—was appropriate for evidence integration due to heterogeneity in dose among the available human evidence, and because a hazard conclusion could be reached without conducting a meta-analysis.

Confidence Rating: Assessment of Body of Evidence

The quality of evidence for neurodevelopmental and cognitive function outcomes was evaluated using the GRADE system for rating the confidence in the body of evidence (Guyatt *et al.* 2011, Rooney *et al.* 2014). More detailed guidance on reaching confidence ratings in the body of evidence as "high," "moderate," "low," or "very low" is provided in the OHAT Handbook for Conducting a Literature-Based Health Assessment (http://ntp.niehs.nih.gov/go/38673, see STEP 5). In brief, available human and animal studies on a particular health outcome were initially grouped by key study design features, and each grouping of studies was given an initial confidence rating by those features. Starting at this initial rating (see column 1 of **Figure 1**), potential downgrading of the confidence rating was considered for factors that decrease confidence in the results (see column 2 of **Figure 1** [risk of bias, unexplained inconsistency, indirectness or lack of applicability, imprecision, and publication bias]); and potential upgrading of the confidence rating was considered for factors that increase confidence in the results (see column 3 of **Figure 1** [large magnitude of effect, dose response, consistency across study designs/populations/animal models or species, consideration of residual confounding, and other factors that increase our confidence in the association or effect]). Consideration of consistency across study designs, human populations, or animal species is not included in the GRADE guidance (Guyatt *et al.* 2011); however, it is considered in the modified version of GRADE used by OHAT (Rooney *et al.* 2014, NTP 2015).

Figure 1. Assessing Confidence in the Body of Evidence

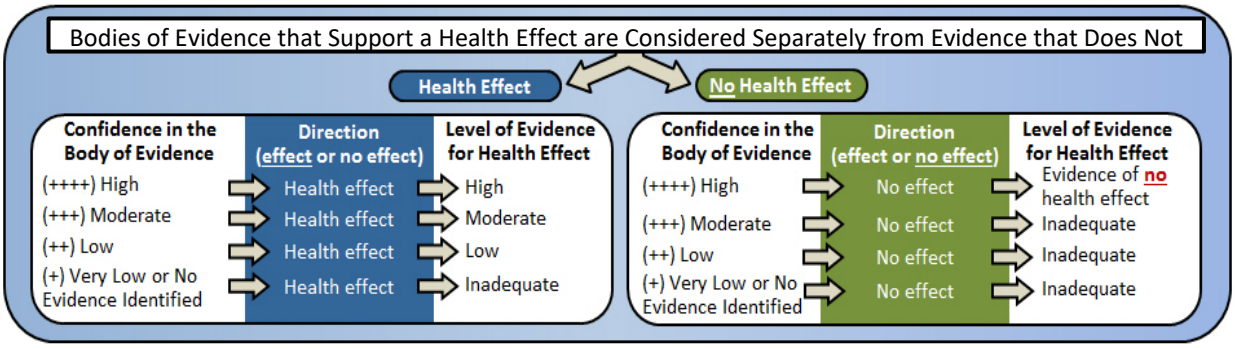


Confidence ratings were assessed by the evaluation team for accuracy and consistency, and discrepancies were resolved by consensus and consultation with technical advisors as needed. Confidence ratings for the primary outcomes are summarized in evidence profile tables for each outcome.

Preparation of Level of Evidence Conclusions

The confidence ratings were translated into level of evidence of health effects for each type of health outcome separately according to one of four statements: (1) High, (2) Moderate, (3) Low, or (4) Inadequate (see **Figure 2**). The descriptor "evidence of no health effect" is used to indicate confidence that the substance is not associated with a health effect. Because of the inherent difficulty in proving a negative, the conclusion "evidence of no health effect" is only reached when there is high confidence in the body of evidence.

Figure 2. Translate Confidence Ratings into Evidence of Health Effect Conclusions



Evidence Descriptors	Definition
High Level of Evidence	There is high confidence in the body of evidence for an association between exposure to fluoride and the health outcome(s).
Moderate Level of Evidence	There is moderate confidence in the body of evidence for an association between exposure to fluoride and the health outcome(s).
Low Level of Evidence	There is low confidence in the body of evidence for an association between exposure to fluoride and the health outcome(s), or no data are available.
Inadequate Evidence	There is insufficient evidence available to assess if exposure to fluoride is associated with the health outcome(s).
Evidence of No Health Effect	There is high confidence in the body of evidence that exposure to fluoride is not associated with the health outcome(s).

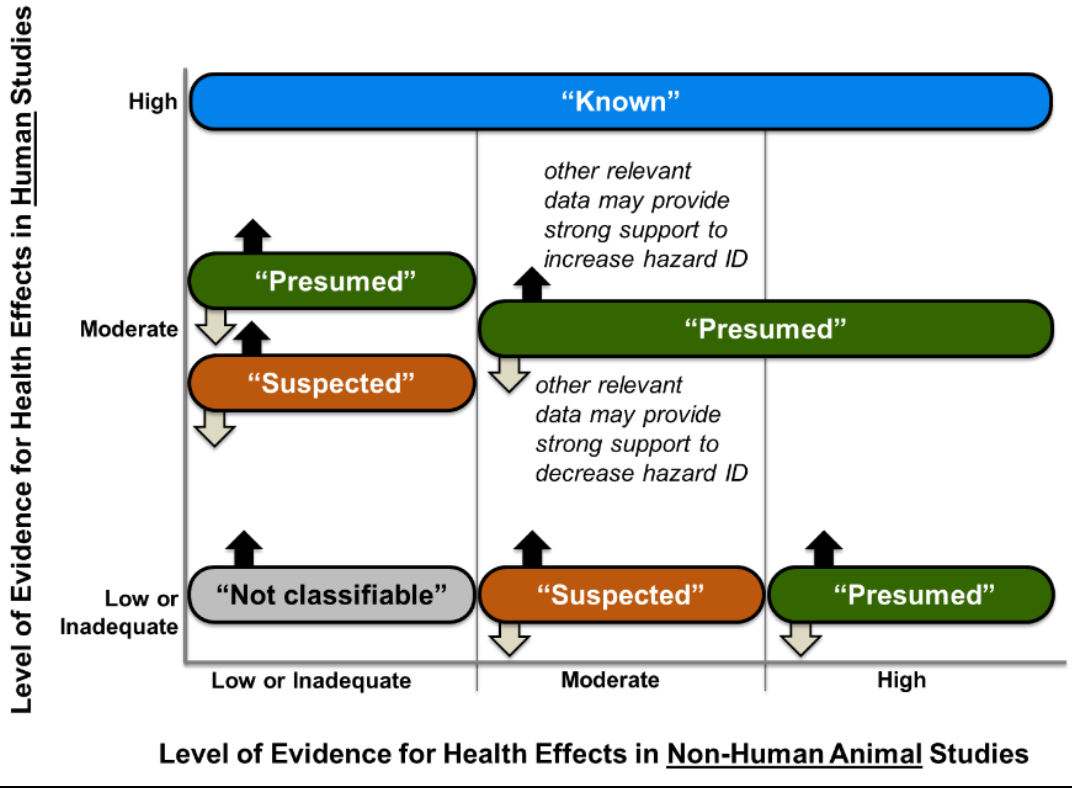
Integrate Evidence to Develop Hazard Identification Conclusions

Finally, the levels of evidence ratings for human and animal data were integrated with consideration of in vitro/mechanistic data to reach one of five possible hazard identification categories: (1) Known, (2) Presumed, (3) Suspected, (4) Not classifiable, or (5) Not identified to be a neurodevelopmental hazard to humans (see **Figure 3**).

Consideration of Human and Animal Data

Initial hazard identification conclusions were attempted by integrating the highest level-of-evidence conclusion for neurodevelopmental effects in children and cognitive effects in adults for the human and the animal evidence streams. The level-of-evidence conclusion for human data for neurodevelopmental or cognitive effects were considered with the level of evidence for non-human animal data to reach one of four initial hazard identification conclusions: Known, Presumed, Suspected, or Not classifiable. When either the human or animal evidence stream was characterized as "Inadequate Evidence," then conclusions were based on the remaining evidence stream alone (which is equivalent to treating the missing evidence stream as "Low" in **Figure 3**). If a moderate level-of-evidence conclusion for human data was reached with "Inadequate or Low Evidence" for the animal evidence stream, a hazard identification conclusion of either "suspected to be a hazard to humans" or "presumed to be a hazard to humans" could be reached based on scientific judgement as to the robustness of the body of evidence that supports moderate confidence in the human data and consideration of the potential impact of additional studies (NTP 2019).

Figure 3. Hazard Identification Scheme for Neurodevelopmental or Cognitive Effects



Consideration of Mechanistic Data

There is no requirement to consider mechanistic or mode-of-action data to reach a hazard identification conclusion regarding neurodevelopmental or cognitive health effects. However, when available, this and other relevant supporting types of evidence may be used to raise (or lower) the category of the hazard identification conclusion. Mechanistic data can come from a wide variety of studies that are not intended to identify a disease phenotype. This source of experimental data includes in vitro and in vivo

laboratory studies directed at cellular, biochemical, genetic, and molecular mechanisms that attempt to explain how a chemical produces particular adverse effects.

For the evaluation of toxicity associated with fluoride exposure, NTP was interested in mechanistic or in vitro measures that may support the biological plausibility of corresponding neurological outcomes reported from in vivo studies in animals or humans. The PECO statement in **Table 3** provides the specific endpoints considered including neuronal electrophysiology; mRNA, gene, or protein expression; cell proliferation or death in brain or thyroid tissue/cells; neuronal signaling; or synaptogenesis. In general, the mechanisms for fluoride-associated neurodevelopmental or cognitive effects are not well understood at this time. Mechanistic data from in vivo studies were used when feasible to examine the biological plausibility of the primary health outcomes considered in developing a hazard conclusion.

The factors outlined for increasing or decreasing confidence that the mechanistic data support biological plausibility are conceptually similar to those used to rate confidence in bodies of evidence for human or animal in vivo studies are listed below and described in depth in the protocol (https://ntp.niehs.nih.gov/go/785076). Four factors were considered that contribute to increased confidence: potency, dose-response, consistency in terms of cellular events observed at the same or lower doses than in vivo health effects, and consistency across cellular targets on the same functional pathway. Three factors were considered that contribute to decreased confidence: unexplained inconsistency across studies of the same endpoint, indirectness/applicability of the pathway for human health or concentrations for human exposure, and publication bias. Evaluations of the strength of evidence provided by mechanistic data were made on an outcome-specific basis based on discussion by the evaluation team and consultation with technical advisors as needed.

- If mechanistic data provided strong support for biological plausibility of the relationship between exposure and the health effect, the hazard identification conclusion may be upgraded (indicated by black “up” arrows in **Figure 3**) from that initially derived by considering the human and non-human animal evidence together.
- If mechanistic data provided strong opposition for biological plausibility of the relationship between exposure and the health effect, the hazard identification conclusion may be downgraded (indicated by gray “down” arrows in **Figure 3**) from that initially derived by considering the human and non-human animal evidence together.

Although it is envisioned that strong evidence for a relevant neurological effect from mechanistic data alone could indicate a potential that the substance is a neurodevelopmental hazard to humans, for this evaluation the mechanistic data were only considered to inform the biological plausibility of observed outcomes from in vivo exposure studies in humans or animals because of a general lack of understanding of the mechanistic basis for neurological outcomes.

RESULTS AND EVIDENCE SYNTHESIS

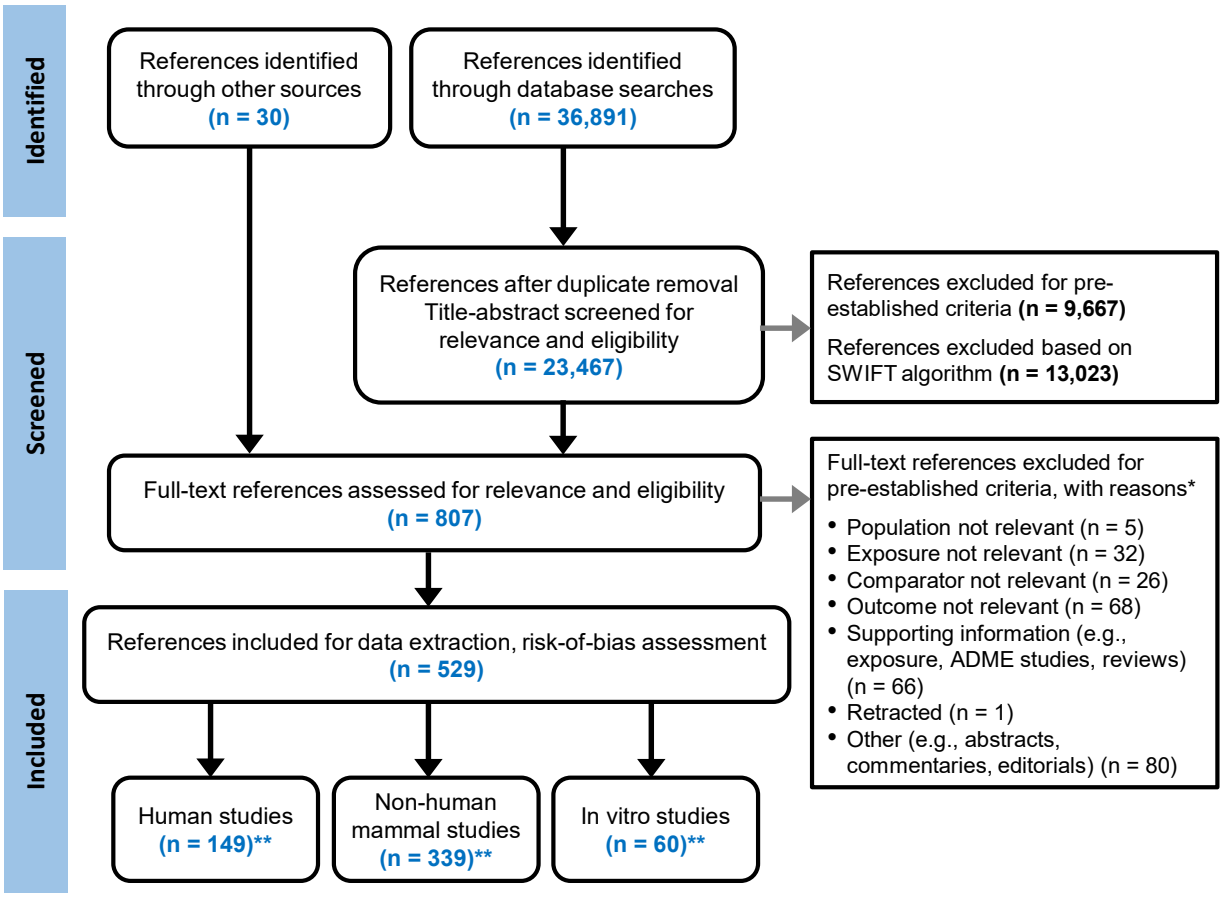
Literature Search Results

The electronic database searches (final updated search conducted on April 1, 2019 with manual searches conducted through August 20, 2019) retrieved 23,467 unique references, and 30 additional references were identified by technical advisors or from reviewing reference lists in published reviews and included studies. Approximately 44% of the studies were manually screened in duplicate at the title and abstract level to reach an estimated ≥98% predicted recall using the statistical algorithm in SWIFT-Active Screener. Eight hundred and seven references were moved to full-text review, 9,667 were excluded during manual screening for not satisfying the PECO criteria, and an additional 13,023 were not screened and excluded based on the SWIFT algorithm. In addition, 278 references were excluded during the full-text review for not satisfying the PECO criteria. These screening results are outlined in a study selection diagram with reasons for exclusion documented at the full text review stage (see **Figure 4**) [using reporting practices outlined in Moher *et al.* (2009)]. After full-text review, 529 studies were considered relevant with primary neurological outcomes, secondary neurological outcomes, and/or outcomes related to thyroid function (see **Appendix 2)**. A few studies assessed data for more than one evidence stream (human, non-human mammal, and/or in vitro), and several human and animal studies assessed more than one type of outcome (e.g., primary and secondary outcomes). The number of included studies is summarized below.

- 149 human studies (68 primary only, 13 secondary only, 5 primary and secondary, 6 primary and thyroid, 2 secondary and thyroid, and 55 thyroid only);
- 339 non-human mammal studies (7 primary only; 187 secondary only; 66 primary and secondary; 6 primary, secondary, and thyroid; 4 secondary and thyroid; and 69 thyroid only); and
- 60 in vitro/mechanistic studies (48 neurological and 12 thyroid).

One publication contained human, experimental non-human mammal, and in vitro data. Three publications contained both human and experimental non-human mammal data. Fourteen publications contained data relevant to both experimental non-human mammal studies and in vitro studies.

Figure 4. Study Selection Diagram



* Studies may have been excluded for more than one reason; the first reason identified by the screener was recorded.

** One publication contained human, experimental non-human mammal, and in vitro data. Three publications contained both human and experimental non-human mammal data. Fourteen publications contained data relevant to both experimental non-human mammal studies and in vitro studies.

Neurodevelopmental and Cognitive Health Effects Results

All the neurodevelopmental and cognitive data were initially considered and evaluated, with more in-depth analysis where similar endpoints were evaluated across multiple studies (e.g., IQ). Hazard conclusions were developed separately for two different age groups (i.e., children and adults) to address potential differences in the health impact based on exposure during development compared to adulthood. Although the data cover a wide array of endpoints (see **Figure 5**), the hazard conclusion covers a single category for each age group. The largest bodies of evidence were for IQ (n = 63 studies), learning and memory (n = 8 studies), as well as other cognitive development effects (e.g., total neurobehavioral scores and total mental capacity index in children and cognitive impairment in adults; n = 12 studies)[4]. Due to heterogeneity in the endpoints examined and the limited number of human or animal studies, congenital neurological malformations and neurological complications of fluorosis were not evaluated because the body of evidence was inadequate to evaluate these potential effects. These

[4]Some studies are included in more than endpoint category (e.g., IQ and other cognitive developmental effects); therefore, these counts are not mutually exclusive.

health outcomes are not further discussed in this assessment. To the extent possible, human and animal data were grouped into similar categories (e.g., IQ in humans was considered comparable to learning and memory in animals). NTP had previously assessed animal data related to effects on learning and memory associated with fluoride exposure (NTP 2016). Therefore, to update the conclusions of the NTP (2016) systematic review, only more recent animal studies were evaluated in this assessment. Although the previous NTP (2016) report was conducted through January 14, 2016, the current assessment included studies published from 2015 onward and considered studies from the NTP (2016) report. Thirty-five animal studies have been identified that met this criteria, including 23 studies with learning and memory endpoints (Banala and Karnati 2015, Shalini and Sharma 2015, Mesram *et al.* 2016, Pulungan *et al.* 2016, Zheng *et al.* 2016, Dong *et al.* 2017, Sudhakar *et al.* 2017, Zhu *et al.* 2017, Banala *et al.* 2018, Bartos *et al.* 2018, Chen *et al.* 2018, Ge *et al.* 2018a, Ge *et al.* 2018b, McPherson *et al.* 2018, Nageshwar *et al.* 2018, Niu *et al.* 2018, Sharma *et al.* 2018, Sun *et al.* 2018, Wang *et al.* 2018, Yang *et al.* 2018, Raju *et al.* 2019, Yuan *et al.* 2019, Zhao *et al.* 2019) and 12 studies with only motor and sensory endpoints (Adedara *et al.* 2017a, Ahmad *et al.* 2017, Nageshwar *et al.* 2017, Agustina *et al.* 2018, Kinawy and Al-Eidan 2018, Nkpaa and Onyeso 2018, Sudhakar *et al.* 2018b, a, Jia *et al.* 2019, Li *et al.* 2019, Lu *et al.* 2019, Manusha *et al.* 2019). Consistent with the NTP (2016) assessment, only learning and memory studies have been considered in the development of hazard identification conclusions. The additional motor and sensory studies have been considered, along with information on motor and sensory effects reported in the learning and memory studies, to provide evidence of possible indirectness related to the learning and memory assessments.

Risk-of-bias Considerations

Risk-of-bias ratings for each individual study for all risk-of-bias questions are available in **Appendix 3**. The risk of bias of individual studies in the body of evidence was considered in developing confidence ratings. The key risk-of-bias questions (i.e., confounding, exposure characterization, and outcome assessment for human studies and randomization, exposure characterization, and outcome assessment for experimental animal studies) are discussed in the consideration of the body of evidence. For this assessment, the key risk-of-bias questions, if not addressed appropriately, are considered to potentially have the greatest impact on the results. In addition, for developmental studies in animals, controlling for potential litter effects (i.e., adjusting for similarities in responses between littermates) was also a key risk-of-bias concern. The other risk-of-bias questions were also taken into consideration and were used to identify any other risk-of-bias concerns that may indicate serious issues with the studies. No study was excluded based on concerns for risk of bias, but, confidence conclusions were considered with and without high risk-of-bias studies (i.e., studies rated probably high or definitely high risk of bias for at least two key risk-of-bias questions) to assess the impact of the high risk-of-bias studies.

Human Neurodevelopmental and Cognitive Data

While there were several neurodevelopmental and cognitive endpoints assessed (see **Figure 5**), most of the available studies evaluated intelligence (e.g., IQ) in children. Other measures of neurodevelopment or cognitive function in children, such as general cognitive index (GCI), mental capacity, mental development index (MDI), or neonatal behavioral neurological assessment (NBNA) were also assessed. However, because the majority of studies evaluated intelligence, the discussion focuses primarily on IQ in children with a separate discussion on other measures of cognitive function and neurobehavioral effects in children and cognitive effects in adults. The available body of literature that evaluates the association between fluoride exposure and neurodevelopmental and cognitive effects is relatively robust (n = 82) and confidence considerations in the body of evidence and hazard conclusions are focused on the studies with the least potential for bias (n = 20). Studies with higher potential for bias

(n = 62) have also been evaluated and determined to have little impact on the confidence and hazard conclusions. All evaluated studies can be found in **Appendix 2**.

This section is organized to present and explain NTP's two confidence ratings in the bodies of evidence from epidemiological studies that fluoride exposure is associated with cognitive neurodevelopmental effects in children and cognitive effects in adults. These confidence ratings were determined as described in **Figure 1**.

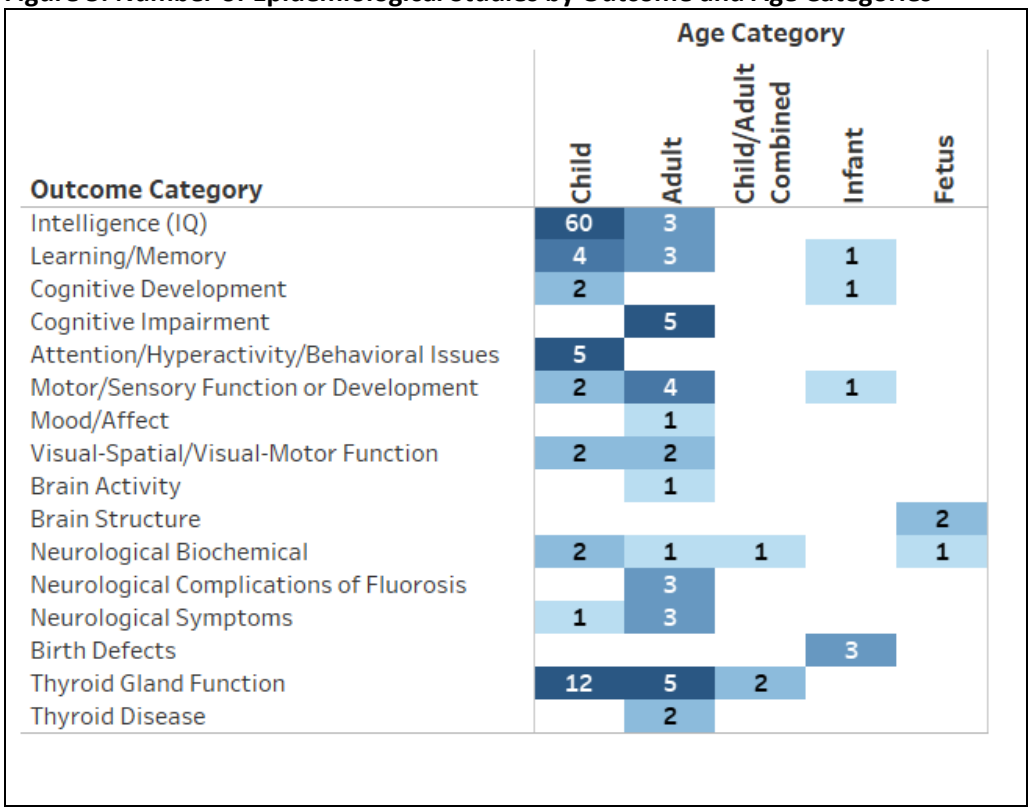
Summary: There is moderate confidence in the body of evidence that fluoride exposure is associated with cognitive neurodevelopmental effects in children, and low confidence in the body of evidence that fluoride exposure is associated with cognitive effects in adults. The moderate confidence rating is supported by consistent evidence from the available studies of an association between high-fluoride exposure (mainly >1.5 ppm in water, but also high exposure via fluoridated salt and food) and decreased IQ or lower cognitive function in children. There is also a recent study of decreased IQ in children living in areas where drinking water fluoride concentrations are <1.5 ppm. Specifically, a study conducted in Canada observed a significant decrease in IQ in boys and girls associated with higher estimated total maternal consumption of fluoride during pregnancy from drinking water and other water-based beverages including black and green tea (Green *et al.* 2019). Another study conducted in Mexico with similar maternal urinary fluoride concentrations during pregnancy as seen in Green *et al.* (2019) observed a significant decrease in IQ in boys and girls associated with higher perinatal exposure to fluoride (Bashash *et al.* 2017). Although the body of evidence in children supports decreased IQ with fluoride exposure, there is a lack of evidence of an association between exposure to fluoride and cognitive effects in adults (Jacqmin *et al.* 1994, Li *et al.* 2016). The body of evidence available to examine the association between exposure to fluoride and cognitive effects in adults is limited to two lower risk-of-bias cross-sectional studies; due to the limited number of studies and a lack of an observed effect, this body of evidence is considered inadequate to evaluate whether fluoride exposure is associated with cognitive effects in adults (see **Table 7**).

Most of the available epidemiological studies that evaluated the association between fluoride exposure and cognitive neurodevelopmental effects assessed IQ and other measures of cognitive function in children (see **Figure 5**). Confidence conclusions are based on those studies with the lowest potential for bias (n = 20) (see **Table 6**). Most of these studies measured fluoride levels in drinking water or urine. All but two of the studies were conducted in infants or children. The two studies in adults were conducted in older adult populations (≥60 years old; one in France and the other in a fluorosis-endemic area of China) to evaluate the effects of fluoride on cognitive impairment. The studies in children were conducted in multiple populations. Of the 18 studies in children, 8 were conducted in China, 5 were conducted in Mexico, 2 were conducted in India, 2 were conducted in Canada, and 1 was conducted in Iran. The IQ studies used many different tests to measure IQ. The IQ tests used often differed by population as not all IQ tests are appropriate for all populations (e.g., western vs. Asian populations). In some cases, different IQ tests were used to study similar populations. Overall, studies used IQ or cognitive tests appropriate for the population and were age appropriate. The different tests conducted and the populations on which the tests were conducted are indicated in **Table 6**.

The lower risk-of-bias studies showing associations with cognitive neurodevelopmental effects in children include 4 prospective cohort studies (Bashash *et al.* 2017, Valdez Jimenez *et al.* 2017, Bashash *et al.* 2018, Green *et al.* 2019) and 14 cross-sectional studies (Xiang *et al.* 2003, Rocha-Amador *et al.* 2007, Li *et al.* 2008a, Rocha-Amador *et al.* 2009, Ding *et al.* 2011, Xiang *et al.* 2011, Saxena *et al.* 2012, Seraj *et al.* 2012, Choi *et al.* 2015, Zhang *et al.* 2015b, Das and Mondal 2016, Barberio *et al.* 2017b, Cui *et*

al. 2018, Yu *et al.* 2018) (see **Figure D1** through **Figure D12**). One limitation of the 14 cross-sectional studies was the lack of direct evidence that exposure to fluoride occurred prior to the development of the neurodevelopmental outcomes. However, several studies (n = 5) indicated that a large portion of the exposed children had dental fluorosis (ranging from 43–100%) at the time of the assessment (Ding *et al.* 2011, Seraj *et al.* 2012, Choi *et al.* 2015, Das and Mondal 2016, Yu *et al.* 2018). Because dental fluorosis occurs when fluoride is consumed during enamel formation usually during the first 6–8 years of life, the presence of dental fluorosis suggests that exposures to fluoride occurred prior to the outcome assessment. Four studies (including Yu *et al.* (2018) listed above) excluded subjects that had not lived in the study area for a specified period of time, sometimes since birth (Rocha-Amador *et al.* 2007, Rocha-Amador *et al.* 2009, Saxena *et al.* 2012, Yu *et al.* 2018). Another study evaluated fluoride exposure in mothers and included urine levels just prior to birth and assessed children a few days after birth (Li *et al.* 2008a). Because these areas were generally known to be fluoride-endemic areas for long periods of time, it can generally be assumed that in these nine cross-sectional studies, exposure occurred prior to the outcome and, therefore, they were considered functionally prospective in nature. These exposure concerns were not an issue for the prospective studies because fluoride levels were measured prenatally. Therefore, the moderate confidence in the body of evidence in children is primarily based on the consistency of findings across different populations in the four lower risk-of-bias prospective cohort studies and the nine cross-sectional studies considered functionally prospective with an initial and final rating of moderate confidence. NTP also considered publication bias when evaluating the confidence in bodies of evidence. This was assessed independently by Choi *et al.* (2012) for 27 studies, all of which were evaluated in this assessment, who found no indication of publication bias based on lack of clear asymmetry in the Begg's funnel plot.

Figure 5. Number of Epidemiological Studies by Outcome and Age Categories*



*Interactive figure and additional study details in Tableau®
(https://public.tableau.com/profile/ntp.visuals#!/vizhome/Fluoride_Epi_UPDATE/Figure5)

Table 6. Studies on Neurodevelopmental and Cognitive Function in Humans[a,b]

Study	Study design (Location/Study) [n]	Exposure measures and summary statistics	Assessment Timing	Outcome assessed and analysis method	Neurological outcome summary
Barberio *et al.* (2017b)	Cross-sectional Canadian Health Measures Survey (Cycles 2 and 3) [2,221]	Children's urine Mean Cycle 2: 32.06 (95% CI: 29.65, 34.46) µmol/L Mean Cycle 3: 26.17 (95% CI: 22.57, 29.76) µmol/L	Children (ages 3–12 years)	Learning disability, ADHD (Cycle 2 only): Parent or child self-report	Significant increase in adjusted OR for learning disability (adjusted OR = 1.02; 95% CI: 1.00, 1.03) only when Cycle 2 and 3 were combined using urinary fluoride Adjusted for age, sex, household income adequacy, and highest attained education in the household; not adjusted for creatinine or specific gravity; associations no longer significant once adjusted for creatinine and specific gravity; no significant associations found between urinary fluoride and ADHD
Bashash *et al.* (2017)	Cohort (prospective) Mexico/Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) participants [299] IQ analysis [211] GCI analysis [287]	Maternal urine during pregnancy Mean (SD): 0.90 (0.35) mg/L Children's urine Mean (SD): 0.82 (0.38) mg/L	Children (ages 4, 6–12 years)	IQ: WASI General cognitive index (GCI): MSCA	Significant effect between maternal urinary fluoride and offspring IQ score (adjusted β = -2.50; 95% CI: -4.12, -0.59) and GCI score (adjusted β = -3.15; 95% CI: -5.42, -0.87); associations with children's urine not significant Adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked during the pregnancy vs. nonsmoker), marital status (married vs not married), age at delivery, IQ, education, and cohort

Table 6. Studies on Neurodevelopmental and Cognitive Function in Humans[a,b]

Study	Study design (Location/Study) [n]	Exposure measures and summary statistics	Assessment Timing	Outcome assessed and analysis method	Neurological outcome summary
Bashash *et al.* (2018)	Cohort (prospective) Mexico/Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) participants [214]	Maternal urine during pregnancy Mean 0.85 (95% CI: 0.81, 0.90) mg/L	Children (ages 6–12 years)	ADHD: Conners' Rating Scales-Revised (CRS-R)	Significant associations between maternal urinary fluoride and CRS-R scores including Cognitive Problems + Inattention Index (adjusted β = 2.54; 95% CI: 0.44, 4.63), DSM-IV Inattention Index (adjusted β = 2.84; 95% CI: 0.84, 4.84), DSM-IV ADHD Total Index (adjusted β = 2.38; 95% CI: 0.42, 4.34), and ADHD Index (adjusted β = 2.47; 95% CI: 0.43, 4.50) Adjusted for gestational age, birth weight, sex, parity, age at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. nonsmoker), marital status (married vs. not married), education, socioeconomic status, and cohort
Choi *et al.* (2015)	Cross-sectional China/first-grade children [51]	Drinking water GM: 2.20 mg/L Children's urine GM: 1.64 mg/L Severity of fluorosis (Dean Index)	Children (ages 6–8 years)	Learning and memory: Neuropsychological tests including WRAML IQ: WISC-IV Visual motor ability: WRAVMA Motor ability: Finger tapping task Manual dexterity: Grooved pegboard test	Compared to normal/ questionable fluorosis, moderate/severe fluorosis significantly associated with lower total (adjusted β = -4.28; 95% CI: -8.22, -0.33) and backward digit span scores (adjusted β = -2.13; 95% CI: -4.24, -0.02); linear correlation between fluoride in urine (adjusted β = -1.67; 95% CI: -5.46, 2.12) and in drinking water (adjusted β = -1.39; 95% CI: -6.76, 3.98) with total digit span was observed but not significant; other outcomes not significantly associated with fluoride exposure Adjusted for child's sex, age, parity, illness before 3 years old, household income last year, and caretaker's age and education
Cui *et al.* (2018)	Cross-sectional China/school children [323]	Children's urine Range (log-transformed): -1.2–2.2	Children (ages 7–12 years)	IQ: Combined Raven's Test for Rural China	Significant correlation between IQ score and urinary fluoride (adjusted β = -2.47) Adjusted for child age, mother's education, family member smoking, stress, and anger

Table 6. Studies on Neurodevelopmental and Cognitive Function in Humans[a,b]

Study	Study design (Location/Study) [n]	Exposure measures and summary statistics	Assessment Timing	Outcome assessed and analysis method	Neurological outcome summary
Das and Mondal (2016)	Cross-sectional India/primary school children [149]	Drinking water Mean (SD): 2.11 (1.64) mg/L Children's urine Range: 0.45–17.00 mg/L	Children (ages 6–18 years)	IQ: Combined Raven's Test for Rural China	Significant correlation between IQ score and water fluoride (r = -0.343) and urinary fluoride (r = -0.751); IQ also generally decreased with increasing dental fluorosis severity No statistical adjustment for confounders
Ding *et al.* (2011)	Cross-sectional China (Inner Mongolia)/ elementary school children [331]	Drinking water Mean (SD): 1.31 (1.05) mg/L Children's urine Range: 0.1–3.55 mg/L	Children (ages 7–14 years)	IQ: Combined Raven's Test for Rural China	Significant association between urinary fluoride and IQ score (each increase in urinary fluoride of 1 mg/L was associated with a decrease in IQ of 0.59 points (95% CI: -1.09, -0.08); dose response relationship between fluoride and dental fluorosis ($p < 0.00001$) Adjusted for age
Green *et al.* (2019)	Cohort (prospective) Canada/Maternal-Infant Research on Environmental Chemicals (MIREC) [512] Non-Fluoridated [238] Fluoridated [162] Boys [248] Girls [264]	Maternal urine during pregnancy Mean (SD): 0.51 (0.36) mg/L (0.40 [0.27] mg/L in non-fluoridated areas and 0.69 [0.42] mg/L in fluoridated areas) Maternal fluoride intake during pregnancy Mean (SD): 0.54 (0.44) mg/day (0.30 [0.26] and 0.93 [0.43] mg/day, respectively) Drinking water Mean (SD): 0.31 (0.23) mg/L (0.13 [0.06] and 0.59 [0.08] mg/L, respectively)	Children (age 3 years)	IQ: full scale, performance, and verbal using Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III)	Significant decrease in full-scale IQ (adjusted β = -4.49; 95% CI: -8.38, -0.60) and performance IQ (adjusted β = -4.63; 95% CI: -9.01, -0.25) per 1-mg/L increase in maternal urine in boys, but not girls (adjusted β = 2.40; 95% CI: -2.53, 7.33 and adjusted β = 4.51; 95% CI: -1.02, 10.05, respectively); significant decrease in full-scale IQ (adjusted β = -3.66; 95% CI: -7.16, -0.15) per 1-mg increase in maternal fluoride intake (no sex interaction); significant decrease in full-scale IQ (adjusted β = -5.29; 95% CI: -10.39, -0.19) per 1-mg/L increase in water fluoride concentration (no sex interaction); no significant decreases observed in verbal IQ Adjusted for city, HOME score, maternal education, race, child's sex, and prenatal secondhand smoke exposure
Jacqmin *et al.* (1994)	Cross-sectional France/elderly adults [3,490]	Drinking water Range: 0.03–2.03 mg	Adults (ages >65 years)	Cognitive function: Mini-Mental State (MMS) Examination	No significant increase in the prevalence of cognitive impairment with increasing fluorine quartiles No statistical adjustment for confounders

Table 6. Studies on Neurodevelopmental and Cognitive Function in Humans[a,b]

Study	Study design (Location/Study) [n]	Exposure measures and summary statistics	Assessment Timing	Outcome assessed and analysis method	Neurological outcome summary
Li *et al.* (2008a)	Cross-sectional China/neonates [91]	Drinking water Range: 0.5–1.0 mg/L (control); 1.7–6.0 mg/L (high) Maternal urine during pregnancy Mean (SD): 1.74 (0.96) mg/L (control); 3.58 (1.47) mg/L (high)	Neonates (24–72 hours after delivery)	Neurodevelopmental: Neonatal behavioral neurological assessment (NBNA)	Significant differences in neurobehavioral assessment total scores between high-fluoride (36.48 ± 1.09) and control groups (38.28 ± 1.10); significant differences in total neurobehavioral capacity scores as measured by non-biological visual orientation reaction and biological visual and auditory orientation reaction between the two groups (11.34 ± 0.56 in controls compared to 10.05 ± 0.94 in high-fluoride group) No statistical adjustment for confounders
Li *et al.* (2016)	Cross-sectional China/adults [511]	Drinking water intake and urinary fluoride Means (SD) reported for a subset of subjects with normal scores (2.23 [2.23] mg and 1.46 [1.04] mg/L, respectively) and subjects with cognitive impairment (3.62 [6.71] mg and 2.47 [2.88] mg/L, respectively)	Adults (ages ≥ 60 years)	Cognitive function: MMS Examination	Results suggested that degree of fluoride exposure was consistent with severity of skeletal fluorosis, and fluoride exposure may be a risk factor for cognitive impairment; however, neither water fluoride intake (adjusted ORs = 0.94 [95% CI: 0.85, 1.04] and 0.86 [95% CI: 0.69, 1.06] in the moderate and severe cognitive impairment groups, respectively) nor urinary fluoride levels (adjusted ORs = 1.12 [95% CI: 0.89, 1.42] and 1.25 [95% CI: 0.87, 1.81] in the moderate and severe cognitive impairment groups, respectively) were significantly correlated with cognitive impairment Adjusted for sex, age, education, marital status (married vs. not married), alcohol consumption (non-drinkers, light drinkers, moderate to heavy drinkers), smoking history (never smoker, ex-smoker, light smoker, heavy smoker), and serum homocysteine levels

Table 6. Studies on Neurodevelopmental and Cognitive Function in Humans[a,b]

Study	Study design (Location/Study) [n]	Exposure measures and summary statistics	Assessment Timing	Outcome assessed and analysis method	Neurological outcome summary
Rocha-Amador *et al.* (2007)	Cross-sectional Mexico/elementary school children [132]	Drinking water Mean (SD): 0.8 (1.4), 5.3 (0.9), 9.4 (0.9) mg/L (3 rural areas) Children's urine Mean (SD): 1.8 (1.5), 6.0 (1.6), 5.5 (3.3) mg/L (3 rural areas)	Children (ages 6–10 years)	IQ: WISC-Revised Mexican Version	Significant associations between fluoride and IQ scores (full IQ adjusted βs of -10.2 with water and -16.9 with urine; CIs not reported); arsenic also present, but the effect was smaller (full IQ adjusted βs of -6.15 with water and -5.72 with urine; CIs not reported) Adjusted for blood lead, mother's education, SES, height-for-age z-scores, and transferrin saturation
Rocha-Amador *et al.* (2009)	Cross-sectional Mexico/elementary school children [80]	Children's urine GM (SD): 5.6 (1.7) mg/L	Children (ages 6–11 years)	Visuospatial organization and visual memory: Rey-Osterrieth Complex Figure Test, children's version	Significant correlation between urinary fluoride and visuospatial organization (r = -0.29) and visual memory scores (r = -0.27); no significant correlation with arsenic Adjusted for age
Saxena *et al.* (2012)	Cross-sectional India/school children [170]	Drinking water Mean: >1.5 mg/L (high fluoride group) Children's urine Range: 1.7–8.4 mg/L	Children (age 12 years)	IQ: Raven's Standard Progressive Matrices	Significant correlation between water (r = 0.534; p = 0.000) and urinary fluoride (r = 0.542; p = 0.000) levels and IQ score; no significant differences in the levels of urinary lead or arsenic in children from the different groups Confounders included in the analysis were not reported
Seraj *et al.* (2012)	Cross-sectional Iran/school children [293]	Drinking water Mean (SD): 0.8 (0.3) (normal), 3.1 (0.9) (medium), 5.2 (1.1) (high) mg/L	Children (ages 6–11 years)	IQ: Raven's Colored Progressive Matrices	Significant correlation between water fluoride and IQ score (adjusted β = -3.865; CIs not reported); significantly higher IQ score in normal area (97.77 ± 18.91) compared with medium (89.03 ± 12.99) and high (88.58 ± 16.01) areas Adjusted for age, gender, child's education level, mother's education level, father's education level, and fluorosis intensity
Valdez Jimenez *et al.* (2017)	Cohort (Prospective) Mexico/infants [65]	Drinking water Range: 0.5–12.5 mg/L (all trimesters) Maternal urine Range: 0.16–8.2 mg/L (all trimesters)	Infants (ages 3–15 months)	Mental development index (MDI): Bayley Scales of Infant Development II (BSDI-II)	Significant correlation between maternal urinary fluoride and MDI score during first trimester (adjusted β = -19.05; SE = 8.9) and second trimester (adjusted β = -19.34; SE = 7.46) Adjusted for gestational age, age of child, marginality index, and type of drinking water

Table 6. Studies on Neurodevelopmental and Cognitive Function in Humans[a,b]

Study	Study design (Location/Study) [n]	Exposure measures and summary statistics	Assessment Timing	Outcome assessed and analysis method	Neurological outcome summary
Xiang *et al.* (2003)	Cross-sectional China/school children [512]	Drinking water Mean (SD): 0.36 (0.15) (control), 2.47 (0.79) (high fluoride) mg/L Children's urine Mean (SD): 1.11 (0.39) (control), 3.47 (1.95) (high fluoride) mg/L Village of residence (non-endemic v. endemic fluorosis)	Children (ages 8–13 years)	IQ: Combined Raven's Test for Rural China	Significant dose-related effect of fluoride on IQ score based on quintile levels with significant decreases in IQ scores observed with water fluoride at 1.53 mg/L or higher; Pearson correlation coefficient of -0.164 with urinary fluoride; IQ scores for children in non-endemic region (100.41 ± 13.21) significantly higher than endemic region (92.02 ± 13.00); calculated a lower-bound confidence limit benchmark concentration (BMCL) of 1.85 mg/L
Xiang *et al.* (2011)	Cross-sectional China/school children [512]	Children's serum Mean (SD): 0.041 (0.009) (control), 0.081 (0.019) (high fluoride) mg/L	Children (ages 8–13 years)	IQ: Combined Raven's Test for Rural China	Significant trend on association between quartiles of serum fluoride and children's IQ score < 80 (adjusted ORs for Q1 and Q2; Q1 and Q3; and Q1 and Q4, respectively: 1; 2.22 [95% CI: 1.43, 3.47]; and 2.48 [95% CI: 1.85, 3.32]); significant effects at >0.05 ppm fluoride Adjusted for age and gender
Yu *et al.* (2018)	Cross-sectional China/children [2,886]	Drinking water Mean (SD): 0.50 (0.27) (normal), 2.00 (0.75) (high) mg/L Children's urine Mean (SD): 0.41 (0.49) (normal), 1.37 (1.08) (high) mg/L	Children (ages 7–13 years)	IQ: Combined Raven's Test for Rural China	Significant difference (p = 0.036) in mean IQ scores in high water fluoride area (>1.0 mg/L; 106.4 ± 12.3 IQ) compared to the normal area (≤1.0 ppm; 107.4 ± 13.0 IQ); distribution of the IQ scores also significantly different (p = 0.003); every 0.5-mg/L increase in water fluoride was associated with a 4.29 decrease in IQ score (95% CI: -8.09, -0.48) between 3.40 and 3.90 mg/L Adjusted for age, sex, maternal education, paternal education, and low birth weight

Table 6. Studies on Neurodevelopmental and Cognitive Function in Humans[a,b]

Study	Study design (Location/Study) [n]	Exposure measures and summary statistics	Assessment Timing	Outcome assessed and analysis method	Neurological outcome summary
Zhang *et al.* (2015b)	Cross-sectional China/school children [180]	Drinking water Mean: 0.63 (control), 1.40 (endemic fluorosis) mg/L (SD not reported) Children's urine Mean (SD): 1.1 (0.67) (control), 2.4 (1.01) (endemic fluorosis) mg/L Children's serum Mean (SD): 0.06 (0.03) (control), 0.18 (0.11) (endemic fluorosis) mg/L	Children (ages 10–12 years)	IQ: Combined Raven's Test for Rural China	Significant correlation between IQ score and serum fluoride (r = -0.47) and urinary fluoride (r = -0.45); significant difference in IQ score for high-fluoride area (>1 ppm; 102.33 ± 13.46) compared with control area (109.42 ± 13.30) Adjusted for age and gender, if applicable

[a] Includes lower risk-of-bias studies

[b] Definitions: **ADHD**: attention-deficit/hyperactivity disorder; **GCI**: General Cognitive Index; **GM**: geometric mean; **HOME**: Home Observation Measurement of the Environment; **IQ**: intelligence quotient; **MSCA**: McCarthy Scales of Children's Abilities; **WASI**: Wechsler Abbreviated Scale of Intelligence (Spanish version); **WISC-IV**: Wechsler Intelligence Scale for Children-Revised; **WRAML**: Wide Range Assessment of Memory and Learning; **WRAVMA**: Wide Range Assessment of Visual Motor Ability

Overall Risk-of-bias Discussion of the Body of Evidence

The confidence rating for the body of evidence in humans was based on studies with the lowest potential for bias (i.e., studies rated probably low or definitely low risk of bias for at least two of the three key risk-of-bias questions). Each of these 20 studies (including 18 studies in children and 2 in adults) had little or no risk-of-bias concerns, and confidence in the body of evidence was not downgraded for risk of bias. However, the remaining studies in the human body of evidence were rated as probably high or definitely high risk of bias for at least two key risk-of-bias questions. Risk-of-bias ratings for individual studies for all questions are available in **Figure A3-1** and **Figure A3-3**. Among the studies with lower potential for bias (see **Figure A3-1** and **Figure A3-2**), the key risk-of-bias question with the most potential for bias was the potential for confounding. Potential confounding was a concern for 6 of the 18 lower risk-of-bias studies in children (see *Confounding* for further discussion). Among the studies with higher potential for bias, there were a number of risk-of-bias concerns, including potential confounding, poor exposure characterization, poor outcome assessment, and, in many cases, potential concern with participant selection (see **Figure A3-3** and **Figure A3-4**). Many of the studies (n = 49) included in the entire human body of evidence were initially published in a foreign language (mainly Chinese) and translated by the Fluoride Action Network (http://fluoridealert.org/researchers/translations/complete_archive/). Most of these studies were considered to have high potential of bias due to lack of information across many key risk-of-bias questions. Therefore, in order to assess if the lack of information relevant to key risk-of-bias concerns was the result of a loss in translation, the original Chinese publications and the translated versions of the five studies that had the most potential for being included in the lower risk-of-bias group of studies were reviewed to determine if any of the risk-of-bias concerns could be addressed (An *et al.* 1992, Chen *et al.* 2008, Du *et al.* 2008, Guo *et al.* 2008a, Li *et al.* 2009). For all five studies, it was determined that there was no additional information that could be obtained from the original Chinese publication that impacted the key risk-of-bias concerns.

Confounding

The list of potential confounding variables and/or effect modifiers considered important for this evaluation (depending on the study population and outcome) included age; child's sex; race/ethnicity; maternal demographics (e.g., maternal age, body mass index [BMI]); parental behavioral and mental health disorders (e.g., ADHD, depression); socioeconomic status (e.g., maternal education, household income, marital status, crowding); smoking (e.g., maternal smoking status, secondhand tobacco smoke exposure); reproductive factors (e.g., parity); nutrition (e.g., BMI, growth, anemia); iodine deficiency/excess; minerals and other chemicals in water associated with neurotoxicity (e.g., arsenic, lead); maternal and paternal IQ; and quantity and quality of caregiving environment (e.g., Home Observation Measurement of the Environment [HOME] score). To be assigned a rating of probably low risk of bias for the key risk-of-bias question regarding confounding, studies were not required to address every potential confounder listed; however, studies were required to address the potential for co-exposures (e.g., arsenic and lead, both of which could affect cognitive function) and any potential confounders considered important for the specific study population and outcome. For example, studies of populations in China, India, and Mexico, where there is concern for exposures to high fluoride and high arsenic, were required to address arsenic, and smoking needed to be addressed in studies of adults when dementia was evaluated.

Among studies with lower risk-of-bias concerns, 14 of the 20 studies were considered to have lower potential for bias due to confounding. Only two lower risk-of-bias studies did not consider any potential confounders, and only four studies did not account for indicators of socioeconomic status (e.g., parental education, household income). Six of the 20 lower risk-of-bias studies accounted for maternal or family member smoking. Potential confounding related to important co-exposures that may impact neurological functioning (e.g., arsenic and lead) was addressed in some lower risk-of-bias studies by either accounting for arsenic or lead in the analyses (three studies for arsenic and six studies for lead) or indicating that arsenic or lead levels in the study areas were very low (four studies for arsenic and six studies for lead). Most of the remaining studies did not consider co-exposures to lead (n = 12) or arsenic (n = 7); however, these co-exposures were considered less likely to be an issue in study populations where there was no evidence that lead or arsenic was prevalent or occurring in relation to fluoride. Studies with reason for concern about arsenic or lead specifically that did not address arsenic or lead as a co-exposure were rated as probably high risk of bias for the key question of confounding.

Although there is variability in the potential confounders considered and differences in populations evaluated, the consistency of the results among the lower risk-of-bias studies indicates that confounding is not a major concern in this body of evidence. Even though 6 of 18 lower risk-of-bias studies in children are considered to have higher potential for bias due to confounding that could not be ruled out for that specific population and outcome (see **Figure 6**), results were consistent across multiple populations; all lower risk-of-bias studies in children reported that higher fluoride exposure is associated with at least one measure of decreased IQ or other cognitive effect. A few lower risk-of-bias studies confirmed the robustness of the results by conducting sensitivity analyses (Bashash *et al.* 2017, Bashash *et al.* 2018, Yu *et al.* 2018, Green *et al.* 2019). None of the sensitivity analyses adjusting for additional confounders found meaningful shifts in the association between fluoride exposure and IQ or other measures of cognitive function. Bashash *et al.* (2017) found that adjusting for HOME score increased the association between maternal urinary fluoride and children's IQ. Bashash *et al.* (2018) examined several potential confounders in sensitivity analyses involving subsets of participants, including HOME scores, child contemporaneous fluoride exposure measured by child urinary fluoride adjusted for specific gravity, and maternal lead and mercury exposures. The authors reported that no sensitivity analyses indicated appreciable changes in the fluoride-related association with behaviors related to ADHD, nor did they

find evidence of effect modification between sex and maternal urinary fluoride. Green *et al.* (2019) found that adjusting for lead, mercury, manganese, perfluorooctanoic acid, and arsenic concentrations did not substantially alter the associations with IQ. Sensitivity analyses by Yu *et al.* (2018) that adjusted for covariates (including age, sex, and socioeconomic status) did not find differences in the results compared to the primary analyses.

As previously mentioned, most of the higher risk-of-bias studies in the human body of evidence did not address the potential confounders of greatest concern. Many of these studies conducted only simple statistical analyses without accounting for any potential confounders (49 of 59 higher risk-of-bias studies), and many studies did not report whether the study subjects were from areas of similar socioeconomic status or environmental conditions (n = 19 higher risk-of-bias studies). Potential confounding related to important co-exposures (e.g., arsenic and lead) was often not addressed in higher risk-of-bias studies. In studies where there was high exposure to fluoride via drinking water with high naturally-occurring fluoride or from the use of coal-containing fluoride, most researchers did not account for potential exposures to arsenic, which is commonly found in coal and drinking water in fluoride-endemic areas of China and Mexico. In general, researchers did not account for potential exposures to lead; however, studies reporting lead levels in fluoride-endemic areas, including areas in China, often reported low levels of lead (Xiang *et al.* 2011, Choi *et al.* 2012, Saxena *et al.* 2012, Seraj *et al.* 2012, Choi *et al.* 2015, Yu *et al.* 2018). Therefore, lead is not assumed to be a common exposure in fluoride-endemic areas. Most of the studies did not account for smoking or socioeconomic status, nor did they provide information to lessen the risk-of-bias concern (e.g., list of study characteristics indicating no significant differences between comparison groups). However, as noted for the lower risk-of-bias studies, given the consistency of the evidence, confounding is not likely a major concern among higher risk-of-bias studies.

Figure 6. Potential Confounders Considered in Lower Risk-of-bias Studies Conducted in Children

Study (Location)[1]	Potential Confounding Factors Considered[2]																Notes	Reported Effect of Fluoride[4]
	Subject Characteristics				Other Exposures					Socioeconomic Factors		Parental Characteristics				Other[3]		
	Age	Sex	Race/Ethnicity	Health Factors[3]	Arsenic	Smoking	Iodine	Lead	Other (in Water)[3]	SES	Caregiving Environment (e.g., HOME score)	Demographics[3]	Reproductive Factors[3]	Health Factors[3]	IQ			
Overall RoB Rating for Confounding: Probably Low																		
Barberio 2017b (Canada)	√	√	–	–	√	–	–	√	–	√	–	–	–	–	–	–	–	Yes
Bashash 2017 (Mexico)	√	√	–	–	√	√	–	√	√	√	√	√	√	–	√	√	Other (in water): Hg, Ca Demographics: maternal age Reproductive: parity, birth order, birth weight, gestational age at delivery Other: cohort	Yes
Bashash 2018 (Mexico)	√	√	–	–	–	√	–	√	√	√	√	√	√	–	–	√	Other (in water): Hg, Ca Demographics: maternal age Reproductive: parity, birth order, birth weight, gestational age at delivery Other: cohort	Yes
Choi 2015 (China)	√	√	–	√	√	–	–	√	–	√	–	√	√	√	–	√	Health: subject Fe deficiency, illnesses before age 3, medical history of subject and caretakers Demographics: parental age Reproductive: parity Other: residential history	Yes
Cui 2018 (China)	√	√	√	√	√	√	√	–	–	√	–	√	√	√	–	–	Health: subject BMI, stress/anger/anxiety/depression, psychological trauma, having a cold, in relatives: thyroid diseases, cancer, mental retardation Demographics: maternal age and education Reproductive: abnormal birth Other: alcohol consumption, proximity to factory, physical activity, various dietary factors	Yes
Green 2019 (Canada)	√	√	√	–	√	√	–	√	√	√	√	√	√	–	–	√	Other exposures: Hg, Mn, PFOA Demographics: parental age, pre-pregnancy BMI Reproductive: parity, weeks of gestation, birth weight, maternal chronic condition during pregnancy Other: alcohol consumption, birth country, voiding interval in urine sampling, breastfeeding duration	Yes
Rocha-Amador 2007 (Mexico)	√	–	–	√	√	–	–	√	–	√	–	–	–	–	–	–	Health: subject height and weight by age, transferrin saturation	Yes
Saxena 2012 (India)	√	√	–	√	√	–	–	√	–	√	–	–	–	–	–	√	Health: subject height for age ratio, weight for height ratio, medical history Other: residential history	Yes
Seraj 2012 (Iran)	√	√	–	–	–	–	√	√	–	√	–	–	–	–	–	√	Other: fluorosis intensity	Yes
Xiang 2011 (China)	√	√	–	–	–	–	√	√	–	√	–	–	–	–	–	–	–	Yes
Yu 2018 (China)	√	√	–	√	√	√	√	√	√	√	–	–	√	–	–	√	Health: subject BMI Reproductive: disease history during pregnancy, delivery conditions Other: dental fluorosis prevalence, consanguineous marriage	Yes
Zhang 2015b (China)	√	√	–	–	√	–	√	√	√	√	–	–	–	–	–	√	Other: thyroid hormone levels, residential history, having knowledge of fluorosis, COMT genotype	Yes

Study (Location)[1]	Potential Confounding Factors Considered[2]																Notes	Reported Effect of Fluoride[4]
	Subject Characteristics				Other Exposures					Socioeconomic Factors		Parental Characteristics				Other[3]		
	Age	Sex	Race/Ethnicity	Health Factors[3]	Arsenic	Smoking	Iodine	Lead	Other (in Water)[3]	SES	Caregiving Environment (e.g., HOME score)	Demographics[3]	Reproductive Factors[3]	Health Factors[3]	IQ			
Overall RoB Rating for Confounding: Probably High																		
Das and Mondal 2016 (India)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Yes
Ding 2011 (China)	√	–	–	–	√	–	√	√	–	–	–	–	–	–	–	–	–	Yes
Li 2008a (China)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Yes
Rocha-Amador 2009 (Mexico)	√	√	–	√	√	–	–	√	–	–	–	–	–	–	–	–	Health: subject height and weight by age	Yes
Valdez Jimenez 2017 (Mexico)	√	√	–	–	–	–	–	–	–	√	–	√	√	√	–	√	Demographics: maternal age Health: pre-pregnancy history of drugs, vaccines, diseases Reproductive: prenatal history, parity, type of birth, week of birth, weight and length at birth, gestational age, Apgar and health conditions of the baby during the first month of life Other: infant feeding type (breastfeeding, formula)	Yes
Xiang 2003 (China)	√	√	–	–	–	–	√	–	–	√	–	–	–	–	–	–	–	Yes

Notes:

[1]Includes all lower risk-of-bias studies in children. Studies are organized as those with an overall risk-of-bias rating for confounding as probably low (green) followed by those with an overall risk-of-bias rating for confounding as probably high (yellow).

[2]Potential confounding factors and/or effect modifiers represented here are those considered important for this evaluation. See study details provided in HAWC for information on additional confounders.

Factors outlined in blue (subject age, subject sex, arsenic, SES) are considered key confounders.

A √ indicates that a factor was considered (and may or may not have been adjusted for in final model). For 'Other Exposures', a √ might also be used when a co-exposure was not expected to be an issue because there is no evidence to indicate that the co-exposure was prevalent or occurring in relation to fluoride. See risk-of-bias explanations in HAWC for details. A hyphen (–) indicates that the factor was not considered.

[3]See the "Notes" column for additional details.

[4]Extent of reported effects varies by study. "Yes" indicates that study authors reported one or more significant effects on IQ or other cognitive functions associated with fluoride exposure.

Exposure assessment

Exposure was assessed using a variety of methods in the human body of evidence. Studies provided varying levels of details on the methods used and employed different exposure characterization methods to group study subjects into exposed and reference groups. Exposure metrics included spot urine (from children or mothers during at least one trimester), 24-hour urine, serum, individual drinking water, municipal drinking water (with residence information), area of residence (endemic versus a non-endemic fluorosis area with or without individual validation of exposure), burning coal (with or without fluoride), and occupation type. Analytical methods to measure fluoride in biological or water samples also varied, some of which included atomic absorption, ion selective electrode methods, colorimetric methods, or the hexamethyldisiloxane microdiffusion method. Individual-level measures of exposure were generally considered more accurate than group-level measures; however, using group-level measures (e.g., endemic versus non-endemic area) in an analysis was less of a concern if the study provided water or urine fluoride levels from individuals to verify that there were differences in the fluoride exposure between groups. Studies that provided results by area but also reported individual urinary or serum fluoride concentrations or other biochemical measures, including dental fluorosis in the children or urinary levels in mothers during pregnancy, were considered to have probably low risk of bias.

In general, there were few or no risk-of-bias concerns regarding exposure assessment in the lower risk-of-bias studies. Many of the lower risk-of-bias studies used individual urine or water measures with appropriate analyses. Urinary fluoride levels include all ingested fluoride and are considered a valid measure to estimate fluoride exposure (Watanabe *et al.* 1995, Villa *et al.* 2010); however, some concerns exist. Urinary fluoride is thought to reflect recent exposure but can be influenced by the timing of exposure (e.g., when water was last consumed, when teeth were last brushed). When compared to 24-hour urine samples, spot urine samples are more prone to these influences and can also be affected by differences in dilution; however, many studies attempted to account for dilution either using urinary creatinine or specific gravity. Strong correlations between 24-hour samples and urinary fluoride concentrations from spot samples adjusted for urinary dilution have been described (Zohouri *et al.* 2006). Despite potential issues with spot urine samples, if authors made appropriate efforts to reduce the concern for bias, studies that used this metric were generally considered to have probably low risk of bias for exposure.

Although there are concerns related to using maternal urine samples, many studies provide evidence to suggest that urinary fluoride is a reasonable measure of exposure. Using three methods to account for urine dilution, Till *et al.* (2018) reported that adjusted risk estimates did not differ from unadjusted estimates. Analyzing the same study population as Till *et al.* (2018), Green *et al.* (2019) found that adjusting for time of urine collection or time of collection since last void during pregnancy did not substantially affect associations with IQ results in either boys or girls. In addition, adjusting the maternal urinary fluoride for creatine did not substantially alter the association observed (Green *et al.* 2019). To provide a more accurate and sensitive measurement of maternal urinary fluoride than a single measurement provides, Green *et al.* (2019) only included participants with valid fluoride measurements at each trimester in their analysis. Several other studies also measured urinary fluoride multiple times throughout pregnancy (Bashash *et al.* 2017, Valdez Jimenez *et al.* 2017, Bashash *et al.* 2018, Green *et al.* 2019). Other studies demonstrated correlations between the urinary fluoride and fluoride in the drinking water or estimated dose based on water (Saxena *et al.* 2012, Zhang *et al.* 2015b, Das and Mondal 2016, Green *et al.* 2019). Till *et al.* (2018) demonstrated that there was a linear association between urinary fluoride concentrations in pregnant women and drinking water fluoride concentrations regardless of method to correct for urine dilution or whether or not adjustments were made for

dilution. Bashash *et al.* (2017) excluded exposure outliers but found that doing so did not change the results in a meaningful way. Taken together, these studies suggest that urinary fluoride is a reasonable measure of exposure despite some of the potential issues.

A frequent critical limitation among the higher risk-of-bias studies was lack of information regarding exposure or poor exposure characterization. Many of the higher risk-of-bias studies only compared subjects living in two regions with differing levels of fluoride exposure, and while most of them did provide some differentiation in levels of fluoride between the areas, limited or no individual exposure information was reported. Among studies that provided drinking water levels of fluoride in two areas being compared, sufficient information to determine if the individual study subjects were exposed to these levels was often not reported. Some studies also lacked information on fluoride analysis methods and timing of the exposure measurements. In some cases (n = 4), study areas that were considered endemic for dental and/or skeletal fluorosis were compared to non-endemic areas, or high-fluoride areas were compared to low-fluoride areas, with no other information provided on fluoride levels in the areas. While living in an area endemic for fluorosis could be an indicator of exposure, these studies did not specify if the study subjects themselves had fluorosis. Another study used only dental fluorosis as a measure of fluoride exposure in subjects that were all from an endemic area with similar drinking water fluoride levels.

Outcome assessment

Studies included in this evaluation used a wide variety of methods to measure IQ and other cognitive effects. Measures of IQ were generally standardized tests of IQ; however, for these standardized methods to be considered low potential for bias they needed to be conducted in the appropriate population or modified for the study population. Because results of these tests can be subjective, it was important that the outcome assessors were blind to the fluoride exposure when evaluating the results of the tests. If the study reported that the assessor was blind to the exposure, this was assumed to mean that the outcome assessor did not have any knowledge of the exposure, including whether the study subjects were from high-fluoride communities.

The lower risk-of-bias studies have few concerns regarding outcome assessment. Only one study (Barberio *et al.* 2017b) had concerns for potential bias in the outcome assessment and that was due to the fact that the learning disability was self-reported. Blinding was not a concern in this study as fluoride was assessed in the urine, and it was not possible for the self-assessment to be based on knowledge of exposure. The remainder of the studies used appropriate measures of IQ or other cognitive effects for the study population. Twelve of the studies reported blinding of the outcome assessors. For the remainder of the studies, it was assumed that the outcome assessors were most likely blind because exposure was assessed via urine or drinking water obtained at the same time as the outcome assessment.

Among the studies with higher risk of bias, the main limitation in the outcome assessment was the lack of reporting on whether the outcome was assessed without knowledge of exposure. Although there is little concern that the children's knowledge of their own exposure would bias the way they took the IQ tests, there is potential for bias if the tests were administered by an interviewer, or if the scoring of results could be subjective (e.g., drawing tests), and the interviewer or scorer had knowledge of the children's exposure. Most of the studies did not provide sufficient information on the person scoring or administering the tests or other information on the assessment methods to alleviate concerns for potential interviewer or reviewer bias. In some cases, the outcomes were not considered sensitive measures (e.g., Seguin Form Board Test to test for IQ), or the test was not considered appropriate for

the study population (e.g., a test validated in a western population was used on a rural Chinese population).

IQ in Children

The results from 13 studies (2 prospective cohort and 11 cross-sectional studies) with lower potential for bias that evaluated IQ in children (Xiang *et al.* 2003, Rocha-Amador *et al.* 2007, Ding *et al.* 2011, Xiang *et al.* 2011, Saxena *et al.* 2012, Seraj *et al.* 2012, Choi *et al.* 2015, Zhang *et al.* 2015b, Das and Mondal 2016, Bashash *et al.* 2017, Cui *et al.* 2018, Yu *et al.* 2018, Green *et al.* 2019) provide consistent evidence that exposure to fluoride is associated with decreased IQ (see **Figure D1** through **Figure D7**); however, the analyses performed and the specific results varied by study. Higher fluoride exposure was associated with at least one measure of decreased IQ in each of the 13 studies. In several studies, a significant correlation was observed between decreased IQ level and increased concurrent fluoride levels in single serum samples (Zhang *et al.* 2015b) or single spot urine samples in children (Xiang *et al.* 2003, Rocha-Amador *et al.* 2007, Ding *et al.* 2011, Saxena *et al.* 2012, Zhang *et al.* 2015b, Das and Mondal 2016, Cui *et al.* 2018, Yu *et al.* 2018) (see **Figure D6** and **Figure D7**). Bashash *et al.* (2017) observed a significant inverse association between children's IQ and maternal urinary fluoride during pregnancy (measured during all three trimesters and included if at least one measurement was available; an increase of 0.5 mg/L of maternal urinary fluoride was associated with a 2.5-point decrease [95% CI: −4.12, −0.59] in IQ) in boys and girls combined (see **Figure D7**); however, the association between IQ level and children's urinary fluoride levels, while inverse, was not significant (single spot urine sample; an increase of 0.5 mg/L of child urinary fluoride was associated with a 0.89-point decrease [95% CI: −2.63, 0.85] in IQ) (Bashash *et al.* 2017). Green *et al.* (2019) also observed a significant decrease in IQ for boys associated with maternal urinary fluoride averaged across trimesters (a significant 4.49-point decrease [95% CI: −8.38, −0.60] in IQ per 1-mg/L increase in maternal urinary fluoride); results were not significant in girls (2.40-point increase [95% CI: −2.53, 7.33] in IQ) or in boys and girls combined (1.95-point decrease in IQ per 1-mg/L increase; 95% CI: −5.19, 1.28). Other measures of prenatal exposure (maternal fluoride intake or water fluoride concentrations) were associated with decreases in IQ in boys and girls combined although the authors did not report boys and girls separately, as they found no significant effect measure modification between child sex and fluoride exposure in these analyses (Green *et al.* 2019). Specifically, when evaluating the association between estimated maternal fluoride intake based on maternal water and beverage consumption during pregnancy and IQ in children, a 1-mg increase in daily maternal consumption of fluoride during pregnancy was associated with a significant decrease of 3.66 IQ points in boys and girls combined (95% CI: −7.16, −0.15). Similarly, water fluoride concentrations for pregnant women from fluoridated areas (mean water fluoride levels of 0.59 ± 0.08 mg/L) versus pregnant women from non-fluoridated areas (mean water fluoride levels of 0.13 ± 0.06 mg/L) were associated with a significant 5.29-point IQ decrease per 1-mg/L increase in fluoride in both boys and girls combined (95% CI: −10.39, −0.19) (Green *et al.* 2019).

Choi *et al.* (2015) conducted a pilot study with 51 children in an area of China with a wide range of fluoride concentrations in the drinking water. Aside from observing no association between the square root block design test score and fluoride exposure from drinking water, the authors observed consistent negative associations between IQ measures and fluoride in children's single spot urine or drinking water and significant associations between specific tasks from an omnibus IQ test (i.e., significant decrease in WISC-IV backward and total digit span scores) and fluoride exposure based on moderate or severe dental fluorosis in children (see **Figure D7**). Rocha-Amador *et al.* (2007) observed significant negative correlations between IQ and both water and children's single spot urinary fluoride levels in a population in Mexico (adjusted β = −10.2 per log fluoride increase [CIs not reported] and −16.9 per log fluoride increase [CIs not reported], respectively) (see **Figure D7**). The authors also observed a significant inverse

association between IQ and children's drinking water and single spot urinary arsenic levels (adjusted β = −6.15 [CIs not reported] and −5.72 [CIs not reported], respectively). Because fluoride and arsenic were highly correlated in the study area, the authors were not able to adjust for exposure to arsenic when evaluating the effects of fluoride exposure (Rocha-Amador *et al.* 2007). Ding *et al.* (2011) reported a negative dose-response relationship between children's single spot urinary fluoride levels and IQ (see **Figure D4**); after adjusting for age, using multiple linear regression, they found a 0.59 point decrease in IQ score (95% CI: −1.09, −0.08) per 1-mg/L increase in urinary fluoride (p-value < 0.0001) (see **Figure D7**). While observing no association between IQ and low children's single spot urinary fluoride levels (0.01–1.60 mg/L), Yu *et al.* (2018) observed significant negative associations (p values not reported) between IQ and median children's urinary fluoride levels (1.60–2.50 mg/L)—with a decrease in IQ score of 2.67 (95% CI: −4.67, −0.68) for every 0.5-mg/L increment of urinary fluoride—and high children's urinary fluoride levels at 2.50–5.54 mg/L with a decrease in IQ score of 0.84 (95% CI: −2.18, 0.50) for every 0.5-mg/L increment of urinary fluoride (see **Figure D7**). The authors also reported a significant negative association between drinking water fluoride levels at 3.40–3.90 mg/L (4.29-point decrease [95% CI: −8.09, −0.48] for every 0.5-mg/L increment of water fluoride); a 0.04-point decrease in IQ (95% CI: −0.33, 0.24) was observed for 0.5-mg/L increments of water fluoride at levels of 0.20–3.40 mg/L. When comparing water fluoride concentrations of >1 mg/L to ≤1 mg/L, there was a greater risk (adjusted OR = 1.25; 95% CI: 0.69, 2.26) for marginal intelligence (i.e., IQ score = 70–79) and a reduced chance (adjusted OR = 0.47; 95% CI: 0.32, 0.71) of excellent intelligence (i.e., IQ score ≥ 130) (see **Figure D4**). Similar results were observed using children's urinary fluoride levels (adjusted OR for marginal intelligence = 1.44; 95% CI: 0.72, 2.91; adjusted OR for excellent intelligence = 0.49; 95% CI: 0.26, 0.93) (Yu *et al.* 2018). Cui *et al.* (2018) observed a significant association between log-transformed children's single spot urine fluoride and decreased IQ scores (2.47 point decrease in IQ [95% CI: −4.93, −0.01] per unit increase in urinary fluoride), and the association was the strongest in subjects with the TT polymorphism in the dopamine receptor D2 gene which, according to the authors, probably results in a reduced D2 receptor density (12.31 point decrease in IQ [95% CI: −18.69, −5.94] per unit increase in urinary fluoride) (Cui *et al.* 2018).

The results from 48 studies with higher potential for bias that evaluated IQ in children provide consistent supporting evidence of decrements in IQ associated with exposures to fluoride. Forty-one of the 48 studies reported an association between high fluoride exposure and decreased IQ in children.

Other Neurodevelopmental or Cognitive Effects in Children

Among the studies with lower potential for bias, the results from three prospective cohort studies (Bashash *et al.* 2017, Valdez Jimenez *et al.* 2017, Bashash *et al.* 2018) and four cross-sectional studies (Li *et al.* 2008a, Rocha-Amador *et al.* 2009, Choi *et al.* 2015, Barberio *et al.* 2017b) provide mostly consistent results for associations of fluoride exposure with cognitive impairment in children other than decrements in IQ, such as hand-eye coordination, neurobehavioral assessment, behavioral capacity, and learning disabilities (see **Figure D8** through **Figure D10**). Because IQ cannot be assessed in infants, other neurodevelopmental tests were conducted. Two studies (Li *et al.* 2008a, Valdez Jimenez *et al.* 2017), based in China and Mexico, evaluated neonates (within 3 days of birth) or infants (3–15 months) (see **Figure D8** and **Figure D10**).

In neonates, the high fluoride group (based on a single maternal urine fluoride level just prior to birth [3.58 ± 1.47 mg/L] compared to controls [1.74 ± 0.96 mg/L]) had significant decreases (p < 0.05) in total neurobehavioral assessment scores (38.28 ± 1.10 in controls compared to 36.48 ± 1.09 in high fluoride group) and total behavioral capacity scores (11.34 ± 0.56 in controls compared to 10.05 ± 0.94 in high fluoride group) as measured by a standard neonatal behavioral neurological assessment (NBNA) method

(Li *et al.* 2008a). In infants, the Mental Development Index (MDI)—which measures functions including hand-eye coordination, manipulation, understanding of object relations, imitation and early language development—was significantly negatively correlated with maternal urinary fluoride in both the first and second trimesters (adjusted βs = −19.05 with standard error of 8.9 for first trimester and −19.34 with standard error of 7.46 for second trimester) (Valdez Jimenez *et al.* 2017). This study did not find an association between maternal fluoride during any trimester and Psychomotor Developmental Index (PDI), which measures gross motor development (adjusted βs = 6.28 and 5.33 for first and second trimesters, respectively; no variance provided) (Valdez Jimenez *et al.* 2017). The General Cognitive Index (GCI) of the McCarthy Scales of Children's Abilities (MSCA) in 4-year-old children was significantly negatively associated with maternal creatinine-adjusted urinary fluoride levels during pregnancy (collected during each trimester) even after adjusting for maternal bone lead (adjusted β = −3.15 [95% CI: −5.42, −0.87] in a model adjusting for main covariates (e.g., gestational age, weight at birth, sex, maternal smoking, and indicators of socioeconomic status); adjusted β = −5.63 [95% CI: −8.53, −2.72] in a model limited to a subset of cases who had data on maternal bone lead and adjusted for main covariates and maternal bone lead) (Bashash *et al.* 2017) (see **Figure D10**). Choi *et al.* (2015), however, evaluated cognitive function endpoints in addition to IQ and found no significant associations between concurrent water or urinary fluoride levels and Wide Range Assessment of Visual Motor Ability (WRAVMA) scores, finger tapping, and the grooved pegboard test although there were some significant associations based on degree of fluorosis (see **Figure D10**). Another study using construction and memory scores in children 6–11 years old observed statistically significant decreases with increasing concurrent child single spot urinary fluoride even after adjusting for age ($p < 0.05$; −0.29 and −0.27 for copy and immediate recall, respectively [CIs not reported]); however, scores were not significantly associated with urinary arsenic levels (−0.05 and 0.02 for copy and immediate recall, respectively [CIs not reported]) (Rocha-Amador *et al.* 2009) (see **Figure** D9).

Barberio *et al.* (2017b) evaluated learning disabilities in children 3–12 years of age, including ADHD, attention deficit disorder (ADD), and dyslexia, as part of the Canadian Health Measures Survey and found a small but significantly increased risk in self-reported (children 12 years of age) or parent- or guardian-reported (children 3–11 years of age) learning disabilities associated with higher spot urinary fluoride levels in children (adjusted OR = 1.02; 95% CI: 1.00, 1.03) (see **Figure D11**); however, significant associations were not observed in analyses using creatinine- or specific gravity-adjusted urinary fluoride (Barberio *et al.* 2017b). Barberio *et al.* (2017b) also reported no associations between single spot urinary fluoride and ADHD in children ages 3 to 12 years. Bashash *et al.* (2018) evaluated behaviors associated with ADHD in children ages 6–12 years using the Conners' Rating Scales-Revised (CRS-R) and observed significant associations between maternal urinary fluoride (measured during each trimester) and ADHD-like symptoms, particularly those related to inattention (an increase in 0.5 mg/L of maternal urinary fluoride was associated with a 2.84-point increase [95% CI: 0.84, 4.84] in the DSM-IV Inattention Index and a 2.54-point increase [95% CI: 0.44, 4.63] in the Cognitive Problems and Inattention Index). These two scales contributed to the global ADHD Index and the DSM-IV ADHD Total Index, which were also associated with higher levels of prenatal fluoride exposure (an increase of 0.5 mg/L in maternal urinary fluoride was associated with a 2.38-point increase [95% CI: 0.42, 4.34] in the DSM-IV ADHD Total Index and a 2.47-point increase [95% CI: 0.43, 4.50] in the ADHD Index) (see **Figure D10)**. Significant associations were not observed between maternal urinary fluoride concentrations during pregnancy and child performance on measures of hyperactivity nor were there any significant results in children using the Connors' Continuous Performance Test (CPT-II, 2nd Edition), a computerized test of sustained attention and inhibitory control (Bashash *et al.* 2018).

Higher risk-of-bias studies (n = 5) also provide some evidence of associations of fluoride exposure with neurodevelopmental or cognitive effects in children other than effects on IQ, but the results are inconsistent with heterogeneous outcomes (Shannon *et al.* 1986, Morgan *et al.* 1998, Li *et al.* 2008b, Malin and Till 2015, Mustafa *et al.* 2018).

Cognitive Effects in Adults

Results from two lower risk-of-bias studies in adults did not find consistent evidence for an association between cognitive impairment (based on the Mini-Mental State Examination) and exposure to fluoride (Jacqmin *et al.* 1994, Li *et al.* 2016). Jacqmin *et al.* (1994) did not find an association between drinking water fluoride and cognitive impairment in populations in France (n = 3,490) and found prevalence rates of cognitive impairment to be the same regardless of fluoride exposure (see **Figure D12**). In an analysis of 38 cognitively-impaired cases and 38 controls matched for several confounders including age, gender, education, alcohol consumption, and smoking, Li *et al.* (2016) did find significantly higher urinary fluoride levels and skeletal fluorosis scores in the cognitively-impaired group compared with the control group; however, the authors found no significant correlation between cognitive impairment and total daily water fluoride intake (adjusted ORs = 0.94 [95% CI: 0.85, 1.04] and 0.86 [95% CI: 0.69, 1.06] in the moderate and severe cognitive impairment groups, respectively) or urinary fluoride levels (adjusted ORs = 1.12 [95% CI: 0.89, 1.42] and 1.25 [95% CI: 0.87, 1.81] in the moderate and severe cognitive impairment groups, respectively) in subjects from fluorosis-endemic areas of China (n = 511).

Higher risk-of-bias studies (n = 7) provide some evidence of cognitive impairment in adults associated with exposure to fluoride. In aluminum factory workers (exposed to gaseous and particular fluoride emissions during the production of aluminum metal), significant decreases in IQ (Duan 1995), diminished performance on several neurobehavioral core battery tests (NCTBs) (Guo *et al.* 2008b), and impaired psychomotor performance and memory were observed (Yazdi *et al.* 2011). One study conducted on adult subjects with fluorosis (dental and skeletal) from a fluorosis-endemic area compared with healthy subjects from a non-endemic area observed significant differences for some cognitive function tests (i.e., tests of speech fluency, recognition, and working memory) but not others and generally did not observe a significant change in IQ except in the operation scores (Shao 2003). One prospective cohort study evaluated exposure to fluoride in children at age of 5 years based on whether or not the children resided in areas with community water fluoridation or used fluoride toothpaste or fluoride tablets, and found no clear differences in IQ scores of the subjects at age 38 years (Broadbent *et al.* 2015). One additional study suggested that populations living in areas with higher drinking water fluoride had lower levels of dementia (Still and Kelley 1980); however, the study was not focused on effects of fluoride, but rather if fluoride was able to reduce the risk associated with aluminum by competing with aluminum and reducing the aluminum bioavailability. Therefore, the study was considered inadequate to evaluate the effects of fluoride on dementia (Still and Kelley 1980). A more recent study in Scotland evaluated dementia rates associated with aluminum and fluoride drinking water concentrations and observed an increase in dementia only in the highest quartile of fluoride (56.3 µg/L) compared to the lowest quartile (<44.4 µg/L), but found a significant increase with all quartiles of aluminum compared with the reference group (Russ *et al.* 2019). In addition to studies that reported on cognitive impairment and exposure to fluoride, two studies were identified that reported effects on motor and sensory function (Rotton *et al.* 1982) and a higher prevalence of self-reported headaches, insomnia, and lethargy (Sharma *et al.* 2009).

Mechanistic Data in Humans

Eight lower risk-of-bias studies were available that evaluated mechanistic data in humans associated with fluoride exposure that was considered potentially relevant to neurological effects, including effects

on thyroid hormones in children (Singh *et al.* 2014, Zhang *et al.* 2015b, Kumar *et al.* 2018), adults (Kheradpisheh *et al.* 2018a, Kheradpisheh *et al.* 2018b, Malin *et al.* 2018), or children and adults combined (Barberio *et al.* 2017a). In addition, some studies evaluated self-reported thyroid conditions in children and adults combined (Barberio *et al.* 2017a) and thyroid diseases in adults (Peckham *et al.* 2015, Kheradpisheh *et al.* 2018b) (see **Figure A3-5** and **Figure A3-6**). Although the lower risk-of-bias studies provide some evidence of mechanistic effects (primarily changes in thyroid stimulating hormone [TSH] levels in children), the studies were too heterogeneous or limited in number to make any determination on mechanism (see **Figure 7**).

Among the seven lower risk-of-bias studies that reported on changes in thyroid hormones, three studies were conducted in children (Singh *et al.* 2014, Zhang *et al.* 2015b, Kumar *et al.* 2018) and reported increases in TSH levels. Zhang *et al.* (2015b) reported significant increases in TSH in children from a fluorosis-endemic area (median fluoride drinking water concentration = 1.40 mg/L; interquartile range = 1.23–1.57 mg/L) compared with a non-fluorosis-endemic area (median fluoride drinking water concentration = 0.63 mg/L; interquartile range = 0.58–0.68 mg/L), while 3,5,3'-triiodothyronine (T_3) or thyroxine (T_4) were not significantly different between the two groups. Similarly, Singh *et al.* (2014) observed significantly higher TSH levels in children without dental fluorosis who lived in a fluorosis-endemic area (fluoride drinking water concentrations of 1.6–5.5 mg/L) compared with children without dental fluorosis who lived in a non-fluorosis-endemic area (fluoride drinking water concentrations of 0.98–1.00 mg/L). Higher TSH levels in children with dental fluorosis from the fluorosis-endemic area compared with children without dental fluorosis from the non-fluorosis-endemic area were observed but did not reach statistical significance. Significant differences in T_4 or T_3 were not observed between groups (Singh *et al.* 2014). Kumar *et al.* (2018) also observed a significant increase in TSH levels in children from a fluorosis endemic area (1.5–5.8 mg/L fluoride) compared with a control area (0.94–1.08 mg/L fluoride). There were also decreases in T_3 and T_4, but results were not statistically significant.

Barberio *et al.* (2017a) evaluated fluoride effects on TSH levels in children and adults combined and found no relationship between fluoride exposure (measures in urine and tap water) and TSH levels. In the one study that evaluated thyroid hormone levels in adults but not children, Kheradpisheh *et al.* (2018b) found a significant increase in TSH associated with higher fluoride concentrations in drinking water in both adults with and without thyroid diseases such as hypothyroidism, hyperthyroidism, thyroid nodules, or thyroid cancer. Significant increases in T_3 were associated with higher fluoride in drinking water in adults without thyroid diseases, but increases in T_3 were not significant in adults with thyroid diseases. A significant association between T_4 and higher fluoride in drinking water was not observed in adults with or without thyroid diseases (Kheradpisheh *et al.* 2018b).

Other than changes in hormone levels, there is limited evidence of fluoride-related mechanistic effects in the three lower risk-of-bias studies that evaluated thyroid-related effects. Barberio *et al.* (2017a) found no relationship between fluoride exposure and self-reported thyroid conditions in children and adults (children were older than 12). Kheradpisheh *et al.* (2018b) also found no association between fluoride exposure and hypothyroidism in an adult population in Iran. One study found a significantly higher prevalence of hypothyroidism in areas with higher fluoride concentrations in drinking water (>0.7 mg/L) compared with areas with lower fluoride drinking water concentrations (≤0.7 mg/L) (Peckham *et al.* 2015).

Several higher risk-of-bias studies were available that evaluated potential mechanistic data in humans associated with fluoride exposure, including effects on thyroid hormones mostly in children (n = 11 studies); catecholamines in adults (Michael *et al.* 1996) or in subjects of unknown ages (Chinoy

and Narayana 1992); acetylcholinesterase (AChE) or serotonin levels in children (Singh *et al.* 2013, Lu *et al.* 2019); brain histopathology or biochemistry in aborted fetuses (Du *et al.* 2008, Yu *et al.* 2008); and mitochondrial fission/fusion molecules in children (Zhao *et al.* 2019). Similar to the lower risk-of-bias studies, the higher risk-of-bias studies provide some evidence of mechanistic effects (primarily changes in TSH levels in children); however, the data are insufficient to identify a clear mechanism by which fluoride causes neurodevelopmental or cognitive effects in humans.

Among higher risk-of-bias studies (see **Figure A3-7** and **Figure A3-8**), varying results were reported in 11 studies that evaluated fluoride exposure and effects on thyroid hormones, and a few of these studies (Lin *et al.* 1991, Wang *et al.* 2001, Yang *et al.* 2008) were complicated by high or low iodine in the high fluoride area. When considering fluoride effects on each of the hormones individually, similar to results from lower risk-of-bias studies, the most consistent evidence of fluoride-associated effects on a thyroid hormone was reported as changes in TSH levels in children, although there was some variation in the direction of effect. Six of the nine higher risk-of-bias studies that evaluated changes in TSH levels in children reported increases in TSH levels with higher fluoride (Lin *et al.* 1991, Yao *et al.* 1996, Wang *et al.* 2001, Susheela *et al.* 2005, Yang *et al.* 2008, Yasmin *et al.* 2013). Two of the nine higher risk-of-bias studies reported decreases in TSH levels in children with higher fluoride (Khandare *et al.* 2017, Khandare *et al.* 2018). One of the nine studies found no significant alterations in TSH levels in children from fluorosis-endemic areas (Hosur *et al.* 2012) (see **Figure 8**).

When considering fluoride-associated effects on TSH, T_3, and T_4 levels together, studies that evaluated changes in all three thyroid hormones reported varying combinations of increases, decreases, or no changes in levels across the three hormones, although among the eight lower and higher risk-of-bias studies that evaluated the effects of fluoride exposure on TSH, T_3, and T_4 levels and reported increases in TSH levels in children, seven of the eight studies found no alterations in T_3 levels (one study found an increase in T_3), and six of the eight studies found no alterations in T_4 levels (two studies found an increase in T_4). Studies also displayed variation by age in fluoride-associated effects on TSH, T_3, and T_4. Due to the dynamic relationship between the thyroid gland, the pituitary gland, and the production and clearance of TSH, T_3, and T_4, the variations in results are not unexpected and do not eliminate the possibility of a mechanistic link between thyroid effects and neurodevelopmental or cognitive effects; however, the data do not support a clear indication that thyroid effects are a mechanism by which fluoride causes these effects in humans.

In addition to evaluating thyroid hormone levels, a few higher risk-of-bias studies evaluated other mechanistic data associated with fluoride exposure; however, the data are insufficient to identify a clear mechanism by which fluoride might cause neurodevelopmental or cognitive effects in humans. Serum epinephrine and norepinephrine were significantly increased in a fluoride-endemic region (not reported whether subjects were children or adults) compared to a non-endemic region (Chinoy and Narayana 1992). Serum adrenaline and noradrenaline were significantly increased in adults in a fluoride-endemic area (fluoride in the drinking water ranged from 1.0–6.53 ppm) compared to a control area (fluoride in the drinking water ranged from 0.56–0.72 ppm) (Michael *et al.* 1996). Serum AChE was significantly reduced in children from a high fluoride region compared to a lower fluoride region (Singh *et al.* 2013). Serum serotonin was significantly increased in children from Turkey who were drinking water containing 2.5 mg/L of fluoride compared to children drinking bottled water or water containing <0.5 mg/L of fluoride (Lu *et al.* 2019). Aborted fetuses from high fluoride areas in China were found to have histological changes in the brain and significant changes in neurotransmitter levels compared to a control area (Du *et al.* 2008, Yu *et al.* 2008).

There are also two more recent lower risk-of-bias studies that evaluated polymorphisms in dopamine-related genes; however, a determination on mechanism cannot be made at this time due to the limited number of studies. For children (10–12 years old) with a Val158Met polymorphism in the *COMT* gene (i.e., catechol-O-methyltransferase), which results in slower degradation and greater availability of dopamine within the brain, a stronger association between increasing urinary fluoride levels and decreasing IQ was reported (Zhang *et al.* 2015b). For children (7–12 years old) with a dopamine receptor-2 (DRD2) Taq 1A polymorphism (which is involved in reduced D2 receptor density and availability) and the TT (variant) genotype, a significant inverse relationship between log urine fluoride and IQ was observed; however, this significant relationship was not observed in children with the CC (wild-type) or CT (hybrid) genotypes (Cui *et al.* 2018).

Figure 7. Number of Lower Risk-of-bias Studies that Evaluated Thyroid Hormones in Children and Adults by Endpoint and Direction of Effect*

Endpoint	Direction of Effect ↑	Direction of Effect NS	Grand Total
serum T3	1	3	4
serum T4		4	4
serum TSH	5	2	7
self-reported thyroid condition		1	1
thyroid disease		1	1
hypothyroidism prevalence	1		1

*Interactive figure and additional study details in Tableau® (https://public.tableau.com/profile/ntp.visuals#!/vizhome/Fluoride_EpiThyroid_UPDATE/Figures6and7). This figure displays study counts for lower risk-of-bias studies in both children and adults, as these counts are most relevant to the summary of fluoride-related mechanistic effects in lower risk-of-bias studies. Counts for higher risk-of bias studies and studies by age (i.e., children, adults, or children/adults combined) can also be accessed in the interactive figure in Tableau®. Study counts are tabulated by significance (unless if study footnotes in Tableau indicate that statistical significance was not tested) —statistically significant increase (↑), statistically significant decrease (↓), or not significant (NS). For example, the "↑" column displays numbers of unique studies with significantly increased results.

Figure 8. Number of Higher Risk-of-bias Studies that Evaluated Thyroid Hormones in Children by Endpoint and Direction of Effect*

Endpoint	Direction of Effect ↑	Direction of Effect ↓	Direction of Effect NS	Grand Total
serum T3	1	1	6	8
serum T4	3		5	8
serum TSH	6	2	1	9

*Interactive figure and additional study details in Tableau® (https://public.tableau.com/profile/ntp.visuals#!/vizhome/Fluoride_EpiThyroid_UPDATE/Figures6and7). This figure displays study counts for higher risk-of-bias studies in children, as these counts are most relevant to the

summary of fluoride-related effects on thyroid hormones in higher risk-of-bias studies. Counts for lower risk-of bias studies, studies in adults, or all studies combined, can also be accessed in the interactive figure in Tableau®. Study counts are tabulated by significance (unless if study footnotes in Tableau indicate that statistical significance was not tested) —statistically significant increase (↑), statistically significant decrease (↓), or not significant (NS). For example, the "↑" column displays numbers of unique studies with significantly increased results.

Animal Learning and Memory Data

In 2016, NTP conducted a systematic review of the available experimental animal studies to develop level-of-evidence conclusions on the association between fluoride exposure and neurobehavioral effects, specifically effects related to learning and memory impairment (NTP 2016). As previously discussed, the evaluation of the animal body of evidence in this assessment is an update to the NTP (2016) systematic review and is consistent with the methodology and format used in that report.

NTP (2016) identified two main issues with the animal body of evidence related to effects of fluoride exposure on learning and memory: indirectness and concerns for risk of bias. The concern related to indirectness was based on the fact that many learning and memory tests rely on a motor response (e.g., latency to achieve the desired effect). Changes in motor function or activity levels associated with fluoride exposures could complicate the interpretation of the results on learning and memory test performance depending on the outcome measured. The directness of the measure as an indicator of learning and memory (i.e., the ability to rule out impaired motor or sensory function) was considered when addressing confidence in the data. Concerns in these studies related to risk of bias included the following factors: lack of randomization, lack of blinding or other methods to reduce potential bias at outcome, lack of exposure information, lack of control for litter effects, lack of expected response in the control animals, and lack of reporting of other key study information such as sample size or sex of the animals.

Since the NTP (2016) report was published, additional experimental animal studies were identified that evaluated learning and memory impairment associated with fluoride exposure, including 12 developmental exposure studies (Banala and Karnati 2015, Mesram *et al.* 2016, Zhu *et al.* 2017, Banala *et al.* 2018, Bartos *et al.* 2018, Chen *et al.* 2018, Ge *et al.* 2018a, Ge *et al.* 2018b, McPherson *et al.* 2018, Sun *et al.* 2018, Wang *et al.* 2018, Zhao *et al.* 2019); 5 Morris water maze study in adults (Zheng *et al.* 2016, Dong *et al.* 2017, Niu *et al.* 2018, Sharma *et al.* 2018, Yang *et al.* 2018); and 7 other maze studies in adults (Shalini and Sharma 2015, Pulungan *et al.* 2016, Sudhakar *et al.* 2017, Nageshwar *et al.* 2018, Sharma *et al.* 2018, Raju *et al.* 2019, Yuan *et al.* 2019). In addition, 12 studies were identified that evaluated motor activity/coordination or sensory effects without evaluating learning and memory impairment (Adedara *et al.* 2017a, Ahmad *et al.* 2017, Nageshwar *et al.* 2017, Agustina *et al.* 2018, Kinawy and Al-Eidan 2018, Nkpaa and Onyeso 2018, Sudhakar *et al.* 2018b, a, Jia *et al.* 2019, Li *et al.* 2019, Lu *et al.* 2019, Manusha *et al.* 2019).

Although Adedara *et al.* (2017a) and Nkpaa and Onyeso (2018) evaluated exploration, the authors concluded that the track plots in the open field novel environment test were consistent with impaired locomotor activity in the fluoride-treated animals. The additional studies reviewed did not address the concern of indirectness and most included risk-of-bias concerns; however, a few of these more recent studies are notable in that they provide results on learning and memory effects that could possibly be distinguished from effects on motor activity. Bartos *et al.* (2018) used a step-down inhibitory avoidance test to evaluate short-term and long-term memory in rat offspring. Although the authors did not discuss activity in the animals, this test would be expected to result in increased latency in animals if there was decreased activity with fluoride exposure. The fluoride-treated female offspring, however, had decreased latency indicating diminished memory of the foot shock. Chen *et al.* (2018) also evaluated

female rat offspring (treatment continued until the offspring were 6 months old) and observed an effect of fluoride on latency to reach the platform and the number of platform crossings in the Morris Water Maze; however, swimming speed was measured, and no changes were observed. The tracks during the spatial probe test were also very different in the two higher exposed groups (i.e., 50 and 100 mg/L NaF), suggesting that the animals did not know the location of the platform. It is not clear if litter effects were addressed in the study.

After further evaluation of the data available in NTP (2016) and in this update, it is concluded that the animal data are inadequate to evaluate the effects of fluoride on learning and memory primarily due to the inability to separate the learning and memory effects from the effects on motor activity or motor coordination. The majority of the studies that evaluated effects of fluoride on learning and memory did not also evaluate a motor activity component to determine if the learning and memory effects could be attributed to motor activity or coordination deficits. Of the studies that did evaluate both learning and memory and motor activity/coordination, studies 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. In addition, studies that found effects on motor activity/coordination or learning and memory often did not provide sufficient indicators of general health of the animals to reliably attribute impaired performance on a task to a specific acquisition of learning and memory or motor activity/coordination. The few studies that provided this information used different test methods or results were inconsistent. Thus, it is difficult to conclude that evidence from experimental animal studies is meaningful when considering the specific question of fluoride's potential influence on human IQ or cognitive function. Based on this consideration, the experimental animal body of evidence does not contribute to confidence in conclusions derived from human epidemiological studies with respect to effects on human IQ. Although the evidence supports an association between fluoride exposure and neurodevelopmental effects, the data are not sufficient to support the primary effect evaluated in children (i.e., IQ) nor is it sufficient to support a conclusion on cognitive effects in adults especially in the absence of additional adult human data.

Mechanistic Data in Animals

There are a wide variety of studies in animals that evaluate mechanistic effects potentially related to neurological changes following oral fluoride exposure (see **Figure 9**). Categories of mechanistic endpoints with the largest amount of available data include changes in biochemical components of the brain or neurons, neurotransmitters, oxidative stress, histopathology, and thyroid function. Limiting the data to studies with at least one exposure at or below 20 ppm fluoride drinking water equivalents (gavage and dietary exposures were back calculated into equivalent drinking water concentrations for comparison) still provided a sufficient number of studies for evaluation of these mechanistic endpoints. Neurotransmitter and biochemical changes in the brain and neurons were considered to be the mechanistic areas with the greatest potential to demonstrate effects of fluoride on the brain of animals in the lower dose range and provide evidence of changes in the brain that may relate to decreased IQ in children (see **Figure 10**). Histological data can be useful in determining whether effects are occurring in the brain at lower fluoride concentrations; however, author descriptions of these effects may be limited thereby making it difficult to directly link histological changes in the brain to learning and memory effects. Oxidative stress is considered a general mechanistic endpoint that cannot be specifically linked to neurodevelopmental or cognitive effects in humans; however, like histopathology, it may help in identifying changes in the brain occurring at lower concentrations of fluoride. Although any effects in the brain or neurological tissue at lower concentrations of fluoride may support reduced IQ in humans,

it may be difficult to distinguish the potential effects of fluoride on learning and memory functions from other neurological outcomes (e.g., locomotor activity in animal models).

Figure 9. Number of Animal Mechanistic Studies for Fluoride by Mechanistic Category and Exposure Level*

Mechanism	Dose Level	
	All	≤20 ppm
Biochemical (brain/neurons)	66	25
Neurotransmitters	53	23
Oxidative stress	78	25
Histopathology	67	30
Apoptosis/cell death	19	7
Inflammation	7	5
Thyroid	50	17
Other	3	2

*Interactive figure and additional study details in Tableau® (https://public.tableau.com/profile/ntp.visuals#!/vizhome/Animal_Mechanisms_All_June2019/Figure8). The number of studies that evaluated mechanistic effects associated with at least one exposure at or below 20 ppm fluoride is tabulated in the "≤20 ppm" column. The total number of studies per mechanistic category are summarized in the "All" column.

The following sections summarize the mechanistic data by category of mechanistic endpoint. Although there is some evidence of consistency in mechanistic effects, overall these data are insufficient to increase confidence or support a change to hazard conclusions.

Neurotransmitters

Twenty of 23 neurotransmitter studies assessed changes in brain cholinesterase activity associated with fluoride exposure at or below 20 ppm fluoride. Acetylcholine is a major neurotransmitter involved in learning, memory, and intelligence (Chen 2012, Gais and Schonauer 2017). AChE is responsible for the breakdown of acetylcholine in the synapses of nerve cells. Changes in cholinesterase, acetylcholine, or AChE could be related to effects on memory. Evidence of an effect varied among the lower risk-of-bias studies that assessed changes in cholinesterase or acetylcholine (n = 11 drinking water studies) (Gao *et al.* 2008a, Sun *et al.* 2008, Gao *et al.* 2009, Chouhan *et al.* 2010, Liu *et al.* 2010, Baba *et al.* 2014, Akinrinade *et al.* 2015a, Mesram *et al.* 2016, Adedara *et al.* 2017a, Khan *et al.* 2017, Nkpaa and Onyeso 2018), with the majority of studies reporting evidence of an effect that is considered inconsistent with the phenotypic outcome. Decreases in cholinesterase will cause increases in acetylcholine, which can have a positive effect on learning and memory; however, long-term decreases in cholinesterase can lead to secondary neuronal damage occurring in the cholinergic region of the brain (Chen 2012).

Five of the 11 studies with lower risk of bias (Gao *et al.* 2009, Baba *et al.* 2014, Adedara *et al.* 2017a, Khan *et al.* 2017, Nkpaa and Onyeso 2018) found statistically significant decreases in cholinesterase or AChE in brain homogenates (with some brains dissected into specific regions prior to homogenizing) with fluoride concentrations in drinking water at or below 20 ppm, and 4 of the 5 studies found statistically significant decreases in cholinesterase or AChE below 10 ppm. The 5 studies were conducted in rats (Wistar or Sprague-Dawley) with exposure ranging from 28 days to 6 months. An additional 2 out

of 11 studies (Gao *et al.* 2008a, Akinrinade *et al.* 2015a) reported decreases in brain homogenate AChE at concentrations at or below 20 ppm fluoride in drinking water, but statistical significance was not reached. These studies were also conducted in rats with exposure for 30 days or 3 months. Gao *et al.* (2008a) reported a dose-dependent decrease in brain homogenate AChE in the low (5 ppm fluoride) and high (50 ppm fluoride) treatment groups compared with the control group, but the decrease was only statistically significant in the high dose group. Similarly, Akinrinade *et al.* (2015a) observed a dose-dependent decrease in percent intensity of AChE immunohistochemistry in the prefrontal cortex associated with 2.1 and 10 ppm sodium fluoride in the drinking water, but neither result was statistically significant. Gao *et al.* (2009) found lower brain homogenate AChE levels in the 5-ppm animals compared with the 50-ppm animals; therefore, the results were not always dose dependent.

Relative to the above-mentioned studies, 2 of the 11 lower risk-of-bias studies observed opposite effects on brain cholinesterase levels. Sun *et al.* (2008) observed a significant increase in brain cholinesterase in Kunming mice associated with fluoride drinking water concentrations from 10 to 100 mg/L, but did not observe a dose response. Chouhan *et al.* (2010) did observe a dose-related increase in AChE levels in brain homogenate of Wistar rats with sodium fluoride concentrations of 1 to 100 ppm for 12 weeks and noted statistically significant results at 1, 50, and 100 ppm but not at 10 ppm.

Mesram *et al.* (2016) did not assess changes in AChE but observed a significant decrease in acetylcholine levels in cerebral cortex homogenate through 30 days of age in rats treated in utero with 20 ppm sodium fluoride, which may suggest an increase in AChE levels. Likewise, Liu *et al.* (2010) did not assess changes in AChE, but measured nicotinic acetylcholine receptors (nAChRs) in brain homogenate of rats following drinking water fluoride exposure, which the authors stated could modulate physiological and pharmacological functions that are involved in learning and memory-related behaviors. Significant decreases in the protein expressions of nAChR subunits at 2.26 ppm fluoride were observed; however, the corresponding receptor subunit mRNAs did not exhibit any changes (Liu *et al.* 2010).

The studies that assessed other neurotransmitters of the brain and neurons were too heterogeneous or limited in number to make any determination on mechanism, even before limiting the review of the data to lower risk-of-bias studies. There were only five studies that evaluated dopamine and/or metabolites (Tsunoda *et al.* 2005, Chouhan *et al.* 2010, Reddy *et al.* 2014, Banala *et al.* 2018, Sudhakar and Reddy 2018). Four of the studies observed decreases in dopamine levels in the brain with exposures less than 20 ppm fluoride (Chouhan *et al.* 2010, Reddy *et al.* 2014, Banala *et al.* 2018, Sudhakar and Reddy 2018); however, the fifth study (Tsunoda *et al.* 2005) observed increased dopamine and metabolites at fluoride exposures below 20 ppm (with statistical significance achieved only for the metabolite homovanillic acid in one brain region). No differences from the control group were observed at levels above 20 ppm fluoride. Other neurotransmitters were evaluated at or below 20 ppm fluoride exposure, but generally only in a couple of studies.

Biochemistry (brain/neurons)

Similar to above, the endpoints measured in brain biochemistry studies were too heterogeneous or limited in number to make any determination on potential relevance of mechanism, even before limiting the review of the data to lower risk-of-bias studies (see **Figure 10**). Endpoints related to biochemical changes in the brain or neurons included carbohydrate or lipid changes, RNA or DNA changes, changes in gene expression, or changes in protein expression. For the most part, only a single study was available for any given endpoint. The largest body of evidence on biochemistry was on protein level in various brain regions. Eleven lower risk-of-bias studies were identified that evaluated protein levels; however, few studies evaluated the same proteins or areas of the brain. In the few cases where

the same protein was evaluated, results were not always consistent. These data are insufficient to increase confidence or support a change to hazard conclusions.

Histopathology

Histopathology of the brain was evaluated in 31 studies with concentrations at or below 20 ppm fluoride, of which 15 studies had a lower potential for bias (Bhatnagar *et al.* 2002, Chouhan *et al.* 2010, Bhatnagar *et al.* 2011, Lou *et al.* 2013, Jiang *et al.* 2014, Akinrinade *et al.* 2015a, Guner *et al.* 2016, Mesram *et al.* 2016, Pulungan *et al.* 2016, Adedara *et al.* 2017b, McPherson *et al.* 2018, Nageshwar *et al.* 2018, Niu *et al.* 2018, Jia *et al.* 2019, Zhao *et al.* 2019). In all but one lower risk-of-bias study [Pulungan *et al.* (2016); gavage], animals were exposed to fluoride via drinking water. All lower risk-of-bias studies were conducted in rodents, and all but three studies were conducted in rats (Wistar [seven studies]; Sprague-Dawley [four studies]; Long-Evans hooded [one study]). Overall, the lower risk-of-bias studies that evaluated histopathology in the brain had low potential for bias for key questions regarding randomization and exposure characterization; however, eight studies were rated as probably high risk of bias for the key risk-of-bias question regarding outcome assessment based on lack of reporting of blinding of outcome assessors and/or inadequate description of outcome measures or lesions. Moreover, low image quality in some of the studies hampered the ability to verify the quality of the data. Further technical review of the 15 lower risk-of-bias studies was conducted by a board-certified pathologist. Based on confidence in the results for each study, the technical reviewer further categorized the lower risk-of-bias studies as studies with higher or lower confidence in the outcome assessment, which is reflected in the following summary of the brain histopathology results. Main limitations of the histopathology data identified by the pathologist included lack of information on methods of euthanasia and fixation. Perfusion fixation is generally considered the best practice for lesions of the central nervous system in addition to complete fixation of the brain prior to its removal from the skull (Garman *et al.* 2016). Four of the lower risk-of-bias studies reported that they used this method (Bhatnagar *et al.* 2002, Bhatnagar *et al.* 2011, Pulungan *et al.* 2016, McPherson *et al.* 2018). Two of the lower risk-of-bias studies handled the brains before fixation was complete, which can produce artifacts that can resemble dead neurons (Nageshwar *et al.* 2018, Zhao *et al.* 2019). Fixation and brain removal details were inadequately described in the remaining lower risk-of-bias studies.

Although there was heterogeneity in the endpoints reported (e.g., cell size, shape, and counts; nuclei fragmentation; increased vacuolar spaces) and some variation in the consistency of the evidence based on the area of the brain evaluated, the majority of the lower risk-of-bias studies (11 of 14 drinking water studies) found some histological change in the brain of rats or mice treated with fluoride at concentrations at or below 20 ppm, of which 8 studies reported histological changes in the brain at or below 10 ppm. Histological changes in the hippocampus (one of the areas of the brain most evaluated for histological changes) associated with fluoride exposures at or below 20 ppm were reported in three of four lower risk-of-bias studies with higher confidence in the outcome assessment (Bhatnagar *et al.* 2002, Bhatnagar *et al.* 2011, Guner *et al.* 2016) and in three of four lower risk-of-bias studies with lower confidence in the outcome assessment (Jiang *et al.* 2014, Nageshwar *et al.* 2018, Niu *et al.* 2018). McPherson *et al.* (2018) was the only drinking water study (with higher confidence in the histopathology outcome assessment) that did not observe any histological changes in hippocampus at 10 or 20 ppm fluoride in male Long-Evans hooded rats exposed in utero through adulthood (>PND80). Although there are too few studies to definitively explain the inconsistency in results, McPherson *et al.* (2018) also did not observe any associations between fluoride exposure and impairments to learning and memory, which is inconsistent with the majority of developmental exposure studies that observed learning and impairments associated with fluoride exposure for other strains of rats. Similarly, histological changes in the cortex were reported in three of the four lower risk-of-bias drinking water studies with higher

confidence in the outcome assessment (Chouhan *et al.* 2010, Bhatnagar *et al.* 2011, Akinrinade *et al.* 2015a) and in three of four lower risk-of-bias studies with lower confidence in the outcome assessment (Lou *et al.* 2013, Mesram *et al.* 2016, Nageshwar *et al.* 2018).

Histological changes were also consistently reported in other areas of the brain in studies with higher confidence in the outcome assessment, including the amygdala, caudate putamen, cerebellum, and hypothalamus, although each of these areas of the brain were only evaluated in one lower risk-of-bias study (Bhatnagar *et al.* 2011, Guner *et al.* 2016). Pulungan *et al.* (2016), one of two lower risk-of-bias studies with higher confidence in the outcome assessment that did not report histological changes in the brain, observed a decreasing trend in the number of pyramidal cells in the prefrontal cortex with increasing dose, but this was not changed at concentrations below 20 ppm (study administered sodium fluoride via gavage; the 5-mg/kg-day dose was considered to be equivalent to 15.3 ppm fluoride in drinking water) nor were any of the results statistically significant.

Oxidative stress

Oxidative stress in the brain was evaluated in 25 studies that examined concentrations at or below 20 ppm fluoride, of which 15 studies had lower potential for bias (Shan *et al.* 2004, Chouhan and Flora 2008, Gao *et al.* 2008b, Gao *et al.* 2009, Chouhan *et al.* 2010, Akinrinade *et al.* 2015b, Zhang *et al.* 2015a, Guner *et al.* 2016, Mesram *et al.* 2016, Adedara *et al.* 2017a, Adedara *et al.* 2017b, Khan *et al.* 2017, Bartos *et al.* 2018, Nageshwar *et al.* 2018, Nkpaa and Onyeso 2018). All of the lower risk-of-bias studies were conducted in rats (mainly Wistar or Sprague-Dawley) and administered fluoride via drinking water with exposure durations ranging from 28 days to 7 months. Although there was heterogeneity in the endpoints reported (i.e., varying measures of protein oxidation, antioxidant activity, lipid peroxidation, and reactive oxygen species [ROS]) and some variation in the consistency of the evidence based on the endpoint, the majority of the studies (13 of 15 studies) (Shan *et al.* 2004, Gao *et al.* 2008b, Gao *et al.* 2009, Akinrinade *et al.* 2015b, Zhang *et al.* 2015a, Guner *et al.* 2016, Mesram *et al.* 2016, Adedara *et al.* 2017a, Adedara *et al.* 2017b, Khan *et al.* 2017, Bartos *et al.* 2018, Nageshwar *et al.* 2018, Nkpaa and Onyeso 2018) found evidence of oxidative stress in the brains of rats treated with fluoride at concentrations at or below 20 ppm, of which 10 studies reported oxidative stress in the brain below 10 ppm fluoride. The most consistent evidence of oxidative stress in the brain was reported through changes in antioxidant activity. Eleven of the 12 lower risk-of-bias studies that evaluated antioxidant activity reported an effect at concentrations at or below 20 ppm (Gao *et al.* 2008b, Gao *et al.* 2009, Akinrinade *et al.* 2015b, Guner *et al.* 2016, Mesram *et al.* 2016, Adedara *et al.* 2017a, Adedara *et al.* 2017b, Khan *et al.* 2017, Bartos *et al.* 2018, Nageshwar *et al.* 2018, Nkpaa and Onyeso 2018). Decreases in antioxidant activity using measures of superoxide dismutase (SOD) activity were reported in seven of eight lower risk-of-bias studies (Akinrinade *et al.* 2015b, Mesram *et al.* 2016, Adedara *et al.* 2017a, Adedara *et al.* 2017b, Khan *et al.* 2017, Nageshwar *et al.* 2018, Nkpaa and Onyeso 2018) and, among these seven studies, all that also measured changes in catalase (CAT) activity (n = 6 studies) also reported decreased activity (Mesram *et al.* 2016, Adedara *et al.* 2017a, Adedara *et al.* 2017b, Khan *et al.* 2017, Nageshwar *et al.* 2018, Nkpaa and Onyeso 2018). A decrease in total antioxidant capacity (T-AOC) as a measure of antioxidant activity was also consistently reported in two lower risk-of-bias studies (Gao *et al.* 2008b, Gao *et al.* 2009), and a decrease in glutathione peroxidase (GPx) activity was reported in two of three lower risk-of-bias studies (Adedara *et al.* 2017b, Nkpaa and Onyeso 2018).

Relative to the above-mentioned studies, 2 of the 15 lower risk-of-bias studies (Chouhan and Flora 2008, Chouhan *et al.* 2010) did not observe statistically significant effects on oxidative stress in the brain with concentrations at or below 20 ppm fluoride; however, the measure of oxidative stress evaluated in Chouhan and Flora (2008) and Chouhan *et al.* (2010) (glutathione [GSH] to oxidized glutathione [GSSG]

ratio as an indication of antioxidant activity and ROS levels) were not evaluated in any other lower risk-of-bias study. Chouhan and Flora (2008) observed a dose-dependent increase in ROS levels associated with 10, 50, and 100 mg/L sodium fluoride in the drinking water; however, results were not statistically significant at any dose. In Chouhan *et al.* (2010), the levels of ROS were significantly higher at 50 ppm sodium fluoride in drinking water, but statistical significance was not met at doses below 20 ppm fluoride (1 and 10 ppm sodium fluoride) or at 100 ppm sodium fluoride; yet, hydrogen peroxide levels as a measure of ROS were found to be significantly increased at 15 ppm sodium fluoride in drinking water in studies conducted by another group of authors (Adedara *et al.* 2017a, Adedara *et al.* 2017b).

Apoptosis/cell death

Seven lower risk-of-bias studies were identified that evaluated apoptosis with concentrations at or below 20 ppm fluoride. Results from these studies were inconsistent and were insufficient for evaluating fluoride-induced apoptosis. These data are insufficient to increase confidence or support a change to hazard conclusions.

Inflammation

Five lower risk-of-bias studies were identified that evaluated potential effects of fluoride on inflammation with concentrations at or below 20 ppm. The inflammation markers were too heterogeneous or limited in number to make any determination on potential relevance of mechanism, even before limiting the review of the data to lower risk-of-bias studies. These data are insufficient to increase confidence or support a change to hazard conclusions.

Thyroid

Seventeen studies were identified that evaluated potential effects of fluoride on the thyroid with concentrations at or below 20 ppm (see **Figure 9**). These animal thyroid data were not evaluated further due to the lack of consistent evidence in the human data to suggest that thyroid effects are a requisite mechanism by which fluoride causes neurodevelopmental or cognitive effects in humans.

Figure 10. Number of Lower Risk-of-bias Animal Studies that Evaluated Biochemical, Neurotransmission, and Oxidative Stress Effects at or Below 20 ppm by Mechanism Subcategory and Direction of Effect*

Mechanism	Mechanism Subcategory	Direction of Effect ↑	Direction of Effect ↓	Direction of Effect NS	Grand Total
Biochemistry (brain/neurons)	Carbohydrate/lipid-related		1	1	2
	Gene expression		1		1
	Protein levels associated with brain function	8	7	7	11
	Other biochemical	2			2
Neurotransmitters	Cholinesterase	2	7	3	11
	Dopamine and metabolites		1		1
	Other neurotransmitters	2	2	1	2
Oxidative stress	Antioxidant activity	1	10	4	12
	Lipid peroxidation byproduct	7		4	11
	Protein oxidation	2		1	2
	ROS	2		2	4

*Interactive figure and additional study details in Tableau® (https://public.tableau.com/profile/ntp.visuals#!/vizhome/Fluoride_Animal_SelectMechanisms_UPDATE/Figure9). This figure displays study counts for lower risk-of-bias studies, as these counts are most relevant to the text in this section. Counts for higher risk-of bias studies or all studies combined can be accessed in the interactive figure in Tableau®. Study

counts are tabulated by significance—statistically significant increase (↑), statistically significant decrease (↓), or not significant (NS). For example, the "↑" column displays numbers of unique studies with at least one endpoint in the mechanistic subcategory with significantly increasing results at fluoride exposure levels of ≤20 ppm. These columns are not mutually exclusive (i.e., a study may report on multiple endpoints with varying results within a single mechanistic subcategory and therefore may be reflected in the counts for the "↑", "↓", and NS columns, but would only be counted once in the Grand Total column). Endpoints, species, strain, sex, and exposure duration are available for each study in the interactive figure in Tableau®.

In Vitro/Mechanistic Data on Neurodevelopmental or Cognitive Effects

Although in vitro data were collected as part of the systematic review process, NTP determined that the information on neurological effects obtained from these studies is too general, and results cannot necessarily be attributed to effects on learning and memory or other cognitive functions at this time. The in vitro data may help support specific mechanisms identified from in vivo mechanistic data, but, as described above, no specific mechanism has been determined for fluoride effects on learning and memory or other neurodevelopmental or cognitive outcomes.

Evidence Synthesis for Neurodevelopmental or Cognitive Effects

There is consistent evidence that exposure to fluoride is associated with cognitive neurodevelopmental effects in children. There is moderate confidence in the human data in children from several well-conducted prospective studies with limited sample sizes, supported by a large number of functionally-prospective cross-sectional studies. The human body of evidence in adults is considered inadequate to evaluate whether fluoride exposure is associated with cognitive effects due to low confidence in the human data in adults, a limited number of studies, and a lack of evidence of an effect (i.e., there is not sufficient evidence of an effect, but the confidence in the data is not high enough to conclude that there is no effect). The animal data are inadequate to evaluate for learning and memory or cognitive effects primarily due to the inability to distinguish effects on learning and memory outcomes from other effects on the nervous system including motor activity. The animal studies are considered inadequate to support the IQ effects in children but provide evidence of other neurodevelopmental effects. There is also evidence from mechanistic studies of adverse neurological effects of fluoride of unknown relationship to learning and memory.

The moderate confidence in the body of evidence in children translates to a moderate level of evidence that fluoride is associated with decreased IQ and other cognitive neurodevelopmental effects in children. The limited and weaker evidence of cognitive effects in adults is considered to provide an inadequate level of evidence that fluoride is associated with cognitive effects in adults. The animal body of evidence is also considered to provide an inadequate level of evidence for cognitive effects in adults.

Integration of these level-of-evidence conclusions supports an initial hazard conclusion of *presumed to be a cognitive neurodevelopmental hazard to humans* because of the extent, consistency, and magnitude of effect in the available data in children. Because most of the available studies evaluated intelligence in children, the primary focus in human data was on IQ and other cognitive neurodevelopmental effects, which is the primary basis for the hazard conclusion. A separate conclusion on other neurodevelopmental effects was not reached based on limited information in humans. It is unlikely that evaluation of additional neurodevelopmental effects would change the hazard conclusion.

The moderate level of evidence in the human data in children supports a hazard conclusion of *presumed* instead of *suspected* due to the relatively large and consistent body of evidence, especially in relation to measures of IQ (all 13 lower risk-of-bias studies that assessed IQ reported that higher fluoride is associated with at least one measure of decreased IQ) across multiple populations. A conclusion of *presumed* is also supported by the relatively large magnitude of effect observed in two well-designed

and well-conducted Canadian and Mexican prospective cohort studies of children where repeated urinary fluoride levels were assessed during pregnancy [i.e., for full-scale IQ, a 4.49-point decrease in boys per 1-mg/L increase in maternal urinary fluoride, a 3.66-point decrease in boys and girls combined per 1-mg increase in maternal total fluoride intake, and a 5.29-point decrease in boys and girls combined per 1-mg/L increase in water fluoride concentration (Green *et al.* 2019); and a 2.5-point decrease in full-scale IQ in boys and girls combined per 0.5-mg/L increase in maternal urinary fluoride (Bashash *et al.* 2017)]. Additional functionally-prospective cross-sectional studies present a consistent pattern of evidence that exposure to fluoride is associated with decreased IQ, which provides further support for the *presumed* hazard conclusion. Furthermore, the *presumed* hazard conclusion is supported by the low expectation that new studies would decrease the hazard conclusion.

Effects in children

- **Human body of evidence:** Moderate Confidence = Moderate Level of Evidence
- **Animal body of evidence:** No studies available to specifically assess effects on learning and memory after exposure during developmental periods separately from other neurological effects including motor activity = Inadequate Level of Evidence
- **Initial hazard conclusion (Moderate Human x Inadequate Animal)** = Presumed to be a Cognitive Neurodevelopmental Hazard to Humans
- **Final hazard conclusion (after consideration of biological plausibility)** = Presumed to be a Cognitive Neurodevelopmental Hazard to Humans

Effects in adults

- **Human body of evidence:** Low Confidence with no discernible effect = Inadequate Level of Evidence
- **Animal body of evidence:** No studies available to specifically assess effects on learning and memory after exposure in adulthood separately from other neurological effects including motor activity = Inadequate Level of Evidence
- **Initial hazard conclusion (Inadequate Human x Inadequate Animal)** = Not classifiable
- **Final hazard conclusion (after consideration of biological plausibility)** = Not classifiable

Table 7. Neurodevelopmental and Cognitive Function Evidence Profile for Fluoride

INITIAL CONFIDENCE for each body of evidence (# of studies)	**Factors decreasing confidence "---" if no concern; "↓" if serious concern to downgrade confidence**					**Factors increasing confidence "---" if not present; "↑" if sufficient to upgrade confidence**				FINAL CONFIDENCE RATING
	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Large Magnitude	Dose Response	Residual Confounding	Consistency Species/Model	
*Human IQ or cognitive function tests in children**										
Initial Moderate (4 prospective cohort studies[a]; 9 cross-sectional studies[b])	---	---	---	---	---	---	---	---	---	**Moderate**
Initial Low (5 cross-sectional studies)[c]	---	---	---	---	---	---	---	---	---	**Low**
*Human IQ or cognitive function tests in adults***										
Initial Low (2 cross-sectional studies)[d]	---	---	---	---	---	---	---	---	---	**Low**
Animal learning and memory or cognitive function										
Inadequate to assess effects in human										

References:

Human: Barberio *et al.* (2017b)[c]; Bashash *et al.* (2017)[a]; Bashash *et al.* (2018)[a]; Choi *et al.* (2015)[b]; Cui *et al.* (2018)[c]; Das and Mondal (2016)[b]; Ding *et al.* (2011)[b]; Green *et al.* (2019)[a]; Jacqmin *et al.* (1994)[d]; Li *et al.* (2008a)[b]; Li *et al.* (2016)[d]; Rocha-Amador *et al.* (2007)[b]; Rocha-Amador *et al.* (2009)[b]; Saxena *et al.* (2012)[b]; Seraj *et al.* (2012)[b]; Valdez Jimenez *et al.* (2017)[a]; Xiang *et al.* (2003)[c]; Xiang *et al.* (2011)[c]; Yu *et al.* (2018)[b]; Zhang *et al.* (2015b)[c]

*This includes learning disabilities, neonatal behavioral neurological assessment, mental development index, memory score for copy, and immediate recall.

**This includes Mini-Mental State Examination scores, psychomotor performance, and memory.

DISCUSSION

Based on the systematic review of the evidence, the NTP concludes that fluoride is *presumed to be a cognitive neurodevelopmental hazard to humans.* This conclusion is based on a moderate level of evidence that higher fluoride exposure is associated with decreased IQ and other cognitive effects in children. Limited and weaker evidence is considered to provide an inadequate level of evidence that fluoride is associated with cognitive effects in adults. The primary focus of the human data was on IQ and cognitive neurodevelopmental effects, which is why the conclusion was based on these data. After further evaluation of the experimental animal data available in NTP (2016) and in this update, it is concluded that the animal data are inadequate to evaluate the effects of fluoride on learning and memory to support the cognitive effects observed in humans primarily due to the inability to separate the learning and memory effects from the effects on motor activity or motor coordination. The animal data do provide evidence for effects of fluoride on neurodevelopment; however, other neurodevelopmental outcomes were not further evaluated because of the limited information in humans. Biological plausibility of effects from mechanistic studies was considered but did not significantly influence the conclusions. Although multiple categories of mechanistic data were evaluated and provide some evidence of adverse effects in the brain, the mechanistic basis for fluoride-associated cognitive neurodevelopmental is not sufficiently understood for these findings to contribute to the overall confidence assessment.

The human body of evidence provides a consistent pattern of findings that higher fluoride exposure is associated with decreased IQ in children. The four lower risk-of-bias prospective cohort studies and nine lower risk-of-bias cross-sectional studies that are considered functionally prospective in nature are the primary basis for the moderate level of evidence from human studies. The evaluation of the animal body of evidence in this assessment is an update to the NTP (2016) systematic review on the association between fluoride exposure and neurobehavioral effects related to learning and memory in animals, which identified a main concern related to indirectness based on the fact that many learning and memory tests rely on a motor response (e.g., latency to achieve the desired effect). The update of the animal data focused on addressing this indirectness concern and the difficulty in distinguishing potential effects of fluoride on motor and sensory functions from effects specifically on learning and memory functions. Further examination of the literature has not provided clarification of this issue. Due to the inability to separate these effects from the other effects on the nervous system, including motor activity or motor coordination, the animal body of evidence is now considered inadequate to contribute to the evaluation of cognitive effects in humans. Although the animal data are not considered sufficient to specifically support the IQ changes observed in children, the data do support possible neurodevelopmental effects.

The NTP conclusion that fluoride is *presumed to be a cognitive neurodevelopmental hazard to humans* is supported by the extent, consistency, and magnitude of the effect in the available data in children. All 13 lower risk-of-bias studies reported that higher fluoride exposure is associated with at least one measure of decreased IQ in children across multiple populations. A conclusion of presumed is also supported by the relatively large magnitude of effect observed in two well-designed and well-conducted Canadian and Mexican prospective cohort studies of children where repeated maternal urinary fluoride levels were assessed during pregnancy [i.e., for full-scale IQ, a 4.49-point decrease in boys per 1-mg/L increase in maternal urinary fluoride, a 3.66-point decrease in boys and girls combined per 1-mg increase in maternal total fluoride intake, and a 5.29-point decrease in boys and girls combined per 1-mg/L increase in water fluoride concentration (Green *et al.* 2019); and a 2.5-point decrease in full-scale IQ in boys and girls combined per 0.5-mg/L increase in maternal urinary fluoride (Bashash *et al.*

2017)]. Additional functionally-prospective cross-sectional studies provided further support for the NTP conclusion, presenting a consistent pattern of evidence that exposure to fluoride is associated with decreased IQ. Furthermore, there is a low expectation that new studies would change the hazard conclusion.

There are few studies in humans and numerous studies in animals that evaluate mechanistic effects related to fluoride exposure. There are sufficient mechanistic data to determine that fluoride exposure at lower concentrations has effects on the nervous system; however, for the cognitive neurodevelopmental outcome evaluated, there are insufficient data to support a specific mechanism or mode of action. Due to the large number of mechanistic studies conducted in animals, evaluation of the mechanistic data in animals focused on studies that had exposures more relevant to humans (i.e., ≤20 ppm in the drinking water). Changes in AChE, which could potentially be related to cognitive effects such as IQ, were evaluated in one study in children and several studies in animals (measured in both the blood and in areas of the brain); however, the majority of these studies, including the study in children, reported results inconsistent with the phenotypic outcome. Animal studies that evaluated changes in other neurotransmitters and other biochemical measures provide some evidence of effects in the brain, but the data are limited due to the heterogeneity of the outcomes measured. Most consistently, studies evaluating histopathology and oxidative stress demonstrated that effects can occur in the brains of animals at or below 20 ppm, which, without supporting a specific mechanism or mode of action relevant to learning and memory impairments, provides evidence of an association between exposure to lower concentrations of fluoride and neurological effects in animals. Therefore, the evidence of neurological effects at exposure levels more relevant to humans that is demonstrated in the mechanistic data supports the NTP conclusion that fluoride is *presumed to be a cognitive neurodevelopmental hazard to humans;* however, it does not provide enough evidence to increase confidence in the human body of evidence or support a higher hazard identification conclusion.

Generalizability to the U.S. Population

For many years, fluoride concentrations were adjusted to levels between 0.8 and 1.2 mg/L in fluoridated community water systems in the United States. The U.S. Public Health Service recommended an adjustment downward to a fluoride concentration of 0.7 mg/L because of evidence of an increase in dental fluorosis in children (US DHHS 2015). In the 2013–2014 National Health and Nutrition Examination Survey (NHANES), a nationwide survey in the United States, data were released for fluoride concentrations in water for U.S. children and adolescents (≤19 years old). Water fluoride levels ranged from approximately 0.03 to 1.5 mg/L in the NHANES data, although the sources of the fluoride (naturally occurring or fluoridated water) are not reported (Jain 2017).

NTP's conclusion that fluoride is *presumed to be a cognitive neurodevelopmental hazard to humans* is based on consistent evidence from 18 lower risk-of-bias studies that evaluated fluoride exposure and effects on children's IQ and other cognitive effects. Although there are many studies that evaluated associations between fluoride in the drinking water and IQ in children, no studies evaluating IQ were conducted in the United States. Generalizing the results from the IQ studies in this evaluation to the U.S. population can be difficult, in part because many studies were conducted in areas with fluoride drinking water concentrations that are much higher than drinking water fluoride concentrations in the United States. Among the human body of evidence evaluated for this assessment (including lower and higher risk-of-bias studies), there are 31 studies that evaluated associations between fluoride in drinking water and IQ in children and compared a reference or low exposure group to higher fluoride-exposed groups. Of these 31 studies, only 8 studies included fluoride exposure groups with fluoride concentrations <1.5 mg/L [i.e., fluoride exposure groups that would potentially be relevant to levels observed in the United

States based on NHANES data (Jain 2017)] (Xu *et al.* 1994, Zhang *et al.* 1998, Xiang *et al.* 2003, Qin *et al.* 2008, Sudhir *et al.* 2009, Broadbent *et al.* 2015, Sebastian and Sunitha 2015, Zhang *et al.* 2015b). Of these eight studies, only two studies were considered to have lower risk of bias (Xiang *et al.* 2003, Zhang *et al.* 2015b).

In addition to these two studies mentioned above, several other studies that evaluated fluoride exposure on a continuous basis could be used to assess generalizability to the United States. This includes studies that examined fluoride exposure levels below 1.5 mg/L for which a dose response could be assessed. **Table 8** provides a summary of children's IQ studies that evaluated lower fluoride exposures (<1.5 mg/L) in drinking water and/or urine (assuming, for comparison purposes, an approximate 1-to-1 equivalence between drinking water fluoride and urinary fluoride concentrations) and provided information to evaluate dose-response in the lower fluoride exposure range (e.g., three or more fluoride exposure groups or dose-response curve provided). Based on review of these studies, there is uncertainty if IQ changes in children occur at lower fluoride levels.

Among studies with lower risk of bias, four of eight studies (Xiang *et al.* 2003, Zhang *et al.* 2015b, Das and Mondal 2016, Green *et al.* 2019) applied regression models to individual exposure outcome measures and observed a linear association between urinary fluoride levels and decreased IQ in children even at the lower fluoride concentrations. However, two of these studies (Xiang *et al.* 2003, Zhang *et al.* 2015b) did not find an association between IQ and drinking water levels below 1.5 mg/L. Xiang *et al.* (2003) observed a significant decrease in IQ comparing endemic and nonendemic villages, but when they grouped children from the endemic villages by exposure level, they did not observe a significant decrease in IQ for children exposed to lower mean exposure levels (0.75 mg/L). Although a significant difference in IQ might not be expected due to the fact that there were only nine children in this group, the difference was less than one point in IQ. Zhang *et al.* (2015b) used a simple correlation and did not observe a significant relationship between fluoride levels in the drinking water (with concentrations up to 1.57 mg/L) and IQ. The other four of eight studies do not provide a clear dose-response at the lower fluoride levels. Bashash *et al.* (2017) concluded that there was no clear association between IQ scores and maternal urinary fluoride below 0.8 mg/L. Yu *et al.* (2018) observed a correlation between decreased IQ in children and fluoride exposure only with concentrations in drinking water above 3.4 mg/L or with urinary fluoride concentrations of 1.6 mg/L or higher. The study authors did note a decreased probability of having an IQ above 130 (i.e., 40% fewer people with high IQ for every 0.5-mg/L increase in fluoride) with water fluoride levels between 0.20 and 1.40 mg/L, but this was not observed with higher levels of fluoride. Although Cui *et al.* (2018) noted that IQ decreased in a "roughly linear manner" with increasing urinary fluoride, this is only apparent in the results for the TT genotype; based on the dose-response, the authors concluded that the "safety threshold" was 1.73 mg/L. Ding *et al.* (2011) looked at mean differences for 10 different exposure groups and found notable decreases from the mean above approximately 1 mg/L.

Although we may have less confidence in the findings from higher risk-of-bias studies, six studies identified with potential dose-response information demonstrated a similar uncertainty at the lower fluoride concentrations. Surprisingly, three of the studies (Xu *et al.* 1994, Qin *et al.* 2008, Aravind *et al.* 2016) found that the lowest IQ scores were in areas with the lowest and the highest fluoride concentrations. In these studies, the lowest fluoride concentrations ranged from 0.1–0.2 mg/L fluoride in Qin *et al.* (2008) to <1.2 mg/L in Aravind *et al.* (2016). Li *et al.* (1995) and Sebastian and Sunitha (2015) only observed a decrease in IQ at concentrations above 2 mg/L. Sudhir *et al.* (2009) observed a significant increase in IQ grade (which is associated with a decrease in IQ) at concentrations of 0.7–1.2 mg/L.

Table 8. Human Studies with Lower Fluoride Exposure and Effects on IQ

Study	Exposure measures [mean ± SD (range)]	Notes
Lower risk-of-bias studies		
Bashash *et al.* (2017)	Maternal urine during pregnancy (mg/L) 0.90 ± 0.35 (0.23–2.36) Children's urine (mg/L) 0.82 ± 0.38 (0.18–2.8)	Authors concluded that the model suggested a nonlinear relationship with no clear association between IQ scores and maternal urine below 0.8 mg/L.
Green *et al.* (2019)	Maternal urine during pregnancy (mg/L) 0.51 ± 0.36 (0.06–2.44) 0.40 ± 0.27 non-fluoridated areas 0.69 ± 0.42 fluoridated areas Maternal intake during pregnancy (mg/day) 0.54 ± 0.44 (0.01–2.65) 0.30 ± 0.26 non-fluoridated areas 0.93 ± 0.43 fluoridated areas Drinking water (mg/L) 0.31 ± 0.23 (0.04–0.87[1]) 0.13 ± 0.06 non-fluoridated areas 0.59 ± 0.08 fluoridated areas	Statistical methods indicated that including quadratic or natural-log effects of maternal urine or intake did not significantly improve the model. In addition, the authors tested separate models with two linear splines to see if the effect of maternal urinary fluoride or maternal fluoride intake significantly differed between lower and higher levels based on knots set at 0.5, 0.8, and 1.0 mg/L for urine and 0.4, 0.8, and 1 mg for intake. There were no differences.
Cui *et al.* (2018)	Drinking water (mg/L)* 0.20–1.00 non-endemic 1.52–2.49 endemic Children's urine Levels not provided; log-transformed with range of approximately −1.2–2.2	Study authors noted that the IQ decreased in a "roughly linear manner as the log-urine fluoride increased." TT genotypes of the dopamine receptor D2 gene had the strongest negative correlation between log-urine fluoride and IQ scores. The study authors determined a safety threshold of urine fluoride levels in subgroup TT as 1.73 mg/L. Drinking water fluoride levels were used to select children from different areas but were not used in the analysis.
Das and Mondal (2016)	Groundwater (mg/L)* 2.11 ± 1.64 (0.25–9.40) Children's urine (mg/L) 0.45–17.00 Children's exposure dose (mg/kg-day) 0.017–0.203	Based on simple regression, there was a steady decline in IQ with increasing urinary fluoride and increasing exposure dose. Groundwater fluoride levels were not used in the analysis but were used in calculating the children's exposure dose.
Ding *et al.* (2011)	Drinking water (mg/L) * 1.31 ± 1.05 (0.24–2.84) Children's urine (mg/L) 0.10–3.55	Although there was a significant correlation between urinary fluoride and IQ score, the main drop in IQ occurred at urinary fluoride levels of approximately 0.7–1.2 mg/L. At levels below 0.7 mg/L, data suggest a plateau with no apparent change in IQ compared with the mean. Drinking water fluoride levels were not used in the analysis.

Table 8. Human Studies with Lower Fluoride Exposure and Effects on IQ

Study	Exposure measures [mean ± SD (range)]	Notes
Xiang *et al.* (2003)	Drinking water (mg/L)* 0.36 ± 0.15 (0.18–0.76) non-endemic village 2.47 ± 0.79 (0.57–4.50) endemic village Endemic subgroups: group A: 0.75 ± 0.14 group B: 1.53 ± 0.27 group C: 2.46 ± 0.30 group D: 3.28 ± 0.25 group E: 4.16 ± 0.22 Children's urine (mg/L) 1.11 ± 0.39 (0.37–2.50) non-endemic village 3.47 ± 1.95 (0.90–12.50) endemic village	IQ in group A in the endemic village was not significantly decreased compared with the non-endemic village, but IQ in all other groups was significantly decreased. Although there were only 9 children in group A, the IQ difference was <1 point. Based on simple regression, there was a steady decline in IQ with increasing urinary fluoride.
Yu *et al.* (2018)	Drinking water (mg/L)* 0.50 ± 0.27 normal 2.00 ± 0.75 high Children's urine (mg/L) 0.41 ± 0.49 normal 1.37 ± 1.08 high	Study authors reported that participants' intelligence presented inverse non-linear dose-response relationships with fluoride content, with obvious decreases at relatively high level of fluoride exposure (drinking water fluoride levels at 3.4–3.90 mg/L and urinary fluoride levels at 1.60–2.50 mg/L). Study authors also note a decreased odds for having IQ ≥ 130 with drinking water fluoride at 0.20–1.40 mg/L (40% decrease with each 0.5-mg/L increase in fluoride), but not at higher concentrations.
Zhang *et al.* (2015b)	Drinking water (mg/L) 0.63 (0.58–0.68) control 1.40 (1.23–1.57) endemic fluorosis Children's urine (mg/L) 1.1 ± 0.67 control 2.4 ± 1.01 endemic fluorosis Children's serum (mg/L) 0.06 ± 0.03 control 0.18 ± 0.11 endemic fluorosis	There was a steady decline in IQ with increasing serum or urinary fluoride levels. A simple correlation did not find drinking water fluoride significantly correlated with IQ.
Higher risk-of-bias studies		
Aravind *et al.* (2016)	Drinking water (mg/L)* <1.2 low fluoride area 1.2–2 medium fluoride area >2 high fluoride area	Mean IQ scores (transformed into percentiles) were highest in the medium fluoride area for both boys and girls.
Li *et al.* (1995)	Children's urine (mg/L) 1.02 non-fluorosis area 1.81 slight fluorosis area 2.01 medium fluorosis area 2.69 severe fluorosis area	A significant decrease in IQ was observed in the medium and severe fluorosis areas compared to the non-fluorosis area. Children's urine was used as an individual measure of exposure to verify that the areas had different fluoride exposure levels; however, analysis was conducted based on residential area.

Table 8. Human Studies with Lower Fluoride Exposure and Effects on IQ

Study	Exposure measures [mean ± SD (range)]	Notes
Qin *et al.* (2008)	Drinking water (mg/L)* 0.1–0.2 low fluoride area 0.5–1.0 normal fluoride area 2.1–4.0 high fluoride area	Average IQ scores (transformed into percentages) were significantly lower in both the low and high fluoride areas compared with the normal fluoride area.
Sebastian and Sunitha (2015)	Drinking water (mg/L)* 0.40 (low fluoride village) 1.2 (normal fluoride village) 2.0 (high fluoride village)	A significant decrease in mean IQ score of children living in the high fluoride area compared with the low and normal fluoride areas was reported. Binary regression models using the low fluoride village as a reference observed an increased odds ratio (1.74) for increased IQ scores in the normal fluoride village and a decreased odds ratio (0.59) in the high fluoride village.
Sudhir *et al.* (2009)	Drinking water (mg/L)* <0.7 (level 1 villages) 0.7–1.2 (level 2 villages) 1.3–4.0 (level 3 villages) >4.0 (level 4 villages)	The number of intellectually impaired children gradually increased with increasing fluoride concentration in the drinking water with an increase in IQ grade (which indicates a decrease in IQ) observed in the 0.7–1.2 mg/L villages. Children were placed in exposure groups based on Water Works Department records. Although children brought in water for verification of strata, it was not collected from all children but only from the first child using a different source of water. Therefore, it is considered group-level data. Note that all groups had a large proportion in the "intellectually impaired" category.
Xu *et al.* (1994)	Drinking water (mg/L)* 0.8 (control area) 0.38 (low fluoride area) 1.8 (high fluoride area)	Both low and high fluoride areas had IQ levels approximately 3 points below the IQ levels in the control area. There was no difference in IQ between the low and high fluoride areas.

*Data are group-level exposure data; exposure data without the asterisks are individual-level exposure data.
[1]Range data were obtained from Till *et al.* (2018).

The conclusion that fluoride is *presumed to be a cognitive neurodevelopmental hazard in children* is based on the consistency of the data; however, most lower risk-of-bias studies observed effects with drinking water concentrations above 1.5 mg/L. As noted above, describing the effects at 1.5 mg/L or below, which is more relevant to the exposures observed in the U.S. population, including from community water fluoridation, is more difficult. In the reviewed studies, when limiting studies to those that evaluated IQ at fluoride levels across a continuum that included the low dose range, results are less consistent.

Limitations of the Evidence Base

Few limitations exist in the lower risk-of-bias epidemiological studies used for the basis of the hazard conclusion. The main limitations in lower risk-of-bias epidemiological studies include:

- Few studies were available that assessed the association between fluoride exposure and the following:

  - Neurodevelopmental or cognitive effects in subjects from communities served by optimally fluoridated versus non-fluoridated water systems.
  - Neurobehavioral (i.e., cognitive) effects (particularly IQ) in adults.
  - Attention-related disorders including ADHD.
- Studies rarely separated the results by gender or provided information to indicate that gender was not a modifying factor, which limits the ability to evaluate how the association between fluoride exposure and cognitive neurodevelopmental effects in children might differ by gender.

Limitations in the epidemiological studies with higher risk of bias include:

- Many of the original publications were in a foreign language and provided limited details on methodology.
- Some studies lacked information regarding exposure and/or had serious limitations in the exposure assessment. Exposure assessment concerns include limited individual exposure information, a lack of information on fluoride sampling methods and timing of the exposure measurements, a lack of quantitation of levels of fluoride in drinking water, and a lack of individual-level information on fluorosis in areas reported to be endemic for fluorosis.
- The comparison groups in studies conducted in areas endemic for fluorosis may have still been exposed to high levels of fluoride or levels similar to those used in water fluoridation in the United States. This factor may have limited the ability to detect true effects.
- Most studies did not provide sufficient direct information (e.g., participation rates) to evaluate selection bias.
- Failure to address potential confounders was a main issue. Many studies conducted simple statistical analyses without accounting for any potential confounders. In cases where adjustments in analyses were made, often these studies did not account for potential confounders considered critical for that study population and outcome.
- Studies conducted in areas with high, naturally-occurring fluoride levels in drinking water often did not account for potential exposures to arsenic or iodine deficiencies in study subjects.
- Many studies did not account for potential exposures to lead as a residual confounder.
- Many studies lacked information on whether the outcome assessors were blind to the exposure group, including studies that examined children in their schools and subjects from high-fluoride communities.

Limitations in the animal studies include:

- The main limitation in the animal studies was the inability to separate possible learning and memory effects from effects on motor activity/coordination or sensory effects.
- Few learning and memory studies in animals evaluated motor activity or sensory effects. Studies that did evaluate motor activity or sensory effects often lacked discussion on general health of the animals when the endpoints measured could be affected by deficits in motor activity or sensory, such as latency to achieve a desired result.

A key data gap in the human and animal bodies of evidence includes the need for mechanistic insight into fluoride-related neurodevelopmental or cognitive changes.

Limitations of the Systematic Review

There are no major limitations of the systematic review. The human body of evidence included a large database of observational studies. Most of the observational studies were cross-sectional; however, nine of these were considered to be functionally prospective in nature. In addition, the systematic review covered a wide range of study designs, populations, and measures of fluoride exposure. The systematic review was designed to cover reports on all potential mechanistic data including effects on the thyroid. After review of the studies evaluating thyroid effects, studies that only evaluated goiters and other effects on thyroid size were not considered in this review. This is not considered a limitation because changes in thyroid size are not functional changes to the thyroid that could specifically indicate a mechanism for thyroid involvement in neurodevelopment. In addition, review of the mechanistic data was limited to in vivo studies with at least one concentration below 20 ppm. This is not considered a limitation for the systematic review since the mechanistic body of evidence was used to evaluate biological plausibility for the effects observed in humans; therefore, data were limited to concentrations that would be more reflective of human exposures. The decision to not more closely evaluate the in vitro data is not considered a limitation because there were sufficient in vivo data, and no key events were identified where in vitro data would provide additional insight.

CONCLUSION

NTP concludes that fluoride is *presumed to be a cognitive neurodevelopmental hazard to humans.* This conclusion is based on a consistent pattern of findings in human studies across several different populations showing that higher fluoride exposure is associated with decreased IQ or other cognitive impairments in children. However, the consistency is based primarily on higher levels of fluoride exposure (i.e., >1.5 ppm in drinking water). When focusing on studies with exposures in ranges typically found in the water distribution systems in the United States (i.e., approximately 0.03 to 1.5 ppm according to NHANES data) that can be evaluated for dose response, effects on cognitive neurodevelopment are inconsistent, and therefore unclear. There is inadequate evidence to determine whether fluoride exposure lowers IQ or impairs cognitive function in adults. The human evidence indicating that fluoride exposure is associated with other neurodevelopmental effects, beyond IQ or other cognitive measures, is limited due to the small number of human studies that evaluated any given neurodevelopmental effect other than IQ or cognitive function. Although conclusions were reached by integrating evidence from human and animal studies with consideration of relevant mechanistic data, the conclusions are based primarily on the human evidence. The evidence from animal studies is inadequate to inform conclusions on cognitive effects, and the mechanisms underlying fluoride-associated cognitive neurodevelopmental effects are not well characterized.

REFERENCES

Adedara IA, Abolaji AO, Idris UF, Olabiyi BF, Onibiyo EM, Ojuade TD, Farombi EO. 2017a. Neuroprotective influence of taurine on fluoride-induced biochemical and behavioral deficits in rats. *Chem Biol Interact* 261: 1-10.

Adedara IA, Olabiyi BF, Ojuade TD, Idris UF, Onibiyo EM, Farombi EO. 2017b. Taurine reverses sodium fluoride-mediated increase in inflammation, caspase-3 activity, and oxidative damage along the brain-pituitary-gonadal axis in male rats. *Can J Phys Pharmacol* 95: 1019-1029.

Agustina F, Sofro ZM, Partadiredja G. 2018. Subchronic administration of high-dose sodium fluoride causes deficits in cerebellar purkinje cells but not motor coordination of rats. *Biol Trace Elem Res* 188(2): 424-433.

Ahmad KR, Noor S, Jabeen S, Nauroze T, Kanwal MA, Raees K, Abbas T. 2017. Amelioration by jambul fruit extract of fluoride-induced hepato-nephronal histopathologies and impaired neuromotor capacity in mice. *Fluoride* 50: 2-14.

Akinrinade ID, Memudu AE, Ogundele OM. 2015a. Fluoride and aluminium disturb neuronal morphology, transport functions, cholinesterase, lysosomal and cell cycle activities. *Pathophysiology* 22: 105-115.

Akinrinade ID, Memudu AE, Ogundele OM, Ajetunmobi OI. 2015b. Interplay of glia activation and oxidative stress formation in fluoride and aluminium exposure. *Pathophysiology* 22: 39-48.

An J, Mei S, Liu A, Fu Y, Wang C. 1992. [Effect of high level of fluoride on children's intelligence]. *Chin J Control Endem Dis* 7(2): 93-94.

Aravind A, Dhanya RS, Narayan A, Sam G, Adarsh VJ, Kiran M. 2016. Effect of fluoridated water on intelligence in 10-12-year-old school children. *J Int Soc Prev Community Dent* 6(Suppl 3): S237-S242.

Baba NA, Raina R, Verma PK, Sultana M. 2014. Alterations in plasma and tissue acetylcholinesterase activity following repeated oral exposure of chlorpyrifos alone and in conjunction with fluoride in Wistar rats. *Proc Indian Natl Sci Acad B Biol Sci* 84: 969-972.

Banala R, Nagapuri K, Mohd K, Reddy M, Karnati P. 2018. Carica Papaya leaf extract as a neuroprotective agent against behavioral and neurotransmitter changes in brain of the rat treated with sodium fluoride in pre- and post-natal periods. *Pharmacogn Mag* 14(55): 123-131.

Banala RR, Karnati PR. 2015. Vitamin A deficiency: An oxidative stress marker in sodium fluoride (NaF) induced oxidative damage in developing rat brain. *Int J Dev Neurosci* 47: 298-303.

Barberio AM, Hosein FS, Quinonez C, McLaren L. 2017a. Fluoride exposure and indicators of thyroid functioning in the Canadian population: Implications for community water fluoridation. *J Epidemiol Community Health* 71: 1019-1025.

Barberio AM, Quinonez C, Hosein FS, McLaren L. 2017b. Fluoride exposure and reported learning disability diagnosis among Canadian children: Implications for community water fluoridation. *Can J Public Health* 108: 229-239.

Bartos M, Gumilar F, Gallegos CE, Bras C, Dominguez S, Monaco N, Esandi MDC, Bouzat C, Cancela LM, Minetti A. 2018. Alterations in the memory of rat offspring exposed to low levels of fluoride during gestation and lactation: Involvement of the alpha7 nicotinic receptor and oxidative stress. *Reprod Toxicol* 81: 108-114.

Bashash M, Thomas D, Hu H, Martinez-Mier EA, Sanchez BN, Basu N, Peterson KE, Ettinger AS, Wright R, Zhang Z, Liu Y, Schnaas L, Mercado-Garcia A, Tellez-Rojo MM, Hernandez-Avila M. 2017. Prenatal fluoride exposure and cognitive outcomes in children at 4 and 6-12 years of age in Mexico. *Environ Health Perspect* 125(9): 1-12.

Bashash M, Marchand M, Hu H, Till C, Martinez-Mier EA, Sanchez BN, Basu N, Peterson KE, Green R, Schnaas L, Mercado-Garcia A, Hernandez-Avila M, Tellez-Rojo MM. 2018. Prenatal fluoride

exposure and attention deficit hyperactivity disorder (ADHD) symptoms in children at 6-12 years of age in Mexico City. *Environ Int* 121(Pt 1): 658-666.

Bhatnagar M, Rao P, Sushma J, Bhatnagar R. 2002. Neurotoxicity of fluoride: Neurodegeneration in hippocampus of female mice. *Indian J Exp Biol* 40: 546-554.

Bhatnagar M, Sukhwal P, Suhalka P, Jain A, Joshi C, Sharma D. 2011. Effects of fluoride in drinking water on NADPH-diaphorase neurons in the forebrain of mice: A possible mechanism of fluoride neurotoxicity. *Fluoride* 44: 195-209.

Broadbent JM, Thomson WM, Moffitt TE, Poulton R. 2015. Community water fluoridation and intelligence response. *Am J Public Health* 105: 3-4.

CDC (Centers for Disease Control and Prevention). 2013. *Community water fluoridation: Fluoridation statistics*. Atlanta, GA. Available: https://www.cdc.gov/fluoridation/statistics/2012stats.htm [accessed 19 August 2019].

Chen J, Niu Q, Xia T, Zhou G, Li P, Zhao Q, Xu C, Dong L, Zhang S, Wang A. 2018. ERK1/2-mediated disruption of BDNF-TrkB signaling causes synaptic impairment contributing to fluoride-induced developmental neurotoxicity. *Toxicology* 410: 222-230.

Chen Y. 2012. Organophosphate-induced brain damage: Mechanisms, neuropsychiatric and neurological consequences, and potential therapeutic strategies. *Neurotox* 33: 391-400.

Chen YX, Han FL, Zhoua ZL, Zhang HQ, Jiao XS, Zhang SC, Huang MC, Chang TQ, Dong YF. 2008. Research on the intellectual development of children in high fluoride areas. *Fluoride* 41: 120-124.

Chinoy NJ, Narayana MV. 1992. Studies on fluorosis in Mehsana District of North Gujarat. *Proc Zool Soc* 45: 157-161.

Choi AL, Sun G, Zhang Y, Grandjean P. 2012. Developmental fluoride neurotoxicity: A systematic review and meta-analysis. *Environ Health Perspect* 120: 1362-1368.

Choi AL, Zhang Y, Sun G, Bellinger DC, Wang K, Yang XJ, Li JS, Zheng Q, Fu Y, Grandjean P. 2015. Association of lifetime exposure to fluoride and cognitive functions in Chinese children: A pilot study. *Neurotoxicol Teratol* 47: 96-101.

Chouhan S, Flora SJS. 2008. Effects of fluoride on the tissue oxidative stress and apoptosis in rats: Biochemical assays supported by IR spectroscopy data. *Toxicology* 254: 61-67.

Chouhan S, Lomash V, Flora SJ. 2010. Fluoride-induced changes in haem biosynthesis pathway, neurological variables and tissue histopathology of rats. *J Appl Toxicol* 30: 63-73.

Cui Y, Zhang B, Ma J, Wang Y, Zhao L, Hou C, Yu J, Zhao Y, Zhang Z, Nie J, Gao T, Zhou G, Liu H. 2018. Dopamine receptor D2 gene polymorphism, urine fluoride, and intelligence impairment of children in China: A school-based cross-sectional study. *Ecotoxicol Environ Saf* 165: 270-277.

Das K, Mondal NK. 2016. Dental fluorosis and urinary fluoride concentration as a reflection of fluoride exposure and its impact on IQ level and BMI of children of Laxmisagar, Simlapal Block of Bankura District, W.B., India. *Environ Monit Assess* 188: 218.

Ding Y, Sun H, Han H, Wang W, Ji X, Liu X, Sun D. 2011. The relationships between low levels of urine fluoride on children's intelligence, dental fluorosis in endemic fluorosis areas in Hulunbuir, Inner Mongolia, China. *J Hazard Mater* 186: 1942-1946.

Dong YT, Wei N, Qi XL, Liu XH, Chen D, Zeng XX, Guan ZZ. 2017. Attenuating effect of vitamin E on the deficit of learning and memory of rats with chronic fluorosis: The mechanism may involve muscarinic acetylcholine receptors. *Fluoride* 50: 354-364.

Du L, Wan C, Cao X, Liu J. 2008. The effect of fluorine on the developing human brain. *Fluoride* 41: 327-330.

Duan JZ, M.; Wang, L.; Fang, D.; Wang, Y.; Wang, W. 1995. A comparative analysis of the results of multiple tests in patients with chronic industrial fluorosis. *Guizhou Med J* 18(3): 179-180.

Gais S, Schonauer M. 2017. Untangling a cholinergic pathway from wakefulness to memory. *Neuron* 94(4): 696-698.

Gao Q, Liu YJ, Wu CX, Long YG, Guan ZZ. 2008a. [Effects of fluoride on learning and memory and cholinesterase activity in rat brains]. *Chin J Endemiol* 27: 128-130.

Gao Q, Liu YJ, Wu CX, Long YG, Guan ZZ. 2008b. [Level of oxidative stress in rat brains and learning and memory function of rats with chronic fluorosis]. *Chin J Endemiol* 27: 371-373.

Gao Q, Liu YJ, Guan ZZ. 2009. Decreased learning and memory ability in rats with fluorosis: Increased oxidative stress and reduced cholinesterase activity in the brain. *Fluoride* 42: 277-285.

Garman RH, Li AA, Kaufmann W, Auer RN, Bolon B. 2016. Recommended Methods for Brain Processing and Quantitative Analysis in Rodent Developmental Neurotoxicity Studies. *Toxicol Pathol* 44(1): 14-42.

Ge QD, Tan Y, Luo Y, Wang WJ, Zhang H, Xie C. 2018a. MiR-132, miR-204 and BDNF-TrkB signaling pathway may be involved in spatial learning and memory impairment of the offspring rats caused by fluorine and aluminum exposure during the embryonic stage and into adulthood. *Environ Toxicol Pharmacol* 63: 60-68.

Ge Y, Chen L, Yin Z, Song X, Ruan T, Hua L, Liu J, Wang J, Ning H. 2018b. Fluoride-induced alterations of synapse-related proteins in the cerebral cortex of ICR offspring mouse brain. *Chemosphere* 201: 874-883.

Green R, Lanphear B, Hornung R, Flora D, Martinez-Mier EA, Neufeld R, Ayotte P, Muckle G, Till C. 2019. Association between maternal fluoride exposure during pregnancy and IQ scores in offspring in Canada. *JAMA Pediatr*: E1-E9.

Guner S, Uyar-Bozkurt S, Haznedaroglu E, Mentes A. 2016. Dental fluorosis and catalase immunoreactivity of the brain tissues in rats exposed to high fluoride pre- and postnatally. *Biol Trace Elem Res* 174: 150-157.

Guo XC, Wang RY, Cheng CF, Wei WS, Tang LM, Wang QS, Tang DX, Liu GW, He GD, Li SL. 2008a. A preliminary investigation of the IQs of 7-13 year-old children from an area with coal burning-related fluoride poisoning. *Fluoride* 41: 125-128.

Guo ZY, He YH, Zhu QX. 2008b. Research on the neurobehavioral function of workers occupationally exposed to fluoride. *Fluoride* 41: 152-155.

Guyatt GH, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, Norris S, Falck-Ytter Y, Glasziou P, Debeer H, Jaeschke R, Rind D, Meerpohl J, Dahm P, Schunemann HJ. 2011. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol* 64(4): 383-394.

Higgins JP, Green S. 2011. *Cochrane handbook for systematic reviews of interventions,* In: The Cochrane Collaboration. Vol 4, New York, NY: John Wiley & Sons.

Hosur MB, Puranik RS, Vanaki S, Puranik SR. 2012. Study of thyroid hormones free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH) in subjects with dental fluorosis. *Eur J Dent* 6: 184-190.

Jacqmin H, Commenges D, Letenneur L, Barberger-Gateau P, Dartigues JF. 1994. Components of drinking water and risk of cognitive impairment in the elderly. *Am J Epidemiol* 139: 48-57.

Jain RB. 2017. Concentrations of fluoride in water and plasma for US children and adolescents: Data from NHANES 2013-2014. *Environ Toxicol Pharmacol* 50: 20-31.

Jia B, Zong L, Lee JY, Lei J, Zhu Y, Xie H, Clemens JL, Feller MC, Na Q, Dong J, McLane MW, Jones-Beatty K, Burd I. 2019. Maternal supplementation of low dose fluoride alleviates adverse perinatal outcomes following exposure to intrauterine inflammation. *Scientific Reports* 9(1): 2575.

Jiang C, Zhang S, Liu H, Guan Z, Zeng Q, Zhang C, Lei R, Xia T, Wang Z, Yang L, Chen Y, Wu X, Zhang X, Cui Y, Yu L, Wang A. 2014. Low glucose utilization and neurodegenerative changes caused by sodium fluoride exposure in rat's developmental brain. *Neuromolecular Med* 16: 94-105.

Jones S, Burt BA, Petersen PE, Lennon MA. 2005. The effective use of fluorides in public health. *Bull World Health Organ* 83: 670-676.

Khan AM, Raina R, Dubey N, Verma PK. 2017. Effect of deltamethrin and fluoride co-exposure on the brain antioxidant status and cholinesterase activity in Wistar rats. *Drug Chem Toxicol* 41(2): 1-5.

Khandare AL, Gourineni SR, Validandi V. 2017. Dental fluorosis, nutritional status, kidney damage, and thyroid function along with bone metabolic indicators in school-going children living in fluoride-affected hilly areas of Doda District, Jammu and Kashmir, India. *Environ Monit Assess* 189: 579.

Khandare AL, Validandi V, Gourineni SR, Gopalan V, Nagalla B. 2018. Dose-dependent effect of fluoride on clinical and subclinical indices of fluorosis in school going children and its mitigation by supply of safe drinking water for 5 years: An Indian study. *Environ Monit Assess* 190: 110.

Kheradpisheh Z, Mahvi AH, Mirzaei M, Mokhtari M, Azizi R, Fallahzadeh H, Ehrampoush MH. 2018a. Correlation between drinking water fluoride and TSH hormone by ANNs and ANFIS. *J Environ Health Sci Eng* 16(1): 11-18.

Kheradpisheh Z, Mirzaei M, Mahvi AH, Mokhtari M, Azizi R, Fallahzadeh H, Ehrampoush MH. 2018b. Impact of drinking water fluoride on human thyroid hormones: A case-control study. *Sci Rep* 8: 2674.

Kinawy AA, Al-Eidan AA. 2018. Impact of prenatal and postnatal treatment of sodium fluoride and aluminum chloride on some hormonal and sensorimotor aspects in rats. *Biol Trace Elem Res*: 1-8.

Kumar V, Chahar P, Kajjari S, Rahman F, Bansal DK, Kapadia JM. 2018. Fluoride, thyroid hormone derangements and its correlation with tooth eruption pattern among the pediatric population from endemic and non-endemic fluorosis areas. *J Contemp Dent Pract* 19(12): 1512-1516.

Li F, Chen X, Huang R, Xie Y. 2009. The impact of endemic fluorosis caused by the burning of coal on the development of intelligence in children. *J Environ Health* 26(4): 838-840.

Li J, Yao L, Shao QL, Wu CY. 2008a. Effects of high fluoride level on neonatal neurobehavioral development. *Fluoride* 41: 165-170.

Li M, Gao Y, Cui J, Li Y, Li B, Liu Y, Sun J, Liu X, Liu H, Zhao L, Sun D. 2016. Cognitive impairment and risk factors in elderly people living in fluorosis areas in China. *Biol Trace Elem Res* 172: 53-60.

Li X, Zhang J, Niu R, Manthari RK, Yang K, Wang J. 2019. Effect of fluoride exposure on anxiety- and depression-like behavior in mouse. *Chemosphere* 215: 454-460.

Li XS, Zhi JL, Gao RO. 1995. Effect of fluoride exposure on the intelligence of children. *Fluoride* 28: 189-192.

Li Y, Li X, Wei S. 2008b. Effects of high fluoride intake on child mental work capacity: Preliminary investigation into the mechanisms involved. *Fluoride* 41: 331-335.

Lin F, Ai H, Zhao H, Lin J, Jhiang J, Maimaiti. 1991. High fluoride and low iodine environment and subclinical cretinism in Xinjiang. *Endem Dis Bull* 6(2): 62-67.

Liu YJ, Gao Q, Wu CX, Guan ZZ. 2010. Alterations of nAChRs and ERK1/2 in the brains of rats with chronic fluorosis and their connections with the decreased capacity of learning and memory. *Toxicol Lett* 192: 324-329.

Lou DD, Guan ZZ, Liu YJ, Liu YF, Zhang KL, Pan JG, Pei JJ. 2013. The influence of chronic fluorosis on mitochondrial dynamics morphology and distribution in cortical neurons of the rat brain. *Arch Toxicol* 87: 449-457.

Lu F, Zhang Y, Trivedi A, Jiang X, Chandra D, Zheng J, Nakano Y, Uyghurturk DA, Jalai R, Guner S, Mentes A, DenBesten PK. 2019. Fluoride related changes in behavioral outcomes may relate to increased serotonin. *Physiol Behav* 206: 76-83.

Malin AJ, Till C. 2015. Exposure to fluoridated water and attention deficit hyperactivity disorder prevalence among children and adolescents in the United States: An ecological association. *Environ Health* 14: 17.

Malin AJ, Riddell J, McCague H, Till C. 2018. Fluoride exposure and thyroid function among adults living in Canada: Effect modification by iodine status. *Environ Int* 121(Pt 1): 667-674.

Manusha S, Sudhakar K, Reddy KP. 2019. Protective effects of allium sativum extract against sodium fluoride induced neurotoxicity. *Int J Pharm Sci Res* 10(2): 625-633.

McPherson CA, Zhang G, Gilliam R, Brar SS, Wilson R, Brix A, Picut C, Harry GJ. 2018. An evaluation of neurotoxicity following fluoride exposure from gestational through adult ages in Long-Evans hooded rats. *Neurotoxicol Res*: 1-18.

Mesram N, Nagapuri K, Banala RR, Nalagoni CR, Karnati PR. 2016. Quercetin treatment against NaF induced oxidative stress related neuronal and learning changes in developing rats. *J King Saud Univ Sci* 29: 221-229.

Michael M, Barot VV, Chinoy NJ. 1996. Investigations of soft tissue functions in fluorotic individuals of North Gujarat. *Fluoride* 29: 63-71.

Miller K, Howard B, Phillips J, Shah M, Mav D, Thayer K, Shah R. 2016. SWIFT-Active screener: Reducing literature screening effort through machine learning for systematic reviews, Cochrane Colloquium Seoul, Seoul, Korea.

Moher D, Liberati A, Tetzlaff J, Altman D. 2009. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med* 6(7): e1000097.

Morgan L, Allred E, Tavares M, Bellinger D, Needleman H. 1998. Investigation of the possible associations between fluorosis, fluoride exposure, and childhood behavior problems. *Pediatr Dent* 20: 244-252.

Mustafa DE, Younis UM, Elhag SAA. 2018. The relationship between the fluoride levels in drinking water and the schooling performance of children in rural areas of Khartoum State, Sudan. *Fluoride* 51(2): 102-113.

Nageshwar M, Sudhakar K, Reddy NCC, Reddy KP. 2017. Neuroprotective effects of curcumin on sodium fluoride induced behavioural and enzymatic changes in brain and muscles of rat. *J Environ Biol* 38: 675-681.

Nageshwar M, Sudhakar K, Reddy KP. 2018. Quercetin ameliorates oxidative stress, neural damage of brain and behavioral impairment of rat with fluoride exposure. *Int J Pharm Sci Res* 9(8): 3247-3256.

Niu Q, Chen J, Xia T, Li P, Zhou G, Xu C, Zhao Q, Dong L, Zhang S, Wang A. 2018. Excessive ER stress and the resulting autophagic flux dysfunction contribute to fluoride-induced neurotoxicity. *Environ Pollut* 233: 889-899.

Nkpaa KW, Onyeso GI. 2018. Rutin attenuates neurobehavioral deficits, oxidative stress, neuro-inflammation and apoptosis in fluoride treated rats. *Neurosci Lett* 682: 92-99.

NRC (National Research Council). 2006. *Committee on fluoride in drinking water, board on environmental studies and toxicology. Fluoride in drinking water: A scientific review of EPA's standards.* Available: http://www.nap.edu/catalog/11571/fluoride-in-drinking-water-a-scientific-review-of-epas-standards [accessed 19 August 2019].

NTP (National Toxicology Program). 2015. *Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration*. Research Triangle Park, NC. Available: http://ntp.niehs.nih.gov/go/38673 [accessed 19 August 2019].

NTP (National Toxicology Program). 2016. *Systematic literature review on the effects of fluoride on learning and memory in animal studies. NTP Research Report 1.* Research Triangle Park, NC. Available: https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/01fluoride_508.pdf [accessed 19 August 2019].

NTP (National Toxicology Program). 2019. *Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration*. Research Triangle Park, NC. Available: https://ntp.niehs.nih.gov/go/systematic_review [accessed 19 August 2019].

OEHHA (California Office of Environmental Health Hazard Assessment). 2011. *Meeting synopsis and slide presentations: carcinogen identification committee meeting held on October 12, 2011*. Available: http://oehha.ca.gov/prop65/public_meetings/cic101211synop.html [accessed 19 August 2019].

Peckham S, Lowery D, Spencer S. 2015. Are fluoride levels in drinking water associated with hypothyroidism prevalence in England? A large observational study of GP practice data and fluoride levels in drinking water. *J Epidemiol Community Health* 69: 619-624.

Pulungan ZS, Sofro ZM, Partadiredja G. 2016. Sodium fluoride does not affect the working memory and number of pyramidal cells in rat medial prefrontal cortex. *Anat Sci Int* 93(1): 128-138.

Qin LS, Huo SY, Chen RL, Chang YZ, Zhao MY. 2008. Using the Raven's Standard Progressive Matrices to determine the effects of the level of fluoride in drinking water on the intellectual ability of school-age children. *Fluoride* 41: 115-119.

Raju S, Sivanesan SK, Gudemalla K. 2019. Cognitive enhancement effect of Ginkgo biloba extract on memory and learning impairments induced by fluoride neurotoxicity. *Int J Res Pharm Sci* 10(1): 129-134.

Reddy YP, Tiwari SK, Shaik AP, Alsaeed A, Sultana A, Reddy PK. 2014. Effect of sodium fluoride on neuroimmunological parameters, oxidative stress and antioxidative defenses. *Toxicol Mech Methods* 24: 31-36.

Rocha-Amador D, Navarro ME, Carrizales L, Morales R, Calderon J. 2007. Decreased intelligence in children and exposure to fluoride and arsenic in drinking water. *Cad Saude Publica* 23 Suppl 4: S579-587.

Rocha-Amador D, Navarro M, Trejo-Acevedo A, Carrizales L, Perez-Maldonado I, Diaz-Barriga F, Calderon J. 2009. Use of the Rey-Osterrieth Complex Figure Test for neurotoxicity evaluation of mixtures in children. *Neurotox* 30: 1149-1154.

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, Thayer KA. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. *Environ Health Perspect* 122(7): 711-718.

Rooney AA, Cooper GS, Jahnke GD, Lam J, Morgan RL, Ratcliffe JM, Kraft AD, Schünemann HJ, Schwingl P, Walker TD, Thayer KA, Lunn RM. 2016. How credible are the study results? Evaluating and applying internal validity tools to literature-based assessments of environmental health hazards. *Environ Int* 92-93: 617-629.

Rotton J, Tikofsky RS, Feldman HT. 1982. Behavioral effects of chemicals in drinking water. *J Appl Psychol* 67: 230-238.

Russ TC, Killin LOJ, Hannah J, Batty GD, Deary IJ, Starr JM. 2019. Aluminium and fluoride in drinking water in relation to later dementia risk. *Brit J Psychol* 14: 1-6.

Saxena S, Sahay A, Goel P. 2012. Effect of fluoride exposure on the intelligence of school children in Madhya Pradesh, India. *J Neurosci Rural Pract* 3: 144-149.

SCHER (Scientific Committee on Health and Environmental Risks). 2011. *Critical review of any new evidence on the hazard profile, health effects, and human exposure to fluoride and the fluoridating agents of drinking water*. European Commission Directorate-General for Health and Consumers Scientific Committees. Available: http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_139.pdf [accessed 19 August 2019].

Sebastian ST, Sunitha S. 2015. A cross-sectional study to assess the intelligence quotient (IQ) of school going children aged 10-12 years in villages of Mysore District, India with different fluoride levels. *J Indian Soc Pedod Prev Dent* 33: 307-311.

Seraj B, Shahrabi M, Shadfar M, Ahmadi R, Fallahzadeh M, Eslamlu HF, Kharazifard MJ. 2012. Effect of high water fluoride concentration on the intellectual development of children in Makoo, Iran. *J Dent* 9: 221-229.

Shalini B, Sharma JD. 2015. Beneficial effects of emblica officinalis on fluoride-induced toxicity on brain biochemical indexes and learning-memory in rats. *Toxicol Int* 22: 35-39.

Shan KR, Qi XL, Long YG, Nordberg A, Guan ZZ. 2004. Decreased nicotinic receptors in PC12 cells and rat brains influenced by fluoride toxicity: A mechanism relating to a damage at the level in post-transcription of the receptor genes. *Toxicology* 200: 169-177.

Shannon FT, Fergusson DM, Horwood LJ. 1986. Exposure to fluoridated public water supplies and child health and behaviour. *New Zeal Med J* 99: 416-418.

Shao Q. 2003. Study of cognitive function impairment caused by chronic fluorosis. *Chin J Endemiol* 22(4): 336-338.

Sharma C, Suhalka P, Bhatnagar M. 2018. Curcumin and resveratrol rescue cortical-hippocampal system from chronic fluoride-induced neurodegeneration and enhance memory retrieval. *Int J Neurosci*: 1-15.

Sharma JD, Sohu D, Jain P. 2009. Prevalence of neurological manifestations in a human population exposed to fluoride in drinking water. *Fluoride* 42: 127-132.

Singh N, Verma KG, Verma P, Sidhu GK, Sachdeva S. 2014. A comparative study of fluoride ingestion levels, serum thyroid hormone & TSH level derangements, dental fluorosis status among school children from endemic and non-endemic fluorosis areas. *Springerplus* 3: 7.

Singh V, Singh C, Sandeep T, Sandeep K, Vikas G, Mukesh T, Anurag T. 2013. A correlation between serum vitamin, acetylcholinesterase activity and IQ in children with excessive endemic fluoride exposure in Rajasthan, India. *Int Res J Medical Sci* 1(3): 12-16.

Still CN, Kelley P. 1980. The incidence of primary degenerative dementia vs. water fluoride content in South Carolina, USA. *Neurotox* 1: 125-132.

Sudhakar K, Nageshwar M, Pratap Reddy K. 2017. Seed extract of Abelmoschus moschatus medik reverses NAF-induced behavioral changes through neurodegeneration and oxidative stress in brain of rat. *Asian J Pharm Clin Res* 10: 165-171.

Sudhakar K, Nageshwar M, Reddy KP. 2018a. Abelmoschus moschatus extract reverses altered pain and neurohistology of a rat with developmental exposure of fluoride. *J Appl Pharm Sci* 8(6): 94-104.

Sudhakar K, Nageshwar M, Reddy KP. 2018b. Protective effect of okra, Abelmoschus moschatus seed extract on developing brain of rats during pre- and post-natal fluoride exposure. *Int J Pharm Sci Res* 9: 1519-1528.

Sudhakar K, Reddy KP. 2018. Protective effects of Abelmoschus moschatus seed extract on neurotransmitter system of developing brain of Wistar rats with gestational and post-natal exposure of sodium fluoride. *Int J Green Pharm* 12: S131-S139.

Sudhir KM, Chandu GN, Prashant GM, Subba Reddy VV. 2009. Effect of fluoride exposure on intelligence quotient (IQ) among 13-15 year old school children of known endemic area of fluorosis, Nalgonda District, Andhra Pradesh. *J Indian Assoc Public Health Dent* 2009(13): 88-94.

Sun Z, Zhang Y, Xue X, Niu R, Wang J. 2018. Maternal fluoride exposure during gestation and lactation decreased learning and memory ability, and glutamate receptor mRNA expressions of mouse pups. *Hum Exp Toxicol* 37: 87-93.

Sun ZR, Liu FZ, Wu LN, Lu Y, Yu DK. 2008. Effects of high fluoride drinking water on the cerebral function of mice. *Fluoride* 41: 148-151.

Susheela AK, Bhatnagar M, Vig K, Mondal NK. 2005. Excess fluoride ingestion and thyroid hormone derangements in children living in Delhi, India. *Fluoride* 38: 98-108.

Till C, Green R, Grundy JG, Hornung R, Neufeld R, Martinez-Mier EA, Ayotte P, Muckle G, Lanphear B. 2018. Community Water Fluoridation and Urinary Fluoride Concentrations in a National Sample of Pregnant Women in Canada. *Environ Health Perspect* 126(10): 107001.

Tsunoda M, Aizawa Y, Nakano K, Liu Y, Horiuchi T, Itai K, Tsunoda H. 2005. Changes in fluoride levels in the liver, kidney, and brain and in neurotransmitters of mice after subacute administration of fluoride. *Fluoride* 38: 284-292.

US DHHS (U.S. Department of Health and Human Services). 2015. *U.S. Public Health Service recommendation for fluoride concentration in drinking water for the prevention of dental caries*. 318-331. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4547570/ [accessed 19 August 2019].

US EPA (U.S. Environmental Protection Agency). 2010. *Fluoride: Exposure and relative source contribution analysis*. 820-R-10-015. Washington, DC. Available: http://www.epa.gov/dwstandardsregulations/fluoride-risk-assessment-and-relative-source-contribution [accessed 19 August 2019].

Valdez Jimenez L, Lopez Guzman OD, Cervantes Flores M, Costilla-Salazar R, Calderon Hernandez J, Alcaraz Contreras Y, Rocha-Amador DO. 2017. In utero exposure to fluoride and cognitive development delay in infants. *Neurotox* 59: 65-70.

Villa A, Anabalon M, Zohouri V, Maguire A, Franco AM, Rugg-Gunn A. 2010. Relationships between fluoride intake, urinary fluoride excretion and fluoride retention in children and adults: an analysis of available data. *Caries Res* 44(1): 60-68.

Wang J, Zhang Y, Guo Z, Li R, Xue X, Sun Z, Niu R. 2018. Effects of perinatal fluoride exposure on the expressions of miR-124 and miR-132 in hippocampus of mouse pups. *Chemosphere* 197: 117-122.

Wang S, Wang L, Hu P, Guo S, Law S. 2001. [Effects of high iodine and high fluorine on children's intelligence and thyroid function]. *Chin J Endemiol* 20(4): 288-290.

Watanabe M, Kono K, Orita Y, Dote T, Usuda K, Takahashi Y, Yoshida Y. 1995. Influence of dietary fluoride intake on urinary fluoride concentration and evaluation of corrected levels in spot urine. *Fluoride* 28(2): 61-70.

Xiang Q, Liang Y, Chen L, Wang C, Chen B, Chen X, Zhou M. 2003. Effect of fluoride in drinking water on children's intelligence. *Fluoride* 36: 84-94.

Xiang Q, Liang Y, Chen B, Chen L. 2011. Analysis of children's serum fluoride levels in relation to intelligence scores in a high and low fluoride water village in China. *Fluoride* 44: 191-194.

Xu Y, Lu C, Zhang X. 1994. Effect of fluoride on children's intelligence. *Endem Dis Bull* 2: 83-84.

Yang L, Jin P, Wang X, Zhou Q, Lin X, Xi S. 2018. Fluoride activates microglia, secretes inflammatory factors and influences synaptic neuron plasticity in the hippocampus of rats. *Neurotox* 69: 108-120.

Yang Y, Wang X, Guo X, Hu P. 2008. The effects of high levels of fluoride and iodine on child intellectual ability and the metabolism of fluoride and iodine. *Fluoride* 41: 336-339.

Yao L, Zhou J, Wang S, Cui K, Lin F. 1996. Analysis of TSH levels and intelligence of children residing in high fluorosis areas. *Lit Inf Prev Med* 2(1): 26-27.

Yasmin S, Ranjan S, D'Souza D. 2013. Effect of excess fluoride ingestion on human thyroid function in Gaya Region, Bihar, India. *Toxicol Environ Chem* 95: 1235-1243.

Yazdi SM, Sharifian A, Dehghani-Beshne M, Momeni VR, Aminian O. 2011. Effects of fluroide on psychomotor performance and memory of aluminum potroom workers. *Fluoride* 44: 158-162.

Yu X, Chen J, Li Y, Liu H, Hou C, Zeng Q, Cui Y, Zhao L, Li P, Zhou Z, Pang S, Tang S, Tian K, Zhao Q, Dong L, Xu C, Zhang X, Zhang S, Liu L, Wang A. 2018. Threshold effects of moderately excessive fluoride exposure on children's health: A potential association between dental fluorosis and loss of excellent intelligence. *Environ Int* 118: 116-124.

Yu YN, Yang WX, Dong Z, Wan CW, Zhang JT, Liu JL, Xiao KQ, Huang YS, Lu BF. 2008. Neurotransmitter and receptor changes in the brains of fetuses from areas of endemic fluorosis. *Fluoride* 41: 134-138.

Yuan J, Li Q, Niu R, Wang J. 2019. Fluoride exposure decreased learning ability and the expressions of the insulin receptor in male mouse hippocampus and olfactory bulb. *Chemosphere* 224: 71-76.

Zhang J, Yao H, Chen Y. 1998. [Effect of high level of fluoride and arsenic on children's intelligence]. *Chin J Public Health* 17(2): 57.

Zhang KL, Lou DD, Guan ZZ. 2015a. Activation of the AGE/RAGE system in the brains of rats and in SH-SY5Y cells exposed to high level of fluoride might connect to oxidative stress. *Neurotoxicol Teratol* 48: 49-55.

Zhang S, Zhang X, Liu H, Qu W, Guan Z, Zeng Q, Jiang C, Gao H, Zhang C, Lei R, Xia T, Wang Z, Yang L, Chen Y, Wu X, Cui Y, Yu L, Wang A. 2015b. Modifying effect of COMT gene polymorphism and a predictive role for proteomics analysis in children's intelligence in endemic fluorosis area in Tianjin, China. *Toxicol Sci* 144: 238-245.

Zhao Q, Niu Q, Chen J, Xia T, Zhou G, Li P, Dong L, Xu C, Tian Z, Luo C, Liu L, Zhang S, Wang A. 2019. Roles of mitochondrial fission inhibition in developmental fluoride neurotoxicity: Mechanisms of action in vitro and associations with cognition in rats and children. *Arch Toxicol* 93(3): 709-726.

Zheng X, Sun Y, Ke L, Ouyang W, Zhang Z. 2016. Molecular mechanism of brain impairment caused by drinking-acquired fluorosis and selenium intervention. *Environ Toxicol Pharmacol* 43: 134-139.

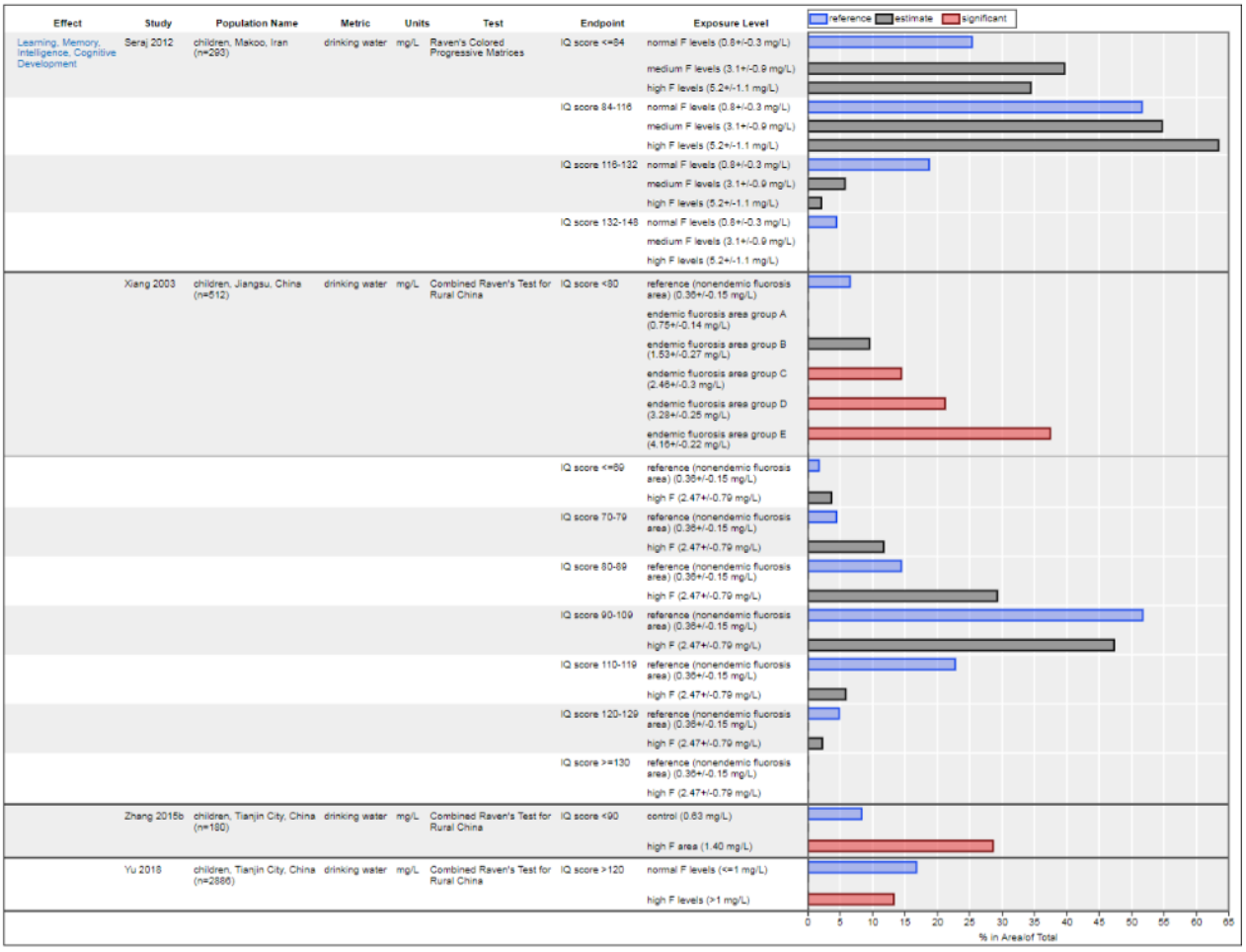
Zhu YP, Xi SH, Li MY, Ding TT, Liu N, Cao FY, Zeng Y, Liu XJ, Tong JW, Jiang SF. 2017. Fluoride and arsenic exposure affects spatial memory and activates the ERK/CREB signaling pathway in offspring rats. *Neurotox* 59: 56-64.

Zohouri FV, Swinbank CM, Maguire A, Moynihan PJ. 2006. Is the fluoride/creatinine ratio of a spot urine sample indicative of 24-h urinary fluoride? *Community Dent Oral Epidemiol* 34(2): 130-138.

DATA FIGURES

Neurodevelopmental or Cognitive Effects and Outcomes

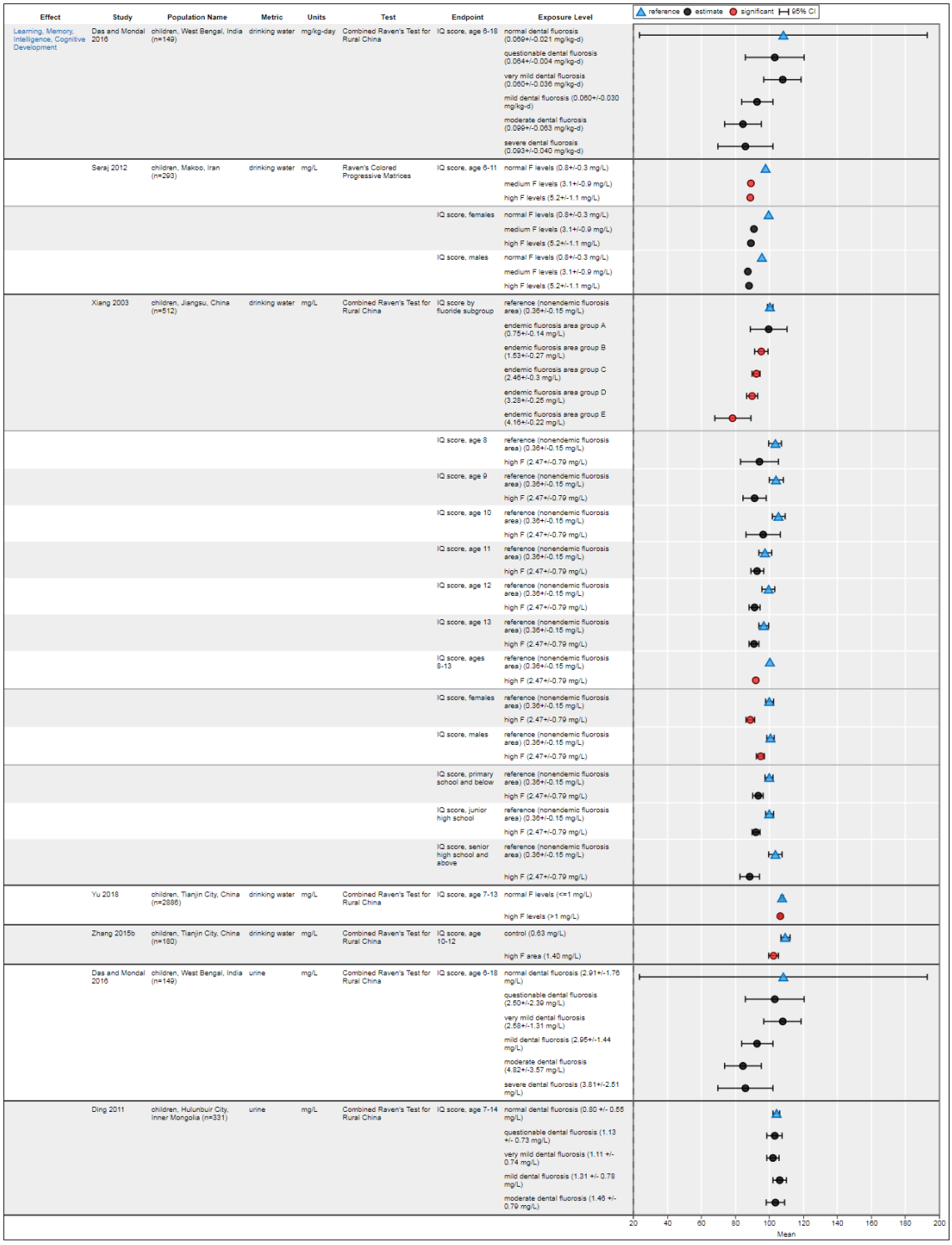
Figure D1. IQ Distribution in Children by Fluoride Exposure (lower risk-of-bias studies; presented as % in area or % of total group)



Interactive figure and additional study details in HAWC here. "F" represents fluoride.

Differences in intelligence between the reference group and treatment groups were statistically significant although significance was not reported separately for each score level.

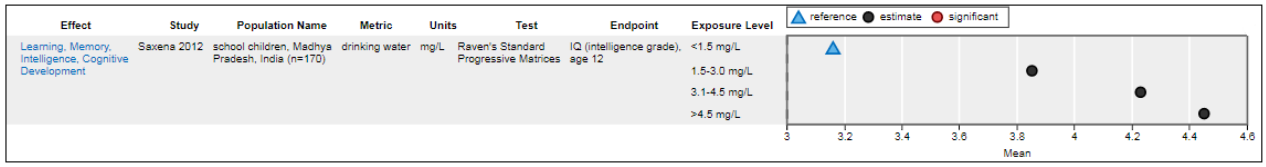
Figure D2. Mean IQ in Children by Fluoride Exposure (lower risk-of-bias studies)



Interactive figure and additional study details in HAWC here. "F" represents fluoride.

For all studies, SDs are available and can be viewed in HAWC by clicking the data points within the plot area; however, 95% CIs could not be calculated for Seraj *et al.* (2012) because Ns are not available for exposure groups.

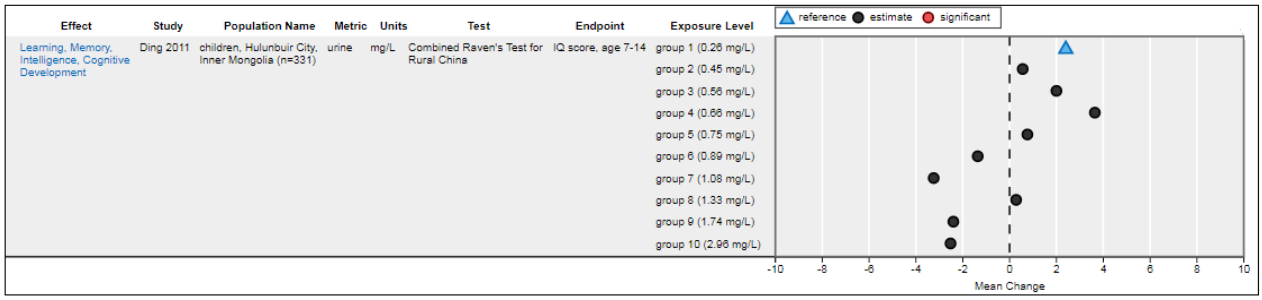
Figure D3. Intelligence Grade in Children by Fluoride Exposure (lower risk-of-bias studies; presented as mean)



Interactive figure and additional study details in HAWC here.

For Saxena *et al.* (2012), children's intelligence was measured using the Raven's Standard Progressive Matrices. Children's scores were converted to percentile and specific grades were allotted based on the percentiles. Grades ranged from intellectually superior (Grade I) to intellectually impaired (Grade V).

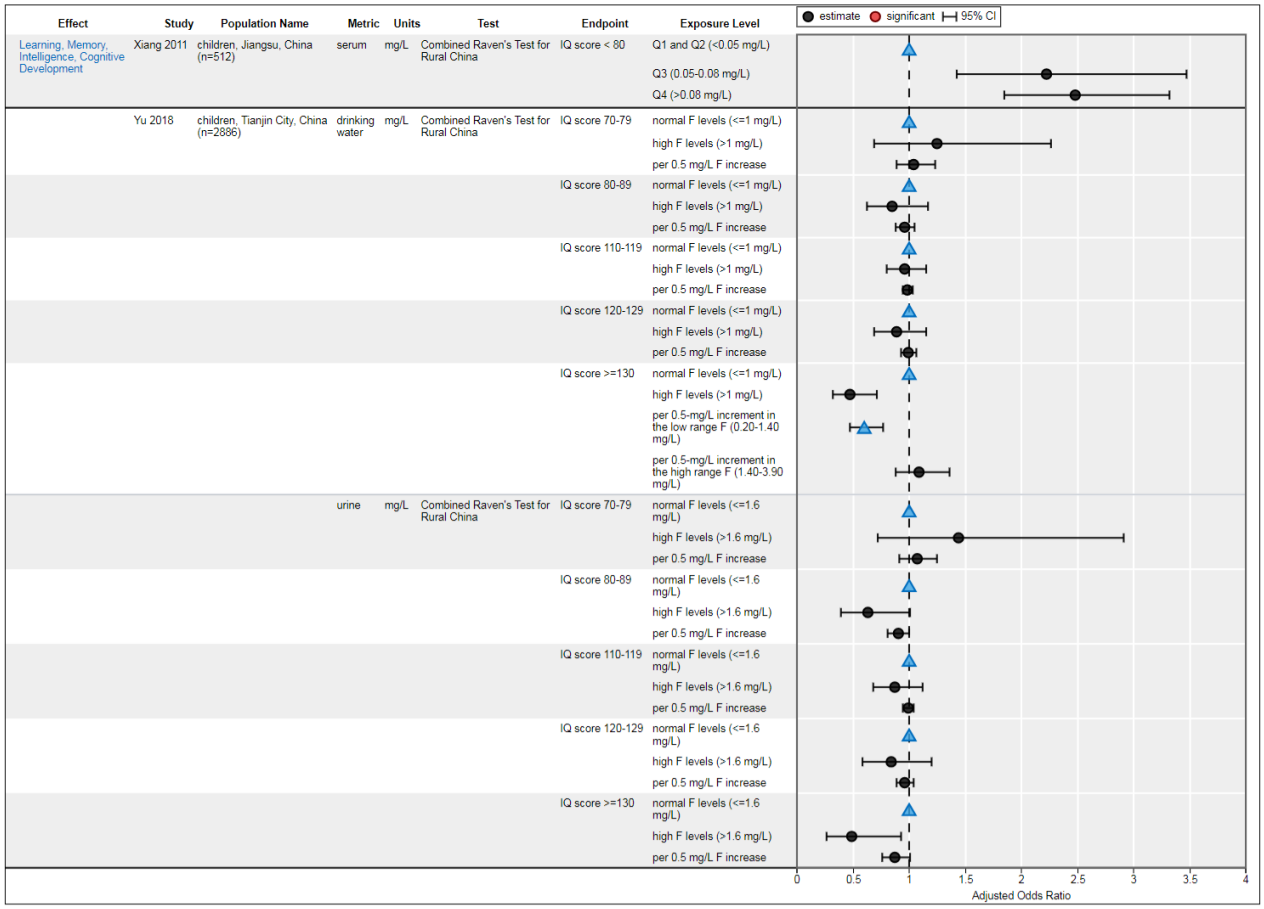
Figure D4. Mean Change in IQ in Children by Fluoride Exposure (lower risk-of-bias studies)



Interactive figure and additional study details in HAWC here.

For Ding *et al.* (2011), SDs are available and can be viewed in HAWC by clicking the data points within the plot area; however, 95% CIs could not be calculated because Ns for each exposure group are not available.

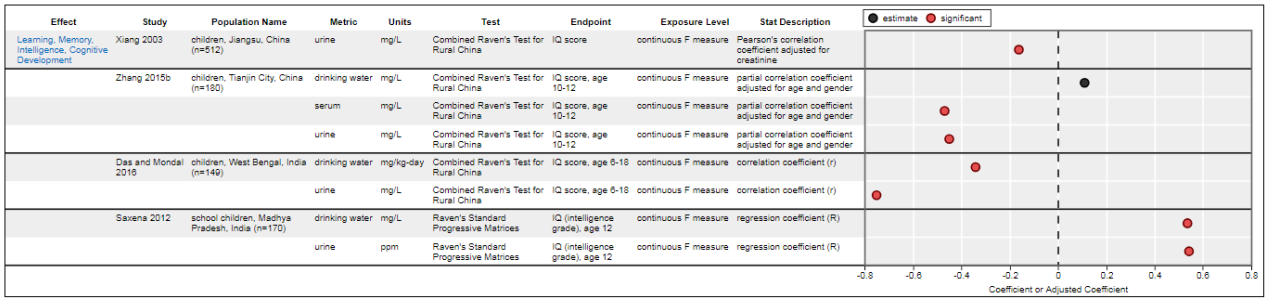
Figure D5. IQ Score in Children by Fluoride Exposure (lower risk-of-bias studies; presented as adjusted OR)



Interactive figure and additional study details in HAWC here.

For Xiang *et al.* (2011), there was a significant linear trend across different levels of serum fluoride for IQ score < 80 ($p < 0.001$). For Yu *et al.* (2018), significance levels by IQ score were not reported.

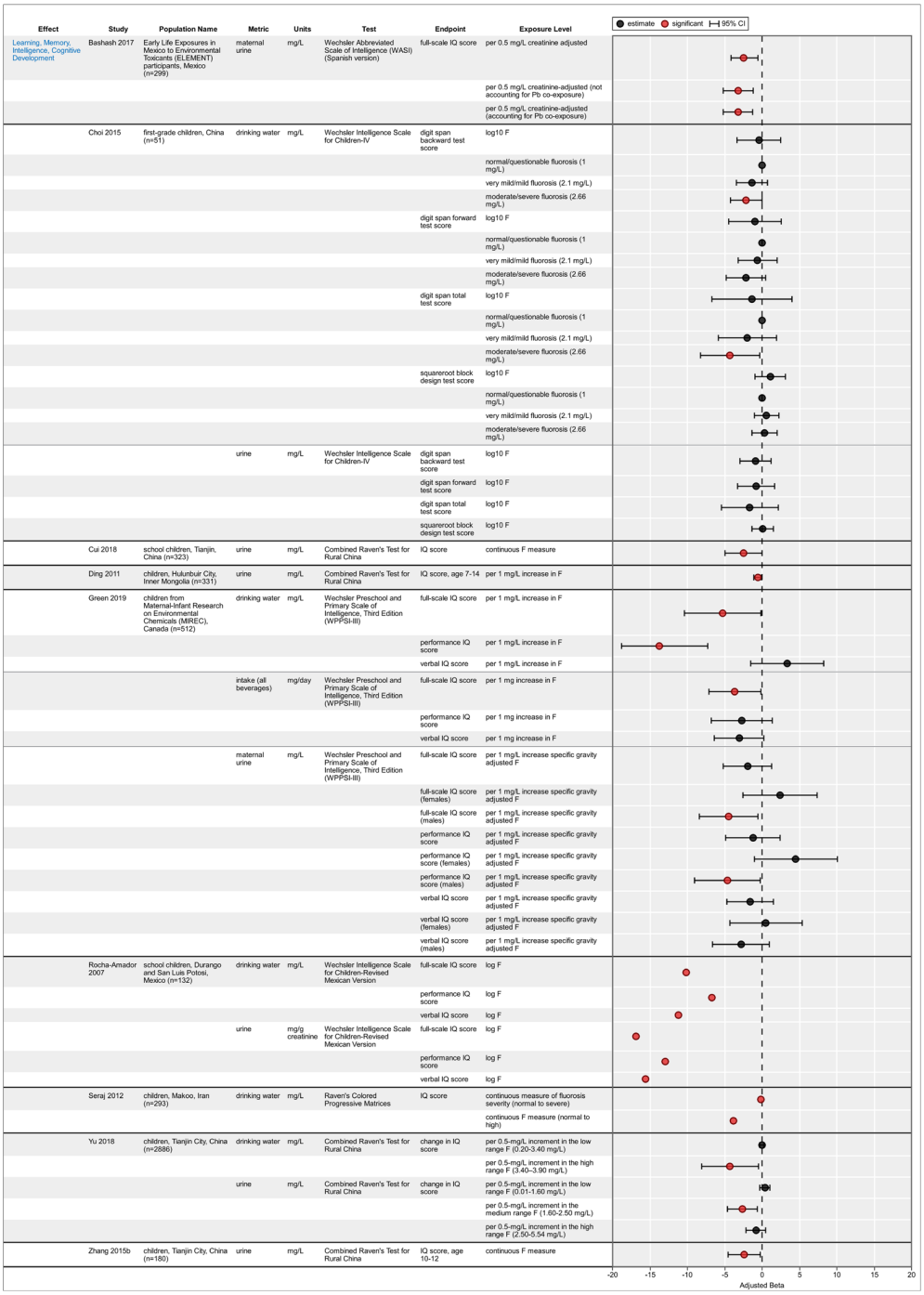
Figure D6. Correlations between IQ Score and Fluoride Exposure in Children (lower risk-of-bias studies; presented as coefficient)



Interactive figure and additional study details in HAWC here. "F" represents fluoride.

For Saxena *et al.* (2012), a significant relationship between water fluoride level and intelligence grade was observed. Increasing intelligence grades reflected increasing levels of impairment (reduced intelligence) in children.

Figure D7. Correlations between IQ Score and Fluoride Exposure in Children (lower risk-of-bias studies; presented as adjusted beta)

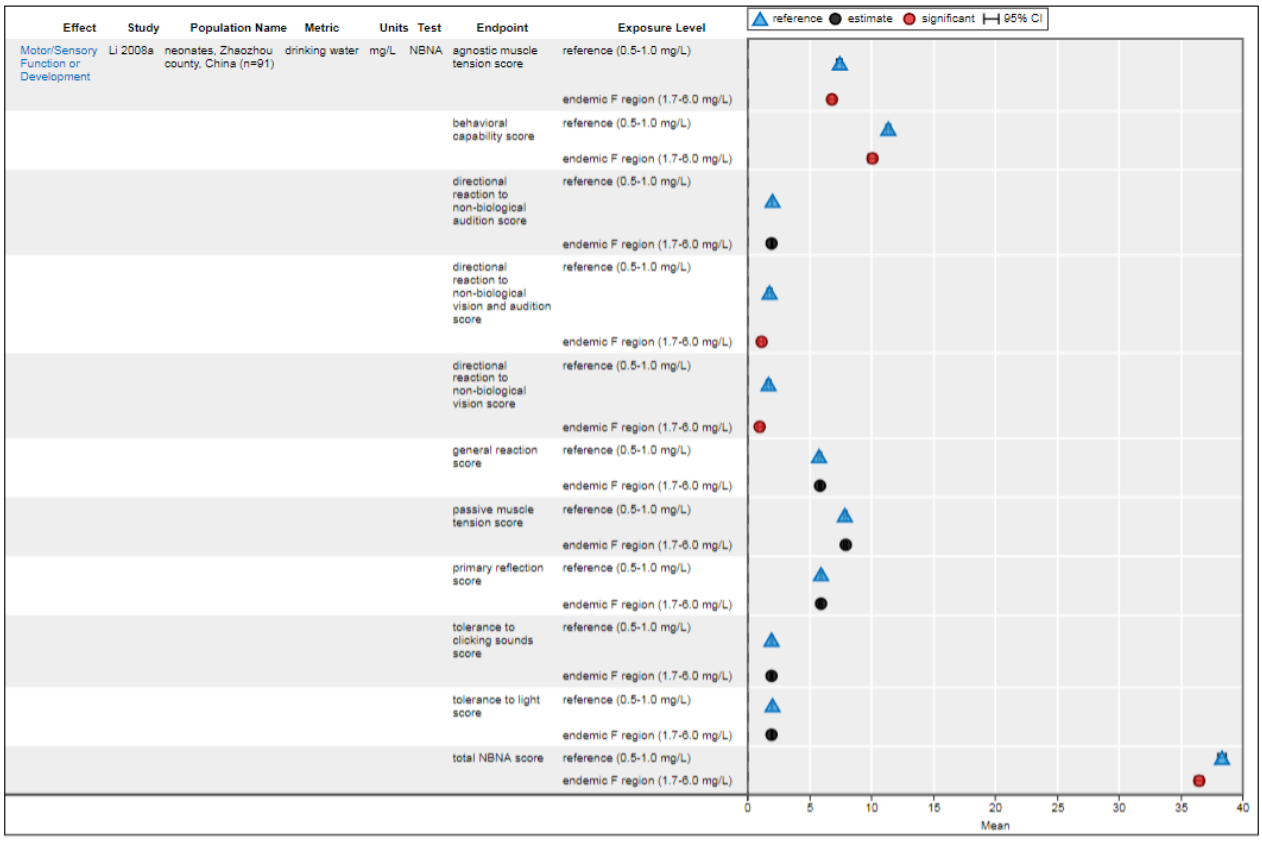


Effect	Study	Population Name	Metric	Units	Test	Endpoint	Exposure Level
Learning, Memory, Intelligence, Cognitive Development	Bashash 2017	Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) participants, Mexico (n=299)	maternal urine	mg/L	Wechsler Abbreviated Scale of Intelligence (WASI) (Spanish version)	full-scale IQ score	per 0.5 mg/L creatinine adjusted
							per 0.5 mg/L creatinine-adjusted (not accounting for Pb co-exposure)
							per 0.5 mg/L creatinine-adjusted (accounting for Pb co-exposure)
	Choi 2015	first-grade children, China (n=51)	drinking water	mg/L	Wechsler Intelligence Scale for Children-IV	digit span backward test score	log10 F
							normal/questionable fluorosis (1 mg/L)
							very mild/mild fluorosis (2.1 mg/L)
							moderate/severe fluorosis (2.66 mg/L)
						digit span forward test score	log10 F
							normal/questionable fluorosis (1 mg/L)
							very mild/mild fluorosis (2.1 mg/L)
							moderate/severe fluorosis (2.66 mg/L)
						digit span total test score	log10 F
							normal/questionable fluorosis (1 mg/L)
							very mild/mild fluorosis (2.1 mg/L)
							moderate/severe fluorosis (2.66 mg/L)
						squareroot block design test score	log10 F
							normal/questionable fluorosis (1 mg/L)
							very mild/mild fluorosis (2.1 mg/L)
							moderate/severe fluorosis (2.66 mg/L)
			urine	mg/L	Wechsler Intelligence Scale for Children-IV	digit span backward test score	log10 F
						digit span forward test score	log10 F
						digit span total test score	log10 F
						squareroot block design test score	log10 F
	Cui 2018	school children, Tianjin, China (n=323)	urine	mg/L	Combined Raven's Test for Rural China	IQ score	continuous F measure
	Ding 2011	children, Hulunbuir City, Inner Mongolia (n=331)	urine	mg/L	Combined Raven's Test for Rural China	IQ score, age 7-14	per 1 mg/L increase in F
	Green 2019	children from Maternal-Infant Research on Environmental Chemicals (MIREC), Canada (n=512)	drinking water	mg/L	Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III)	full-scale IQ score	per 1 mg/L increase in F
						performance IQ score	per 1 mg/L increase in F
						verbal IQ score	per 1 mg/L increase in F
			intake (all beverages)	mg/day	Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III)	full-scale IQ score	per 1 mg increase in F
						performance IQ score	per 1 mg increase in F
						verbal IQ score	per 1 mg increase in F
			maternal urine	mg/L	Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III)	full-scale IQ score	per 1 mg/L increase specific gravity adjusted F
						full-scale IQ score (females)	per 1 mg/L increase specific gravity adjusted F
						full-scale IQ score (males)	per 1 mg/L increase specific gravity adjusted F
						performance IQ score	per 1 mg/L increase specific gravity adjusted F
						performance IQ score (females)	per 1 mg/L increase specific gravity adjusted F
						performance IQ score (males)	per 1 mg/L increase specific gravity adjusted F
						verbal IQ score	per 1 mg/L increase specific gravity adjusted F
						verbal IQ score (females)	per 1 mg/L increase specific gravity adjusted F
						verbal IQ score (males)	per 1 mg/L increase specific gravity adjusted F
	Rocha-Amador 2007	school children, Durango and San Luis Potosi, Mexico (n=132)	drinking water	mg/L	Wechsler Intelligence Scale for Children-Revised Mexican Version	full-scale IQ score	log F
						performance IQ score	log F
						verbal IQ score	log F
			urine	mg/g creatinine	Wechsler Intelligence Scale for Children-Revised Mexican Version	full-scale IQ score	log F
						performance IQ score	log F
						verbal IQ score	log F
	Seraj 2012	children, Makoo, Iran (n=293)	drinking water	mg/L	Raven's Colored Progressive Matrices	IQ score	continuous measure of fluorosis severity (normal to severe)
							continuous F measure (normal to high)
	Yu 2018	children, Tianjin City, China (n=2886)	drinking water	mg/L	Combined Raven's Test for Rural China	change in IQ score	per 0.5-mg/L increment in the low range F (0.20-3.40 mg/L)
							per 0.5-mg/L increment in the high range F (3.40–3.90 mg/L)
			urine	mg/L	Combined Raven's Test for Rural China	change in IQ score	per 0.5-mg/L increment in the low range F (0.01-1.60 mg/L)
							per 0.5-mg/L increment in the medium range F (1.60-2.50 mg/L)
							per 0.5-mg/L increment in the high range F (2.50-5.54 mg/L)
	Zhang 2015b	children, Tianjin City, China (n=180)	urine	mg/L	Combined Raven's Test for Rural China	IQ score, age 10-12	continuous F measure

Interactive figure and additional study details in HAWC here. "F" represents fluoride.

For Yu *et al.* (2018), authors note an obvious decrease in the IQ score at water fluoride exposure levels between 3.40 mg/L and 3.90 mg/L and a similar adverse effect on IQ scores at urinary fluoride exposure levels from 1.60 mg/L to 2.50 mg/L, and so the changes in IQ score are indicated as significant; however, significance levels by change in IQ score were not reported.

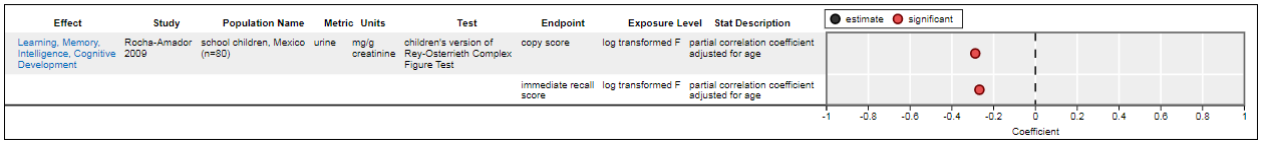
Figure D8. Mean Motor/Sensory Scores in Children by Fluoride Exposure (lower risk-of-bias studies)



Interactive figure and additional study details in HAWC here. "F" represents fluoride.

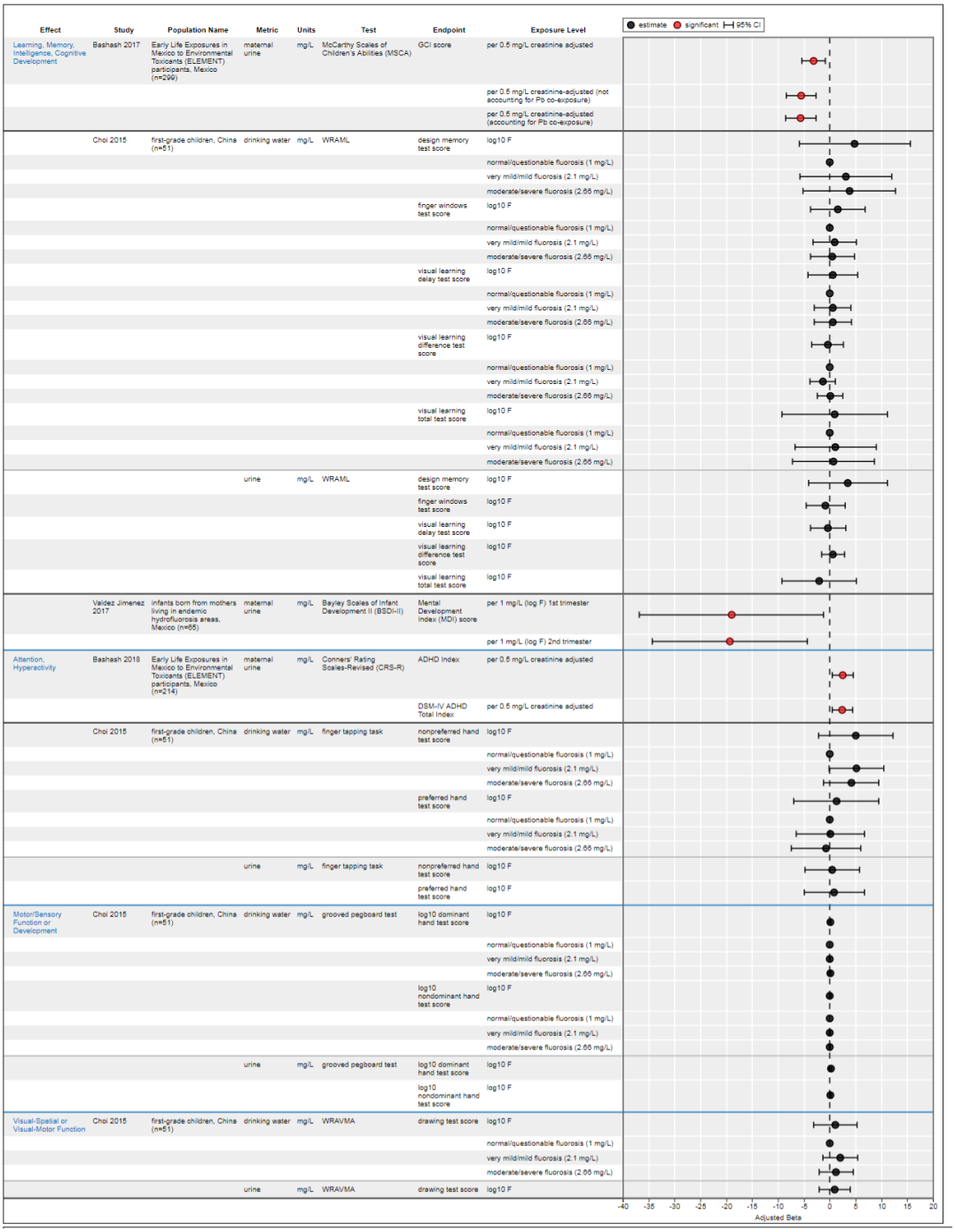
95% CIs are small and are within figure symbols and may be difficult to see. Values for SDs and 95% CIs can be viewed in HAWC by clicking the data points within the plot area.

Figure D9. Correlations between Other Neurological Effects and Fluoride Exposure in Children (lower risk-of-bias studies; presented as coefficient)



Interactive figure and additional study details in HAWC here. "F" represents fluoride.

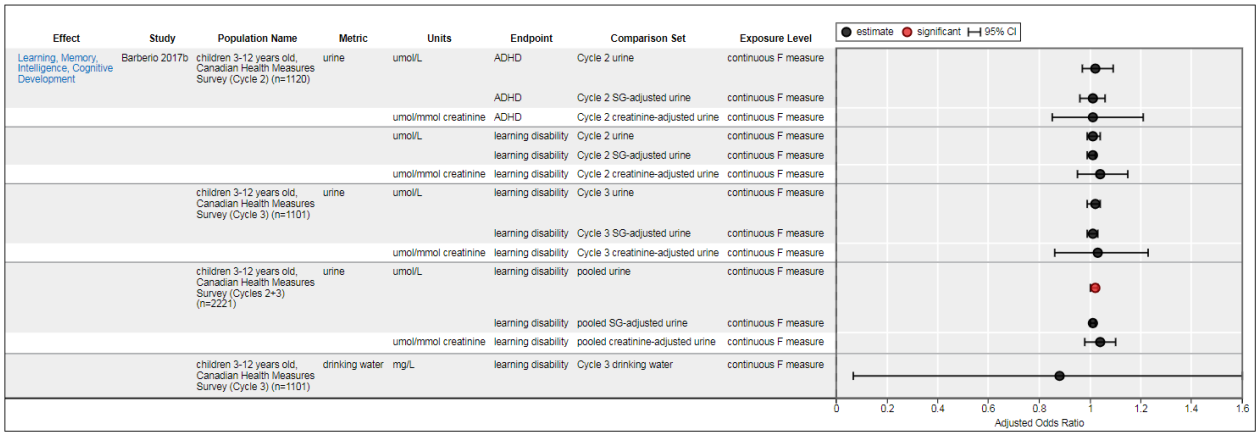
Figure D10. Correlations between Other Neurological Effects and Fluoride Exposure in Children (lower risk-of-bias studies; presented as adjusted beta)



Interactive figure and additional study details in HAWC here. "F" represents fluoride.

Bashash et al. (2018) observed significant associations between maternal urinary fluoride and ADHD-like symptoms related to inattention (an increase in 0.5 mg/L of maternal urinary fluoride was associated with a 2.84-point increase in the DSM-IV Inattention Index and a 2.54-point increase in Cognitive Problems and Inattention Index). These two scales contributed to the global ADHD Index and the DSM-IV ADHD Total Index shown here.

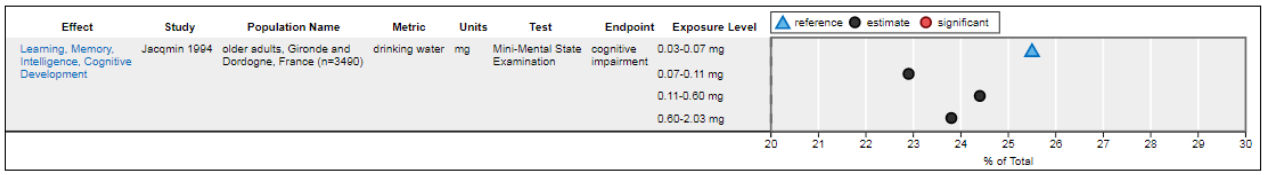
Figure D11. Correlations between Other Neurological Effects and Fluoride Exposure in Children (lower risk-of-bias studies; presented as adjusted OR)



Interactive figure and additional study details in HAWC here. "F" represents fluoride.

Drinking water results for Barberio *et al.* (2017b) have a large confidence interval and are not completely visible in the figure. 95% CIs are 0.068–11.33 and can be viewed in HAWC by clicking the OR within the plot area.

Figure D12. Cognitive Impairment in Adults by Fluoride Exposure (lower risk-of-bias studies; presented as % of total group)



Interactive figure and additional study details in HAWC here.

Results from Li *et al.* (2016) suggested that fluoride exposure may be a risk factor for cognitive impairment in elderly subjects; however, results from the study were not conducive to presentation in this visualization.

ABOUT THIS REVIEW

Sources of Support

National Institute of Environmental Health Sciences/Division of the National Toxicology Program

Contributors

Evaluation Team

The evaluation team is composed of federal staff and contractor staff support.

Name	Affiliation
Kyla Taylor, PhD	NIEHS/DNTP, Project Lead
John Bucher, PhD	NIEHS/DNTP
Andrew Rooney, PhD	NIEHS/DNTP
Vickie Walker	NIEHS/DNTP
Cynthia J. Willson, PhD, DVM, DACVP	ILS
Louise Assem, PhD	ICF [f]
Carlye A. Austin, PhD	ICF [d]
Robyn Blain, PhD	ICF [abcdef]
Natalie Blanton, MPH	ICF [a]
Kristin Bornstein, PhD	ICF [f]
Canden Byrd	ICF [a]
Michelle Cawley	ICF [c]
Anna Engstrom, PhD	ICF [abcdf]
Jeremy S. Frye, MLS	ICF [a]
Susan Goldhaber, MPH	ICF [e]
Ali Goldstone, MPH	ICF [f]
Pamela Hartman, MEM	ICF [abce]
Cara Henning, PhD	ICF [a]
Melinda Hoang, MPH	ICF [d]
Tao Hong	ICF [ef]
Jennifer Hsieh, MSPH	ICF [f]
Penelope Kellar	ICF [a]
Courtney Lemeris	ICF [a]
Alex Lindahl, MPH	ICF [f]
William Mendez, PhD	ICF [b]
Whitney Mitchell	ICF [a]
Revathi Muralidharan	ICF [de]
Johanna Rochester, PhD	ICF [def]
Pam Ross, MSPH	ICF [df]
Jennifer Seed, PhD	ICF [de]
Codi Sharp	ICF [de]
Robert Shin, MHS	ICF [e]
Kelly Shipkowski, PhD	ICF [df]

Name	Affiliation
Christopher Sibrizzi, MPH	ICF [abcdef]
Anna Stamatogiannakis	ICF [a]
Nicole Vetter, MLS	ICF [a]

Note: the roles of individual contractors differed: [a] indicates monograph development; [b] indicates review of data, results, and analyses; [c] indicates database and HAWC support; [d] indicates literature screening, [e] indicates data extraction, [f] indicates risk-of-bias assessment

Peer Reviewers

The peer reviewers were outside experts selected for their experience with fluoride, developmental neurobehavioral toxicity, and systematic review procedures. Peer reviewers were screened for conflict of interest prior to their service and did not report any conflicts of interest. Service as a peer reviewer does not necessarily indicate that the reviewer endorses the final document.

Protocol Reviewers

Name	Affiliation
Joseph Braun, PhD	Brown University
Marie Sutton, PhD	Health Research Board
Thomas Zoeller, PhD	University of Massachusetts, Amherst
Thomas Webster, PhD	Boston University
Gail Wasserman, PhD	Columbia University

no conflicts of interest declared

Technical Review of Draft Monograph

Name	Affiliation
Freya Kamal, PhD	NIEHS (retired)

no conflicts of interest declared

Protocol History and Revisions

Date	Activity or revision
December 14, 2016	**Draft evaluation protocol reviewed:** sent to technical advisors for peer review
April 10, 2017	**Draft human risk-of-bias protocol reviewed;** sent to technical advisors for peer review
May 2, 2017	**Draft animal risk-of-bias protocol reviewed;** sent to technical advisors for peer review
June 2017	**Evaluation protocol finalized:** Review protocol finalized for use and posting

APPENDICES

Appendix 1. Literature Search Strategy

The strategy for this search is broad for the consideration of neurodevelopmental or cognitive endpoints and comprehensive for fluoride as an exposure or treatment in order to ensure inclusion of relevant papers. The search terms for PubMed are provided below. The specific search strategies for other databases are available in the protocol (https://ntp.niehs.nih.gov/go/785076).

Database	Search Terms
PUBMED	((Fluorides[mh:noexp] OR fluorides, topical[mh] OR sodium fluoride[mh] OR Fluorosis, Dental[mh] OR fluorosis[tiab] OR fluorid*[tiab] OR flurid*[tiab] OR fluorin*[tiab] OR florin*[tiab]) NOT (18F[tiab] OR f-18[tiab] OR 19F[tiab] OR f-19[tiab] OR f-labeled[tiab] OR "fluorine-18"[tiab] OR "fluorine-19"[tiab] OR pet-scan[tiab] OR radioligand*[tiab])) AND ((Aryl Hydrocarbon Hydroxylases[mh] OR Aryl Hydrocarbon Receptor Nuclear Translocator[mh] OR Behavior and Behavior Mechanisms[mh] OR Gene Expression Regulation[mh] OR Glucuronosyltransferase[mh] OR Intelligence tests[mh] OR Malate Dehydrogenase[mh] OR Mediator Complex Subunit 1[mh] OR Mental disorders[mh] OR Mental processes[mh] OR Monocarboxylic Acid Transporters[mh] OR Myelin Basic Protein[mh] OR nervous system[mh] OR nervous system diseases[mh] OR nervous system physiological phenomena[mh] OR Neurogranin[mh] OR Oligodendroglia[mh] OR Peroxisome Proliferator-Activated Receptors[mh] OR Psychological Phenomena and Processes[mh] OR Receptors, thyroid hormone[mh] OR Receptors, thyrotropin[mh] OR Retinoid X Receptors[mh] OR thyroid diseases[mh] OR thyroid hormones[mh] OR Thyrotropin-releasing hormone[mh] OR Thyroxine-Binding Proteins[mh] OR Pregnane X Receptor[supplementary concept] OR thyroid-hormone-receptor interacting protein[supplementary concept] OR Constitutive androstane receptor[supplementary concept] OR Academic performance[tiab] OR auditory[tiab] OR cortical[tiab] OR delayed development[tiab] OR developmental impairment[tiab] OR developmental-delay*[tiab] OR developmental-disorder*[tiab] OR euthyroid[tiab] OR gait[tiab] OR glia*[tiab] OR gliogenesis[tiab] OR hyperactiv*[tiab] OR impulse-control[tiab] OR iodide peroxidase[tiab] OR IQ[tiab] OR ischemi*[tiab] OR locomotor[tiab] OR mental deficiency[tiab] OR mental development[tiab] OR mental illness[tiab] OR mental-deficit[tiab] OR mobility[tiab] OR mood[tiab] OR morris-maze[tiab] OR morris-water[tiab] OR motor abilit*[tiab] OR Motor activities[tiab] OR motor performance[tiab] OR nerve[tiab] OR neural[tiab] OR neurobehav*[tiab] OR Neurocognitive impairment[tiab] OR neurodegenerat*[tiab] OR Neurodevelopment*[tiab] OR neurodisease*[tiab] OR neurologic*[tiab] OR neuromuscular[tiab] OR neuron*[tiab] OR neuropath*[tiab] OR obsessive compulsive[tiab] OR OCD[tiab] OR olfaction[tiab] OR olfactory[tiab] OR open-field-test[tiab] OR passive avoidance[tiab] OR plasticity[tiab] OR senil*[tiab] OR sociab*[tiab] OR speech*[tiab] OR spelling[tiab] OR stereotypic-movement*[tiab] OR synap*[tiab] OR tauopath*[tiab] OR Thyroglobulin[tiab] OR Thyroid disease*[tiab] OR thyroid gland[tiab] OR thyroid hormone*[tiab] OR thyronine*[tiab] OR visual motor[tiab] OR Visuospatial processing[tiab] OR water maze[tiab]) OR ((active-avoidance[tiab] OR ADHD[tiab] OR alzheimer*[tiab] OR amygdala[tiab] OR antisocial[tiab] OR anxiety[tiab] OR anxious[tiab] OR asperger*[tiab] OR attention deficit[tiab] OR autism[tiab] OR autistic[tiab] OR behavioral[tiab] OR behaviors[tiab] OR behavioural[tiab] OR behaviours[tiab] OR bipolar[tiab] OR cerebellum[tiab] OR cognition[tiab] OR cognitive[tiab] OR communication-disorder*[tiab] OR comprehension[tiab] OR cranial[tiab] OR dementia[tiab] OR dendrit*[tiab] OR dentate-gyrus[tiab] OR depression[tiab] OR dextrothyroxine[tiab] OR diiodothyronine*[tiab] OR diiodotyrosine[tiab] OR down syndrome[tiab] OR dyslexia[tiab] OR entorhinal cortex[tiab] OR epilep*[tiab] OR gangli*[tiab] OR goiter[tiab] OR graves-disease[tiab] OR hearing[tiab] OR hippocamp*[tiab] OR human development[tiab] OR hyperthyroid*[tiab] OR hypothalam*[tiab] OR hypothyroid*[tiab] OR impulsiv*[tiab] OR Intellectual disability[tiab] OR intelligence[tiab] OR language[tiab] OR learning[tiab] OR lewy bod*[tiab] OR

Database	Search Terms
	long-term potentiation[tiab] OR long-term synaptic depression[tiab] OR memory[tiab] OR mental disorder*[tiab] OR mental recall[tiab] OR monoiodotyrosine[tiab] OR Motor activity[tiab] OR motor skill*[tiab] OR multiple sclerosis[tiab] OR myxedema[tiab] OR Nervous system[tiab] OR nervous-system[tiab] OR neurit*[tiab] OR optic[tiab] OR palsy[tiab] OR panic[tiab] OR parahippocamp*[tiab] OR paranoia[tiab] OR paranoid[tiab] OR parkinson*[tiab] OR perception[tiab] OR perforant*[tiab] OR personality[tiab] OR phobia[tiab] OR problem solving[tiab] OR proprioception[tiab] OR psychomotor[tiab] OR reflex[tiab] OR risk taking[tiab] OR schizophrenia[tiab] OR seizure*[tiab] OR sensation*[tiab] OR sleep[tiab] OR smell[tiab] OR spatial behavior[tiab] OR stroke[tiab] OR substantia-nigra[tiab] OR taste[tiab] OR thyroiditis[tiab] OR thyrotoxicosis[tiab] OR Thyrotropin[tiab] OR thyroxine[tiab] OR triiodothyronine[tiab] OR vision[tiab]) NOT medline[sb]))

Appendix 2. List of Included Studies

Studies in Humans

As described in **Figure 4**, 149 human studies were included; however, full data extraction was only conducted on studies with neurological outcomes or thyroid hormone data. Data extraction was completed using HAWC. Data were extracted from a subset of included studies in humans (n = 106) and are available in HAWC based on outcome. The following lists of references are organized as studies that are available in HAWC followed by studies that are not available in HAWC. Specifically, data for primary neurodevelopmental or cognitive outcomes (learning, memory, and intelligence) and secondary neurobehavioral outcomes (anxiety, aggression, motor activity, or biochemical changes), as well as thyroid hormone level data, were extracted from included human studies and are available in HAWC. Included human studies that only evaluated other thyroid-related effects such as goiters or thyroid size (n = 43) were not extracted and are not available in HAWC.

Studies Available in HAWC

An J, Mei S, Liu A, Fu Y, Wang C. 1992. [Effect of high level of fluoride on children's intelligence]. *Chin J Control Endem Dis* 7(2): 93-94.

Aravind A, Dhanya RS, Narayan A, Sam G, Adarsh VJ, Kiran M. 2016. Effect of fluoridated water on intelligence in 10-12-year-old school children. *J Int Soc Prev Community Dent* 6(Suppl 3): S237-S242.

Bai A, Li Y, Fan Z, Li X, Li P. 2014. [Intelligence and growth development of children in coal-burning-borne arsenism and fluorosis areas: An investigation study]. *Chin J Endemiol* 33(2): 160-163.

Barberio AM, Hosein FS, Quinonez C, McLaren L. 2017. Fluoride exposure and indicators of thyroid functioning in the Canadian population: Implications for community water fluoridation. *J Epidemiol Community Health* 71: 1019-1025.

Barberio AM, Quinonez C, Hosein FS, McLaren L. 2017. Fluoride exposure and reported learning disability diagnosis among Canadian children: Implications for community water fluoridation. *Can J Public Health* 108: 229-239.

Bashash M, Thomas D, Hu H, Martinez-Mier EA, Sanchez BN, Basu N, Peterson KE, Ettinger AS, Wright R, Zhang Z, Liu Y, Schnaas L, Mercado-Garcia A, Tellez-Rojo MM, Hernandez-Avila M. 2017. Prenatal fluoride exposure and cognitive outcomes in children at 4 and 6-12 years of age in Mexico. *Environ Health Perspect* 125(9): 1-12.

Bashash M, Marchand M, Hu H, Till C, Martinez-Mier EA, Sanchez BN, Basu N, Peterson KE, Green R, Schnaas L, Mercado-Garcia A, Hernandez-Avila M, Tellez-Rojo MM. 2018. Prenatal fluoride exposure and attention deficit hyperactivity disorder (ADHD) symptoms in children at 6-12 years of age in Mexico City. *Environ Int* 121(Pt 1): 658-666.

Broadbent JM, Thomson WM, Moffitt TE, Poulton R. 2015. Community water fluoridation and intelligence response. *Am J Public Health* 105: 3-4.

Chen YX, Han FL, Zhoua ZL, Zhang HQ, Jiao XS, Zhang SC, Huang MC, Chang TQ, Dong YF. 2008. Research on the intellectual development of children in high fluoride areas. *Fluoride* 41: 120-124.

Chinoy NJ, Narayana MV. 1992. Studies on fluorosis in Mehsana District of North Gujarat. *Proc Zool Soc* 45: 157-161.

Choi AL, Zhang Y, Sun G, Bellinger DC, Wang K, Yang XJ, Li JS, Zheng Q, Fu Y, Grandjean P. 2015. Association of lifetime exposure to fluoride and cognitive functions in Chinese children: A pilot study. *Neurotoxicol Teratol* 47: 96-101.

Cui Y, Zhang B, Ma J, Wang Y, Zhao L, Hou C, Yu J, Zhao Y, Zhang Z, Nie J, Gao T, Zhou G, Liu H. 2018. Dopamine receptor D2 gene polymorphism, urine fluoride, and intelligence impairment of children in China: A school-based cross-sectional study. *Ecotoxicol Environ Saf* 165: 270-277.

Das K, Mondal NK. 2016. Dental fluorosis and urinary fluoride concentration as a reflection of fluoride exposure and its impact on IQ level and BMI of children of Laxmisagar, Simlapal Block of Bankura District, W.B., India. *Environ Monit Assess* 188: 218.

Ding Y, Sun H, Han H, Wang W, Ji X, Liu X, Sun D. 2011. The relationships between low levels of urine fluoride on children's intelligence, dental fluorosis in endemic fluorosis areas in Hulunbuir, Inner Mongolia, China. *J Hazard Mater* 186: 1942-1946.

Du L, Wan C, Cao X, Liu J. 2008. The effect of fluorine on the developing human brain. *Fluoride* 41: 327-330.

Duan JZ, M.; Wang, L.; Fang, D.; Wang, Y.; Wang, W. 1995. A comparative analysis of the results of multiple tests in patients with chronic industrial fluorosis. *Guizhou Med J* 18(3): 179-180.

Erickson JD, Hay S. 1976. Water fluoridation and congenital malformations: No association. *J Am Dent Assoc* 93: 981-984.

Erickson JD. 1980. Down syndrome, water fluoridation, and maternal age. *Teratology* 21: 177-180.

Eswar P, Nagesh L, Devaraj CG. 2011. Intelligent quotients of 12-14 year old school children in a high and low fluoride village in India. *Fluoride* 44: 168-172.

Fan Z, Dai H, Bai A, Li P, Li T, Li G. 2007. Effect of high fluoride exposure in children's intelligence. *J Environ Health* 24(10): 802-803.

Green R, Lanphear B, Hornung R, Flora D, Martinez-Mier EA, Neufeld R, Ayotte P, Muckle G, Till C. 2019. Association between maternal fluoride exposure during pregnancy and IQ scores in offspring in Canada. *JAMA Pediatr*: E1-E9.

Guo XC, Wang RY, Cheng CF, Wei WS, Tang LM, Wang QS, Tang DX, Liu GW, He GD, Li SL. 2008. A preliminary investigation of the IQs of 7-13 year-old children from an area with coal burning-related fluoride poisoning. *Fluoride* 41: 125-128.

Guo ZY, He YH, Zhu QX. 2008. Research on the neurobehavioral function of workers occupationally exposed to fluoride. *Fluoride* 41: 152-155.

He H, Cheng ZS, Liu WQ. 2008. Effects of fluorine on the human fetus. *Fluoride* 41: 321-326.

He MX, Zhang CN. 2010. [Investigation of children's intelligence quotient and dental fluorosis in drinking water-type of endemic fluorosis area in Pucheng County, Shaanxi Province before and after drinking water change]. *Chin J Endemiol* 29: 547-548.

Hong FG, Cao YX, Yang D, Wang H. 2008. Research on the effects of fluoride on child intellectual development under different environmental conditions. *Fluoride* 41: 156-160.

Hosur MB, Puranik RS, Vanaki S, Puranik SR. 2012. Study of thyroid hormones free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH) in subjects with dental fluorosis. *Eur J Dent* 6: 184-190.

Jacqmin H, Commenges D, Letenneur L, Barberger-Gateau P, Dartigues JF. 1994. Components of drinking water and risk of cognitive impairment in the elderly. *Am J Epidemiol* 139: 48-57.

Karimzade S, Aghaei M, Mahvi AH. 2014. Investigation of intelligence quotient in 9-12 year-old children exposed to high- and low-drinking water fluoride in West Azerbaijan Province, Iran. *Fluoride* 47: 9-14.

Khan SA, Singh RK, Navit S, Chadha D, Johri N, Navit P, Sharma A, Bahuguna R. 2015. Relationship between dental fluorosis and intelligence quotient of school going children in and around Lucknow District: A cross-sectional study. *J Clin Diagn Res* 9(11): 10-15.

Khandare AL, Gourineni SR, Validandi V. 2017. Dental fluorosis, nutritional status, kidney damage, and thyroid function along with bone metabolic indicators in school-going children living in fluoride-affected hilly areas of Doda District, Jammu and Kashmir, India. *Environ Monit Assess* 189: 579.

Khandare AL, Validandi V, Gourineni SR, Gopalan V, Nagalla B. 2018. Dose-dependent effect of fluoride on clinical and subclinical indices of fluorosis in school going children and its mitigation by supply of safe drinking water for 5 years: An Indian study. *Environ Monit Assess* 190: 110.

Kheradpisheh Z, Mahvi AH, Mirzaei M, Mokhtari M, Azizi R, Fallahzadeh H, Ehrampoush MH. 2018. Correlation between drinking water fluoride and TSH hormone by ANNs and ANFIS. *J Environ Health Sci Eng* 16(1): 11-18.

Kheradpisheh Z, Mirzaei M, Mahvi AH, Mokhtari M, Azizi R, Fallahzadeh H, Ehrampoush MH. 2018. Impact of drinking water fluoride on human thyroid hormones: A case-control study. *Sci Rep* 8: 2674.

Kumar V, Chahar P, Kajjari S, Rahman F, Bansal DK, Kapadia JM. 2018. Fluoride, thyroid hormone derangements and its correlation with tooth eruption pattern among the pediatric population from endemic and non-endemic fluorosis areas. *J Contemp Dent Pract* 19(12): 1512-1516.

Kundu H, Basavaraj P, Singla A, Gupta R, Singh K, Jain S. 2015. Effect of fluoride in drinking water on children's intelligence in high and low fluoride areas of Delhi. *J Indian Assoc Public Health Dent* 13(2): 116-121.

Lamberg M, Hausen H, Vartiainen T. 1997. Symptoms experienced during periods of actual and supposed water fluoridation. *Community Dent Oral Epidemiol* 25: 291-295.

Li XS, Zhi JL, Gao RO. 1995. Effect of fluoride exposure on the intelligence of children. *Fluoride* 28: 189-192.

Li J, Yao L, Shao QL, Wu CY. 2008. Effects of high fluoride level on neonatal neurobehavioral development. *Fluoride* 41: 165-170.

Li Y, Li X, Wei S. 2008. Effects of high fluoride intake on child mental work capacity: Preliminary investigation into the mechanisms involved. *Fluoride* 41: 331-335.

Li YP, Jing XY, Chen D, Lin L, Wang ZJ. 2008. Effects of endemic fluoride poisoning on the intellectual development of children in Baotou. *Fluoride* 41: 161-164.

Li F, Chen X, Huang R, Xie Y. 2009. The impact of endemic fluorosis caused by the burning of coal on the development of intelligence in children. *J Environ Health* 26(4): 838-840.

Li X, Hou G, Yu B, Yuan C, Liu Y, L Z. 2010. Investigation and analysis of children's intelligence and dental fluorosis in high fluoride area. *J Med Pest Control* 26(3): 230-231.

Li M, Gao Y, Cui J, Li Y, Li B, Liu Y, Sun J, Liu X, Liu H, Zhao L, Sun D. 2016. Cognitive impairment and risk factors in elderly people living in fluorosis areas in China. *Biol Trace Elem Res* 172: 53-60.

Lin F, Ai H, Zhao H, Lin J, Jhiang J, Maimaiti. 1991. High fluoride and low iodine environment and subclinical cretinism in Xinjiang. *Endem Dis Bull* 6(2): 62-67.

Liu SL, Lu Y, Sun ZR, Wu L, Lu WL, Wang XW, Yan S. 2008. Report on the intellectual ability of children living in high-fluoride water areas. *Fluoride* 41: 144-147.

Lu Y, Sun ZR, Wu LN, Wang X, Lu W, Liu SS. 2000. Effect of high-fluoride water on intelligence in children. *Fluoride* 33: 74-78.

Lu F, Zhang Y, Trivedi A, Jiang X, Chandra D, Zheng J, Nakano Y, Uyghurturk DA, Jalai R, Guner S, Mentes A, DenBesten PK. 2019. Fluoride related changes in behavioral outcomes may relate to increased serotonin. *Physiol Behav* 206: 76-83.

Malin AJ, Till C. 2015. Exposure to fluoridated water and attention deficit hyperactivity disorder prevalence among children and adolescents in the United States: An ecological association. *Environ Health* 14: 17.

Malin AJ, Riddell J, McCague H, Till C. 2018. Fluoride exposure and thyroid function among adults living in Canada: Effect modification by iodine status. *Environ Int* 121(Pt 1): 667-674.

Michael M, Barot VV, Chinoy NJ. 1996. Investigations of soft tissue functions in fluorotic individuals of North Gujarat. *Fluoride* 29: 63-71.

Mondal D, Dutta G, Gupta S. 2016. Inferring the fluoride hydrogeochemistry and effect of consuming fluoride-contaminated drinking water on human health in some endemic areas of Birbhum District, West Bengal. *Environ Geochem Health* 38: 557-576.

Morgan L, Allred E, Tavares M, Bellinger D, Needleman H. 1998. Investigation of the possible associations between fluorosis, fluoride exposure, and childhood behavior problems. *Pediatr Dent* 20: 244-252.

Mustafa DE, Younis UM, Elhag SAA. 2018. The relationship between the fluoride levels in drinking water and the schooling performance of children in rural areas of Khartoum State, Sudan. *Fluoride* 51(2): 102-113.

Nagarajappa R, Pujara P, Sharda AJ, Asawa K, Tak M, Aapaliya P, Bhanushali N. 2013. Comparative assessment of intelligence quotient among children living in high and low fluoride areas of Kutch, India: A pilot study. *Iran J Public Health* 42: 813-818.

Peckham S, Lowery D, Spencer S. 2015. Are fluoride levels in drinking water associated with hypothyroidism prevalence in England? A large observational study of GP practice data and fluoride levels in drinking water. *J Epidemiol Community Health* 69: 619-624.

Poureslami HR, Horri A, Garrusi B. 2011. A comparative study of the IQ of children age 7-9 in a high and a low fluoride water city in Iran. *Fluoride* 44: 163-167.

Qin LS, Huo SY, Chen RL, Chang YZ, Zhao MY. 2008. Using the Raven's Standard Progressive Matrices to determine the effects of the level of fluoride in drinking water on the intellectual ability of school-age children. *Fluoride* 41: 115-119.

Ratan SK, Rattan KN, Pandey RM, Singhal S, Kharab S, Bala M, Singh V, Jhanwar A. 2008. Evaluation of the levels of folate, vitamin B12, homocysteine and fluoride in the parents and the affected neonates with neural tube defect and their matched controls. *Pediatr Surg Int* 24: 803-808.

Razdan P, Patthi B, Kumar JK, Agnihotri N, Chaudhari P, Prasad M. 2017. Effect of fluoride concentration in drinking water on intelligence quotient of 12-14-year-old children in Mathura District: A cross-sectional study. *J Int Soc Prev Community Dent* 7: 252-258.

Ren D, Li K, Liu D. 2008. A study of the intellectual ability of 8-14 year-old children in high fluoride, low iodine areas. *Fluoride* 41: 319-320.

Rocha-Amador D, Navarro ME, Carrizales L, Morales R, Calderon J. 2007. Decreased intelligence in children and exposure to fluoride and arsenic in drinking water. *Cad Saude Publica* 23 Suppl 4: S579-587.

Rocha-Amador D, Navarro M, Trejo-Acevedo A, Carrizales L, Perez-Maldonado I, Diaz-Barriga F, Calderon J. 2009. Use of the Rey-Osterrieth Complex Figure Test for neurotoxicity evaluation of mixtures in children. *Neurotox* 30: 1149-1154.

Rotton J, Tikofsky RS, Feldman HT. 1982. Behavioral effects of chemicals in drinking water. *J Appl Psychol* 67: 230-238.

Russ TC, Killin LOJ, Hannah J, Batty GD, Deary IJ, Starr JM. 2019. Aluminium and fluoride in drinking water in relation to later dementia risk. *Brit J Psychol* 14: 1-6.

Saxena S, Sahay A, Goel P. 2012. Effect of fluoride exposure on the intelligence of school children in Madhya Pradesh, India. *J Neurosci Rural Pract* 3: 144-149.

Sebastian ST, Sunitha S. 2015. A cross-sectional study to assess the intelligence quotient (IQ) of school going children aged 10-12 years in villages of Mysore District, India with different fluoride levels. *J Indian Soc Pedod Prev Dent* 33: 307-311.

Seraj B, Shahrabi M, Falahzade M, Falahzade F, Akhondi N. 2006. Effect of high fluoride concentration in drinking water on children's intelligence. *J Dent Med* 19(2): 80-86.

Seraj B, Shahrabi M, Shadfar M, Ahmadi R, Fallahzadeh M, Eslamlu HF, Kharazifard MJ. 2012. Effect of high water fluoride concentration on the intellectual development of children in Makoo, Iran. *J Dent* 9: 221-229.

Shannon FT, Fergusson DM, Horwood LJ. 1986. Exposure to fluoridated public water supplies and child health and behaviour. *New Zeal Med J* 99: 416-418.

Shao Q. 2003. Study of cognitive function impairment caused by chronic fluorosis. *Chin J Endemiol* 22(4): 336-338.

Sharma JD, Sohu D, Jain P. 2009. Prevalence of neurological manifestations in a human population exposed to fluoride in drinking water. *Fluoride* 42: 127-132.

Shivaprakash PK, Ohri K, Noorani H. 2011. Relation between dental fluorosis and intelligence quotient in school children of Bagalkot District. *J Indian Soc Pedod Prev Dent* 29: 117-120.

Singh A, Jolly SS, Devi P, Bansal BC, Singh SS. 1962. Endemic fluorosis: An epidemiological, biochemical and clinical study in the Bhatinda District of Panjab. *Indian J Med Res* 50: 387-398.

Singh V, Singh C, Sandeep T, Sandeep K, Vikas G, Mukesh T, Anurag T. 2013. A correlation between serum vitamin, acetylcholinesterase activity and IQ in children with excessive endemic fluoride exposure in Rajasthan, India. *Int Res J Medical Sci* 1(3): 12-16.

Singh N, Verma KG, Verma P, Sidhu GK, Sachdeva S. 2014. A comparative study of fluoride ingestion levels, serum thyroid hormone & TSH level derangements, dental fluorosis status among school children from endemic and non-endemic fluorosis areas. *Springerplus* 3: 7.

Still CN, Kelley P. 1980. The incidence of primary degenerative dementia vs. water fluoride content in South Carolina, USA. *Neurotox* 1: 125-132.

Sudhir KM, Chandu GN, Prashant GM, Subba Reddy VV. 2009. Effect of fluoride exposure on intelligence quotient (IQ) among 13-15 year old school children of known endemic area of fluorosis, Nalgonda District, Andhra Pradesh. *J Indian Assoc Public Health Dent* 2009(13): 88-94.

Sun M, Li S, Wang Y, Li F. 1991. Measurement of intelligence by drawing test among the children in the endemic area of Al-F combined toxicosis. *J Guiyang Med Coll* 16(3): 204-206.

Susheela AK, Bhatnagar M, Vig K, Mondal NK. 2005. Excess fluoride ingestion and thyroid hormone derangements in children living in Delhi, India. *Fluoride* 38: 98-108.

Tamboli BL, Mathur GM, Mathur AP, Lalla SK, Goyal OP. 1980. Prevalence of fluorosis in Pratabpura and Surajpura villages, District Ajmer (Rajasthan). *Indian J Med Res* 71: 57-67.

Tripathi P, Sultana N. 2007. Fluoride content of groundwater and prevalence of dental, skeletal and neurological stage of fluorosis in Tehsil Purwa of Unnao. *Indian J Environ Prot* 27: 737-739.

Trivedi MH, Verma RJ, Chinoy NJ, Patel RS, Sathawara NG. 2007. Effect of high fluoride water on intelligence of school children in India. *Fluoride* 40: 178-183.

Trivedi M, Sangai N, Patel R, Payak M, Vyas S. 2012. Assessment of groundwater quality with special reference to fluoride and its impact on IQ of schoolchildren in six villages of the Mundra Region, Kachchh, Gujurat, India. *Fluoride* 45(4): 377-383.

Valdez Jimenez L, Lopez Guzman OD, Cervantes Flores M, Costilla-Salazar R, Calderon Hernandez J, Alcaraz Contreras Y, Rocha-Amador DO. 2017. In utero exposure to fluoride and cognitive development delay in infants. *Neurotox* 59: 65-70.

Wang S, Wang L, Hu P, Guo S, Law S. 2001. [Effects of high iodine and high fluorine on children's intelligence and thyroid function]. *Chin J Endemiol* 20(4): 288-290.

Wang SX, Wang ZH, Cheng XT, Li J, Sang ZP, Zhang XD, Han LL, Qiao XY, Wu ZM, Wang ZQ. 2005. [Investigation and evaluation on intelligence and growth of children in endemic fluorosis and arsenism areas]. *Chin J Endemiol* 24: 179-182.

Wang Z, Wang S, Zhang X, Li J, Zheng X, Hu C. 2006. Investigation of children's growth and development under long-term fluoride exposure. *Chin J Control Endem Dis* 21(4): 239-241.

Wang SX, Wang ZH, Cheng XT, Li J, Sang ZP, Zhang XD, Han LL, Qiao XY, Wu ZM, Wang ZQ. 2007. Arsenic and fluoride exposure in drinking water: Children's IQ and growth in Shanyin County, Shanxi Province, China. *Environ Health Perspect* 115: 643-647.

Wang G, Yang D, Jia F, Wang H. 2008. A study of the IQ levels of four- to seven-year-old children in high fluoride areas. *Fluoride* 41: 340-343.

Wang SY, Zhang HX, Fan W, Fang SJ, Kang PP, Chen XG, Yu MJ. 2008. The effects of endemic fluoride poisoning caused by coal burning on the physical development and intelligence of children. *Fluoride* 41: 344-348.

Wei N, Li Y, Deng J, Xu S, Guan Z. 2014. [The effects of comprehensive control measures on intelligence of school-age children in coal-burning-borne endemic fluorosis areas]. *Chin J Endemiol* 33(2): 320-322.

Xiang Q, Liang Y, Chen L, Wang C, Chen B, Chen X, Zhou M. 2003. Effect of fluoride in drinking water on children's intelligence. *Fluoride* 36: 84-94.

Xiang Q, Liang Y, Chen B, Chen L. 2011. Analysis of children's serum fluoride levels in relation to intelligence scores in a high and low fluoride water village in China. *Fluoride* 44: 191-194.

Xu Y, Lu C, Zhang X. 1994. Effect of fluoride on children's intelligence. *Endem Dis Bull* 2: 83-84.

Yang Y, Wang X, Guo X, Hu P. 2008. The effects of high levels of fluoride and iodine on child intellectual ability and the metabolism of fluoride and iodine. *Fluoride* 41: 336-339.

Yao L, Zhou J, Wang S, Cui K, Lin F. 1996. Analysis of TSH levels and intelligence of children residing in high fluorosis areas. *Lit Inf Prev Med* 2(1): 26-27.

Yao Y. 1997. Comparative assessment of the physical and mental development of children in endemic fluorosis area with water improvement and without water improvement. *Lit Inf Prev Med* 3(1): 42-43.

Yasmin S, Ranjan S, D'Souza D. 2013. Effect of excess fluoride ingestion on human thyroid function in Gaya Region, Bihar, India. *Toxicol Environ Chem* 95: 1235-1243.

Yazdi SM, Sharifian A, Dehghani-Beshne M, Momeni VR, Aminian O. 2011. Effects of fluroide on psychomotor performance and memory of aluminum potroom workers. *Fluoride* 44: 158-162.

Yu YN, Yang WX, Dong Z, Wan CW, Zhang JT, Liu JL, Xiao KQ, Huang YS, Lu BF. 2008. Neurotransmitter and receptor changes in the brains of fetuses from areas of endemic fluorosis. *Fluoride* 41: 134-138.

Yu X, Chen J, Li Y, Liu H, Hou C, Zeng Q, Cui Y, Zhao L, Li P, Zhou Z, Pang S, Tang S, Tian K, Zhao Q, Dong L, Xu C, Zhang X, Zhang S, Liu L, Wang A. 2018. Threshold effects of moderately excessive fluoride exposure on children's health: A potential association between dental fluorosis and loss of excellent intelligence. *Environ Int* 118: 116-124.

Zhang J, Yao H, Chen Y. 1998. [Effect of high level of fluoride and arsenic on children's intelligence]. *Chin J Public Health* 17(2): 57.

Zhang S, Zhang X, Liu H, Qu W, Guan Z, Zeng Q, Jiang C, Gao H, Zhang C, Lei R, Xia T, Wang Z, Yang L, Chen Y, Wu X, Cui Y, Yu L, Wang A. 2015. Modifying effect of COMT gene polymorphism and a predictive role for proteomics analysis in children's intelligence in endemic fluorosis area in Tianjin, China. *Toxicol Sci* 144: 238-245.

Zhao LB, Liang GH, Zhang DN, Wu XR. 1996. Effect of a high fluoride water supply on children's intelligence. *Fluoride* 29: 190-192.

Zhao Q, Niu Q, Chen J, Xia T, Zhou G, Li P, Dong L, Xu C, Tian Z, Luo C, Liu L, Zhang S, Wang A. 2019. Roles of mitochondrial fission inhibition in developmental fluoride neurotoxicity: Mechanisms of action in vitro and associations with cognition in rats and children. *Arch Toxicol* 93(3): 709-726.

Studies Not Available in HAWC

Bachinskii PP, Gutsalenko OA, Naryzhniuk ND, Sidora VD, Shliakhta AI. 1985. [Action of the body fluorine of healthy persons and thyroidopathy patients on the function of hypophyseal-thyroid the system]. *Probl Endokrinol* 31: 25-29.

Balabolkin MI, Mikhailets ND, Lobovskaia RN, Chernousova NV. 1995. [The interrelationship of the thyroid and immune statuses of workers with long-term fluorine exposure]. *Ter Arkh* 67: 41-42.

Baum K, Boerner W, Reiners C, Moll E. 1981. [Bone density and thyroid function in adolescents in relation to fluoride content of drinking water]. *Fortschr Med* 99: 1470-1472.

Berry WTC, Whittles JH. 1963. Absence of effect of fluoride upon the incidence of thyroid enlargements in Wiltshire schoolgirls. *Mon Bull Minist Health Public Health Lab Serv* 22: 50-52.

Cherkinskii SN, Zaslavskaia RM. 1956. [Significance of fluorides in potable water in the development of endemic goiter]. *Probl Endokrinol Gormonoter* 2: 70-75.

Choubisa SL. 2001. Endemic fluorosis in southern Rajasthan, India. *Fluoride* 34: 61-70.

Chuka A, Zhukovskil V, Mirku I, Postel'Niku D. 1964. Prezhdevremennoe starenie naseleniya v zone rasprostraneniya endemicheskogo zoba. *Vestnik Akad Med Nauk Sssr* 19: 23-27.

Dai HX, Zeng P, Wang KY, Zhang XG, Ma ZJ, Zhou YG, Fan ZX, Guo SH. 2013. [Analysis of a survey results of patients with suspected high iodine goiter in Liuji Town Fuping County of Shaanxi Province]. *Chin J Endemiol* 32: 408-411.

Day T, Powell-Jackson P. 1972. Fluoride, water hardness, and endemic goitre. *Lancet* 299(7761): 1135-1138.

Desai VK, Solanki DM, Bansal RK. 1993. Epidemiological study of goitre in endemic fluorosis district of Gujarat. *Fluoride* 26: 187-190.

Díaz-Cadórniga FJ, Delgado E, Tartón T, Valdés MM, Méndez A, Fernández MT, Rojo C. 2003. Endemic goiter associated with high iodine intake in primary school children in the Saharawi Arab Democratic Republic. *Endocrinol Nutr* 50: 357-362.

Eichner R, Borner W, Henschler D, Kohler W, Moll E. 1981. [Osteoporosis therapy and thyroid function. Influence of 6 months of sodium fluoride treatment on thyroid function and bone density]. *Fortschr Med* 99: 342-348.

Fiorentini S, Galeazzi M, Visintin B. 1947. II fluoro in natura come agente morbigeno II. La fluorosi die Campagnano di Roma. III. Un focolaio di fluorosi umana a Campagnano di Roma. IV. Osservazioni radiologiche sui processi alveolari, sulle ossa mascellari, e sul paradenzio degli abitanti die Campagnano. V. Zona fluorotica intorno a Campagnano di Roma. VI. Frequenza e caratteri clinici della carie dentale in soggetti fluorotici. *Rend Ist Superiore Sanita* 10: 721-804.

Gabovich RD, Verzhikovskaia NV. 1958. [Effect of fluoride compounds on absorption of radioactive iodine by the thyroid gland in humans and in experimental conditions]. *Probl Endokrinol Gormonoter* 4: 49-54.

Galletti P, Held HR, Korrodi H, Wegmann T. 1956. [Investigations on the possible damaging effect of fluorine on the thyroid gland]. *Helv Med Acta* 23: 601-605.

Galletti PM, Joyet G, Jallut O. 1957. [Effect of sodium fluoride on thyroid function in Basedow's Disease]. *Helv Med Acta* 24: 209-215.

Galletti PM, Joyet G. 1958. Effect of fluorine on thyroidal iodine metabolism in hyperthyroidism. *J Clin Endocrinol Metab* 18: 1102-1110.

Gas'kov AI, Savchenkov MF, Iushkov NN. 2005. [The specific features of the development of iodine deficiencies in children living under environmental pollution with fluorine compounds]. *Gig Sanit*: 53-55.

Gedalia I, Brand N. 1963. The relationship of fluoride and iodine in drinking water in the occurrence of goiter. *Arch Int Pharmacodyn Ther* 142: 312-315.

Grimm H. 1973. [The physical development of schoolchildren under the influence of drinking water fluoridation in Karl Marx Stadt]. *Dtsch Gesundheitsw* 28: 2363-2369.

Hasling C, Nielsen HE, Melsen F, Mosekilde L. 1987. Safety of osteoporosis treatment with sodium fluoride, calcium phosphate and vitamin D. *Miner Electrolyte Metab* 13: 96-103.

Hidehiko T. 1958. On the relation between the distribution of endemic goiter and the fluorine content of natural water in Hidaka Province, Hokkaido. *Folia Pharmacol Jpn* 54: 225-229.

Hoffmann-Axthelm W. 1953. [Observations on the influence of fluorine on dental enamel and thyroid gland]. *Dtsch Zahnarztl Z* 8: 757-765.

Jentzer A. 1956. [Effect of fluorine on the iodine content of the human thyroid gland]. *Bull Schweiz Akad Med Wiss* 12: 539-543.

Jooste PL, Weight MJ, Kriek JA, Louw AJ. 1999. Endemic goitre in the absence of iodine deficiency in schoolchildren of the Northern Cape Province of South Africa. *Eur J Clin Nutr* 53: 8-12.

Kolomiitseva MG. 1961. [The content of fluorine in the external environment of the Upper Altai autonomous region and its role in the etiology of endemic goiter]. *Gig Sanit* 26: 101-103.

Korrodi H, Wegmann T, Galleti P, Held HR. 1955. [Caries prophylaxis and the untoward effects of fluor on the thyroid gland]. *Schweiz Med Wochenschr* 85: 1016-1019.

Kutlucan A, Kale Koroglu B, Numan Tamer M, Aydin Y, Baltaci D, Akdogan M, Ozturk M, Vural H, Ermis F. 2013. The investigation of effects of fluorosis on thyroid volume in school-age children. *Med Glas* 10: 93-98.

Latham MC, Grech P. 1967. The effects of excessive fluoride intake. *Am J Public Health* 57: 651-660.

Leone NC, Leatherwood EC, Petrie IM, Lieberman L. 1964. Effect of fluoride on thyroid gland: Clinical study. *J Am Dent Assoc* 69: 179-180.

Levi JE, Silberstein HE. 1955. Lack of effect of fluorine ingestion on uptake of iodine 131 by the thyroid gland. *J Lab Clin Med* 45: 348-351.

McGlashan N, Chelkowska E, Sasananan S. 2010. A survey of goiter morbidity in Ban Mae Toen, northwest Thailand. *Southeast Asian J Trop Med Public Health* 41: 1200-1208.

Rathore S, Meena C, Gonmei Z, Dwivedi S, Toteja GS, Bala K. 2018. Study of excess fluoride ingestion and thyroid hormone derangement in relation with different fluoride levels in drinking water among children of Jodhpur District, Rajasthan, India. *Asian J Microbiol Biotechnol Environ Sci* 20: 327-331.

Reisenauer R, Rezler D, Křemenová J, Preininger Q. 1961. [Fluorization of the waters in Czechoslovakia. IV. Endocrinological control of results of two years' fluorization of drinking-water in school children]. *Cesk Stomatol* 61: 91-97.

Romer TEZ, Kowalczyk B, Kacprzak M, Wiktorowski M. 1976. [Incidence of goitre in pubertal girls of the Piotrkow Region and iodide content in drinking water]. *Endokrynol Pol* 27: 373-380.

Savchenkov MF, Efimova NV, Manueva RS, Nikolaeva LA, Shin NS. 2016. [Thyroid gland pathology in children population exposed to the combination of iodine deficiency and fluoride pollution of environment]. *Gig Sanit* 95: 1201-1205.

Shtifanova AK. 1962. [The fluorine content in water, soil and vegetal products of the Alma-Atinsk District areas and its role in the etiology of dental caries and endemic goiter]. *Zdravookhranenie Kazakhstana*: 60-63.

Siddiqui AH. 1969. Incidence of simple goiter in areas of endemic fluorosis in Nalgonda District, Andhra Pradesh, India. *Fluoride* 2: 200-205.

Sidora VD, Shliakhta AI, Iugov VK, Kas'ianenko AS, Piatenko VG. 1983. [Indices of the pituitary-thyroid system in residents of cities with various fluorine concentrations in drinking water]. *Probl Endokrinol* 29: 32-35.

Sung FC, Chen KP, Chen CY, Tai PW, Yang CF. 1973. Studies of the effect of salt iodization on endemic goiter in Taiwan. IV. A survey of drinking water in relation to endemic goiter. *J Fomosan Med Assoc* 72: 96-103.

Tokar VI, Voroshnin VV, Sherbakov SV. 1989. [Chronic effects of fluorides on the pituitary-thyroid system in industrial workers]. *Gig Tr Prof Zabol*: 19-22.

Wespi HJ. 1954. [Iodized-fluoridized salt for the prevention of goiter and caries]. *Schweiz Med Wochenschr* 84: 885-890.

Yu YN. 1985. [Effects of chronic fluorosis on the thyroid gland]. *Chin Med J* 65: 747-7479.

Studies in Non-human Animals

As described in **Figure 4**, 339 non-human mammal studies were included; however, full data extraction was only conducted on studies with primary neurological outcomes and/or secondary functional neurological outcomes (e.g., motor activity). Data extraction was completed using HAWC. Data were extracted from a subset of included studies in animals (n = 123) and are available in HAWC based on outcome. The following lists of references are organized as studies that are available in HAWC followed by studies that are not available in HAWC. Specifically, all primary outcomes and functional neurological secondary outcomes (e.g., motor activity) were extracted from animal studies and are available in HAWC, including studies from the NTP (2016) assessment. Studies are also available in HAWC that evaluated mechanistic effects related to oral fluoride exposure at or below 20 ppm fluoride drinking water equivalents for categories of mechanistic endpoints with the largest amount of available data (i.e., biochemistry of the brain or neurons, neurotransmission, oxidative stress, and histopathology [n = 70]); however, these mechanistic data were generally not extracted. Several animal studies assessed primary neurological outcomes and/or functional neurological secondary outcomes and mechanistic effects in the four mechanistic categories listed above (n = 56). In total, 140 animal studies are available in HAWC (70 with primary neurological outcomes and/or secondary functional neurological outcomes without relevant mechanistic data; 15 with relevant mechanistic data only; and 55 with primary/or secondary functional neurological outcomes with relevant mechanistic data). Studies that evaluated other mechanistic endpoints, as well as studies that only assessed mechanistic effects at fluoride levels above 20 ppm fluoride drinking water equivalents, are not available in HAWC (n = 199).

Studies Available in HAWC

Adedara IA, Abolaji AO, Idris UF, Olabiyi BF, Onibiyo EM, Ojuade TD, Farombi EO. 2017. Neuroprotective influence of taurine on fluoride-induced biochemical and behavioral deficits in rats. *Chem Biol Interact* 261: 1-10.

Adedara IA, Olabiyi BF, Ojuade TD, Idris UF, Onibiyo EM, Farombi EO. 2017. Taurine reverses sodium fluoride-mediated increase in inflammation, caspase-3 activity, and oxidative damage along the brain-pituitary-gonadal axis in male rats. *Can J Phys Pharmacol* 95: 1019-1029.

Agustina F, Sofro ZM, Partadiredja G. 2018. Subchronic administration of high-dose sodium fluoride causes deficits in cerebellar purkinje cells but not motor coordination of rats. *Biol Trace Elem Res* 188(2): 424-433.

Ahmad KR, Noor S, Jabeen S, Nauroze T, Kanwal MA, Raees K, Abbas T. 2017. Amelioration by jambul fruit extract of fluoride-induced hepato-nephronal histopathologies and impaired neuromotor capacity in mice. *Fluoride* 50: 2-14.

Akinrinade ID, Memudu AE, Ogundele OM. 2015. Fluoride and aluminium disturb neuronal morphology, transport functions, cholinesterase, lysosomal and cell cycle activities. *Pathophysiology* 22: 105-115.

Akinrinade ID, Memudu AE, Ogundele OM, Ajetunmobi OI. 2015. Interplay of glia activation and oxidative stress formation in fluoride and aluminium exposure. *Pathophysiology* 22: 39-48.

Baba NA, Raina R, Verma PK, Sultana M. 2014. Alterations in plasma and tissue acetylcholinesterase activity following repeated oral exposure of chlorpyrifos alone and in conjunction with fluoride in Wistar rats. *Proc Indian Natl Sci Acad B Biol Sci* 84: 969-972.

Bagmut I, Kolisnyk I, Titkova A, Babiy L, Filipchenko S. 2018. The antioxidant system enzymes' activity in rats' brain, intoxicated with sodium fluoride in subtoxic doses. *Arch Balkan Med Union* 53(4): 506-511.

Balaji B, Kumar EP, Kumar A. 2015. Evaluation of standardized bacopa monniera extract in sodium fluoride-induced behavioural, biochemical, and histopathological alterations in mice. *Toxicol Ind Health* 31: 18-30.

Balayssac D, Richard D, Authier N, Nicolay A, Jourdan D, Eschalier A, Coudore F. 2002. Absence of painful neuropathy after chronic oral fluoride intake in Sprague-Dawley and Lou/C rats. *Neurosci Lett* 327: 169-172.

Banala RR, Karnati PR. 2015. Vitamin A deficiency: An oxidative stress marker in sodium fluoride (NaF) induced oxidative damage in developing rat brain. *Int J Dev Neurosci* 47: 298-303.

Banala R, Nagapuri K, Mohd K, Reddy M, Karnati P. 2018. Carica Papaya leaf extract as a neuroprotective agent against behavioral and neurotransmitter changes in brain of the rat treated with sodium fluoride in pre- and post-natal periods. *Pharmacogn Mag* 14(55): 123-131.

Banji D, Banji OJ, Pratusha NG, Annamalai AR. 2013. Investigation on the role of spirulina platensis in ameliorating behavioural changes, thyroid dysfunction and oxidative stress in offspring of pregnant rats exposed to fluoride. *Food Chem* 140: 321-331.

Baran-Poesina V, Negres S, Dobrescu D, Dimcevici-Poesina N, Dimcevici-Poesina A, Feghiu A, Soare T, Militaru M. 2013. Experimental pharmacological researches regarding the influence of sodium fluoride in allopathic and homeopathic doses on central nervous system's performances: A correlation between behavioral response in classic maze test and morphological aspects of cerebral cortex. *Farmacia* 61: 781-799.

Bartos M, Gumilar F, Bras C, Gallegos CE, Giannuzzi L, Cancela LM, Minetti A. 2015. Neurobehavioural effects of exposure to fluoride in the earliest stages of rat development. *Physiol Behav* 147: 205-212.

Bartos M, Gumilar F, Gallegos CE, Bras C, Dominguez S, Monaco N, Esandi MDC, Bouzat C, Cancela LM, Minetti A. 2018. Alterations in the memory of rat offspring exposed to low levels of fluoride during gestation and lactation: Involvement of the alpha7 nicotinic receptor and oxidative stress. *Reprod Toxicol* 81: 108-114.

Basha PM, Rai P, Begum S. 2011. Fluoride toxicity and status of serum thyroid hormones, brain histopathology, and learning memory in rats: A multigenerational assessment. *Biol Trace Elem Res* 144: 1083-1094.

Basha PM, Sujitha NS. 2012. Combined impact of exercise and temperature in learning and memory performance of fluoride toxicated rats. *Biol Trace Elem Res* 150: 306-313.

Bataineh HN, Nusier MK. 2006. Impact of 12-week ingestion of sodium fluoride on aggression, sexual behavior, and fertility in adult male rats. *Fluoride* 39: 293-301.

Bera I, Sabatini R, Auteri P, Flace P, Sisto G, Montagnani M, Potenza MA, Marasciulo FL, Carratu MR, Coluccia A, Borracci P, Tarullo A, Cagiano R. 2007. Neurofunctional effects of developmental sodium fluoride exposure in rats. *Eur Rev Med Pharmacol Sci* 11: 211-224.

Bhatnagar M, Rao P, Sushma J, Bhatnagar R. 2002. Neurotoxicity of fluoride: Neurodegeneration in hippocampus of female mice. *Indian J Exp Biol* 40: 546-554.

Bhatnagar M, Sukhwal P, Suhalka P, Jain A, Joshi C, Sharma D. 2011. Effects of fluoride in drinking water on NADPH-diaphorase neurons in the forebrain of mice: A possible mechanism of fluoride neurotoxicity. *Fluoride* 44: 195-209.

Chen H, Geng D. 2011. [The change of cognition induced by chronic fluoride in rats]. *Acta Academiae Medicinae Xuzhou* 31(5): 319-322.

Chen J, Niu Q, Xia T, Zhou G, Li P, Zhao Q, Xu C, Dong L, Zhang S, Wang A. 2018. ERK1/2-mediated disruption of BDNF-TrkB signaling causes synaptic impairment contributing to fluoride-induced developmental neurotoxicity. *Toxicology* 410: 222-230.

Chinoy NJ, Shah SD. 2004. Biochemical effects of sodium fluoride and arsenic trioxide toxicity and their reversal in the brain of mice. *Fluoride* 37: 80-87.

Chioca LR, Raupp IM, Da Cunha C, Losso EM, Andreatini R. 2008. Subchronic fluoride intake induces impairment in habituation and active avoidance tasks in rats. *Eur J Pharmacol* 579: 196-201.

Chouhan S, Flora SJS. 2008. Effects of fluoride on the tissue oxidative stress and apoptosis in rats: Biochemical assays supported by IR spectroscopy data. *Toxicology* 254: 61-67.

Chouhan S, Lomash V, Flora SJ. 2010. Fluoride-induced changes in haem biosynthesis pathway, neurological variables and tissue histopathology of rats. *J Appl Toxicol* 30: 63-73.

Cui YS, Zhong Q, Li WF, Liu ZH, Wang Y, Hou CC. 2017. [Effects of fluoride exposure on thyroid hormone level and intelligence in rats]. *Chin J Ind Hyg Occup Dis* 35: 888-892.

Dabrowska E. 1997. Effect of different fluorine doses on the supraoptic nucleus of the rat. *Folia Histochem Cytobiol* 35: 115-116.

Dong Y, Wang Y, Wei N, Guan Z. 2015. [Expression levels of brain muscarinic acetylcholine receptor in offspring rats of drinking-water borne fluorosis]. *Chin J Endemiol* 34: 326-330.

Dong YT, Wang Y, Wei N, Zhang QF, Guan ZZ. 2015. Deficit in learning and memory of rats with chronic fluorosis correlates with the decreased expressions of M1 and M3 muscarinic acetylcholine receptors. *Arch Toxicol* 89: 1981-1991.

Dong YW, Y. Wei, N. Guan, Z. 2015. [Expression of muscarinic acetylcholine receptors in the brain of rats with chronic fluorosis]. *Chin J Endemiol* 34(2): 84-88.

Dong YT, Wei N, Qi XL, Liu XH, Chen D, Zeng XX, Guan ZZ. 2017. Attenuating effect of vitamin E on the deficit of learning and memory of rats with chronic fluorosis: The mechanism may involve muscarinic acetylcholine receptors. *Fluoride* 50: 354-364.

Ekambaram P, Paul V. 2001. Calcium preventing locomotor behavioral and dental toxicities of fluoride by decreasing serum fluoride level in rats. *Environ Toxicol Pharmacol* 9: 141-146.

Ekambaram P, Paul V. 2002. Modulation of fluoride toxicity in rats by calcium carbonate and by withdrawal of fluoride exposure. *Pharmacol Toxicol* 90: 53-58.

Ekambaram P, Paul V. 2003. Effect of vitamin D on chronic behavioral and dental toxicities of sodium fluoride in rats. *Fluoride* 36: 189-197.

El-lethey HS, Kamel MM, Shaheed IB. 2010. Neurobehavioral toxicity produced by sodium fluoride in drinking water of laboratory rats. *J Am Sci* 6(5): 54-63.

El-lethey HS, Kamel MM. 2011. Effects of black tea in mitigation of sodium fluoride potency to suppress motor activity and coordination in laboratory rats. *J Am Sci* 7(4): 243-254.

El-lethey HS, Shaheed IB. 2011. Potential health impact of black tea against Na-F-induced alterations in territorial aggression, sexual behaviour and fertility of male rats. *Life Sci J* 8: 828-839.

Elliott L. 1967. Lack of effect of administration of fluoride on the central nervous system of rats. *Acta Pharmacol Toxicol (Copenh)* 25: 323-328.

Flace P, Benagiano V, Vermesan D, Sabatini R, Inchingolo AM, Auteri P, Ambrosi G, Tarullo A, Cagiano R. 2010. Effects of developmental fluoride exposure on rat ultrasonic vocalization, acoustic startle reflex and pre-pulse inhibition. *Eur Rev Med Pharmacol Sci* 14: 507-512.

Gabovich RD. 1962. [On the problem of the effect of fluorine in drinking water on the functional state of the central nervous system]. *Gig Sanit* 27: 10-12.

Gao Q, Liu YJ, Wu CX, Long YG, Guan ZZ. 2008. [Level of oxidative stress in rat brains and learning and memory function of rats with chronic fluorosis]. *Chin J Endemiol* 27: 371-373.

Gao Q, Liu YJ, Wu CX, Long YG, Guan ZZ. 2008. [Effects of fluoride on learning and memory and cholinesterase activity in rat brains]. *Chin J Endemiol* 27: 128-130.

Gao Q, Liu YJ, Guan ZZ. 2009. Decreased learning and memory ability in rats with fluorosis: Increased oxidative stress and reduced cholinesterase activity in the brain. *Fluoride* 42: 277-285.

Gao Y, Liu L, Young L, Huan L, Jin H. 2009. Effects of learning and memory of fluoride and the antagonism of selenium in rats. *Studies of Trace Elements and Health* 26(2): 1-3.

Ge QD, Tan Y, Luo Y, Wang WJ, Zhang H, Xie C. 2018. MiR-132, miR-204 and BDNF-TrkB signaling pathway may be involved in spatial learning and memory impairment of the offspring rats caused by fluorine and aluminum exposure during the embryonic stage and into adulthood. *Environ Toxicol Pharmacol* 63: 60-68.

Ge Y, Chen L, Yin Z, Song X, Ruan T, Hua L, Liu J, Wang J, Ning H. 2018. Fluoride-induced alterations of synapse-related proteins in the cerebral cortex of ICR offspring mouse brain. *Chemosphere* 201: 874-883.

Gopal K, Saxena R, Gupta GSD, Rana MD, Agrawal D. 2006. Fluoride induced alterations in neurobehavioural and cardiovascular responses in rats. *J Adv Zool* 27: 1-7.

Gui CZ, Ran LY, Wu CX, Long YG, He J, Zhang H, Guan ZZ. 2009. [Changes in learning and memory ability and brain cholinesterase activity in the rats with coal burning fluorosis]. *Chin J Endemiol* 28: 497-500.

Gui CZ, Ran LY, Li JP, Guan ZZ. 2010. Changes of learning and memory ability and brain nicotinic receptors of rat offspring with coal burning fluorosis. *Neurotoxicol Teratol* 32: 536-541.

Gui CZ, Ran LY, Guan ZZ. 2011. [Expression levels of brain nicotinic acetylcholine receptor mRNA and protein in coal-burning type of fluorosis rats]. *Chin J Endemiol* 30: 239-242.

Guner S, Uyar-Bozkurt S, Haznedaroglu E, Mentes A. 2016. Dental fluorosis and catalase immunoreactivity of the brain tissues in rats exposed to high fluoride pre- and postnatally. *Biol Trace Elem Res* 174: 150-157.

Han H, Du W, Zhou B, Zhang W, Xu G, Niu R, Sun Z. 2014. Effects of chronic fluoride exposure on object recognition memory and mRNA expression of SNARE complex in hippocampus of male mice. *Biol Trace Elem Res* 158: 58-64.

Hong JH, Ge YM, Ning HM, Wang JD. 2005. [Effects of High Fluoride and Low Iodine on Learning-Memory and TchE of Brain in Offspring Rats]. *Chin Prev Med* 6: 489-491.

Inkielewicz I, Krechniak J. 2004. Fluoride effects on glutathione peroxidase and lipid peroxidation in rats. *Fluoride* 37: 7-12.

Jain A, Mehta VK, Chittora RA, Mahdi A, Bhatnagar M. 2015. Melatonin ameliorates fluoride induced neurotoxicity in young rats: An in vivo evidence. *Asian J Pharm Clin Res* 8: 164-167.

Jetti R, Raghuveer CV, Mallikarjuna RC. 2016. Protective effect of ascorbic acid and ginkgo biloba against learning and memory deficits caused by fluoride. *Toxicol Ind Health* 32: 183-187.

Jia B, Zong L, Lee JY, Lei J, Zhu Y, Xie H, Clemens JL, Feller MC, Na Q, Dong J, McLane MW, Jones-Beatty K, Burd I. 2019. Maternal supplementation of low dose fluoride alleviates adverse perinatal outcomes following exposure to intrauterine inflammation. *Sci Rep* 9(1): 2575.

Jiang C, Zhang S, Liu H, Guan Z, Zeng Q, Zhang C, Lei R, Xia T, Wang Z, Yang L, Chen Y, Wu X, Zhang X, Cui Y, Yu L, Wang A. 2014. Low glucose utilization and neurodegenerative changes caused by sodium fluoride exposure in rat's developmental brain. *Neuromolecular Med* 16: 94-105.

Jiang S, Su J, Yao S, Zhang Y, Cao F, Wang F, Wang H, Li J, Xi S. 2014. Fluoride and arsenic exposure impairs learning and memory and decreases mGluR5 expression in the hippocampus and cortex in rats. *PLoS One* 9(4): e96041.

Khan AM, Raina R, Dubey N, Verma PK. 2017. Effect of deltamethrin and fluoride co-exposure on the brain antioxidant status and cholinesterase activity in Wistar rats. *Drug Chem Toxicol* 41(2): 1-5.

Kinawy AA, Al-Eidan AA. 2018. Impact of prenatal and postnatal treatment of sodium fluoride and aluminum chloride on some hormonal and sensorimotor aspects in rats. *Biol Trace Elem Res*: 1-8.

Kivrak Y. 2012. Effects of fluoride on anxiety and depression in mice. *Fluoride* 45: 302-306.

Li M, Cui J, Gao YH, Zhang W, Sun LY, Liu XN, Liu Y, Sun DJ. 2015. Pathological changes and effect on the learning and memory ability in rats exposed to fluoride and aluminum. *Toxicol Res* 4: 1366-1373.

Li X, Zhang J, Niu R, Manthari RK, Yang K, Wang J. 2019. Effect of fluoride exposure on anxiety- and depression-like behavior in mouse. *Chemosphere* 215: 454-460.

Liu WX. 1989. [Experimental study of behavior and cerebral morphology of rat pups generated by fluorotic female rat]. *Chin J Pathol* 18: 290-292.

Liu YJ, Gao Q, Wu CX, Long YG, Guan ZZ. 2009. [Modified expression of extracellular signal-regulated protein kinase signal transduction in rat brains and changed capacity of learning and memory of rats with chronic fluorosis]. *Chin J Endemiol* 28: 32-35.

Liu YJ, Gao Q, Wu CX, Guan ZZ. 2010. Alterations of nAChRs and ERK1/2 in the brains of rats with chronic fluorosis and their connections with the decreased capacity of learning and memory. *Toxicol Lett* 192: 324-329.

Liu YJ, Gao Q, Long YG, Yu YN, Guan ZZ. 2011. [Influence of chronic fluorosis on expression of phospho-Elk-1 in rat brains]. *Chin J Endemiol* 30: 251-255.

Liu F, Ma J, Zhang H, Liu P, Liu YP, Xing B, Dang YH. 2014. Fluoride exposure during development affects both cognition and emotion in mice. *Physiol Behav* 124: 1-7.

Lou DD, Guan ZZ, Liu YJ, Liu YF, Zhang KL, Pan JG, Pei JJ. 2013. The influence of chronic fluorosis on mitochondrial dynamics morphology and distribution in cortical neurons of the rat brain. *Arch Toxicol* 87: 449-457.

Lu F, Zhang Y, Trivedi A, Jiang X, Chandra D, Zheng J, Nakano Y, Uyghurturk DA, Jalai R, Guner S, Mentes A, DenBesten PK. 2019. Fluoride related changes in behavioral outcomes may relate to increased serotonin. *Physiol Behav* 206: 76-83.

Ma J, Liu F, Liu P, Dong YY, Chu Z, Hou TZ, Dang YH. 2015. Impact of early developmental fluoride exposure on the peripheral pain sensitivity in mice. *Int J Dev Neurosci* 47: 165-171.

Manusha S, Sudhakar K, Reddy KP. 2019. Protective effects of allium sativum extract against sodium fluoride induced neurotoxicity. *Int J Pharm Sci Res* 10(2): 625-633.

McPherson CA, Zhang G, Gilliam R, Brar SS, Wilson R, Brix A, Picut C, Harry GJ. 2018. An evaluation of neurotoxicity following fluoride exposure from gestational through adult ages in Long-Evans hooded rats. *Neurotoxicol Res*: 1-18.

Mesram N, Nagapuri K, Banala RR, Nalagoni CR, Karnati PR. 2016. Quercetin treatment against NaF induced oxidative stress related neuronal and learning changes in developing rats. *J King Saud Univ Sci* 29: 221-229.

Mullenix PJ, Denbesten PK, Schunior A, Kernan WJ. 1995. Neurotoxicity of sodium fluoride in rats. *Neurotoxicol Teratol* 17: 169-177.

Nageshwar M, Sudhakar K, Reddy NCC, Reddy KP. 2017. Neuroprotective effects of curcumin on sodium fluoride induced behavioural and enzymatic changes in brain and muscles of rat. *J Environ Biol* 38: 675-681.

Nageshwar M, Sudhakar K, Reddy KP. 2018. Quercetin ameliorates oxidative stress, neural damage of brain and behavioral impairment of rat with fluoride exposure. *Int J Pharm Sci Res* 9(8): 3247-3256.

Nian W, Wang X, Shao D, Yu Q, Ouyang W, Zhang Z, Ruan Q. 2018. Effects of subchronic exposure to fluorine on hippocampal injury in mice and its molecular mechanism. *Acta Sci Circumst* 38(11): 4512-4519.

Niu R, Sun Z, Wang J, Cheng Z. 2008. Effects of fluoride and lead on locomotor behavior and expression of nissl body in brain of adult rats. *Fluoride* 41: 276-282.

Niu R, Sun Z, Cheng Z, Li Z, Wang J. 2009. Decreased learning ability and low hippocampus glutamate in offspring rats exposed to fluoride and lead. *Environ Toxicol Pharmacol* 28: 254-258.

Niu R, Liu S, Wang J, Zhang J, Sun Z. 2014. Proteomic analysis of hippocampus in offspring male mice exposed to fluoride and lead. *Biol Trace Elem Res* 162: 227-233.

Niu R, Xue X, Zhao Y, Sun Z, Yan X, Li X, Feng C, Wang J. 2015. Effects of fluoride on microtubule ultrastructure and expression of Tubalpha1a and Tubbeta2a in mouse hippocampus. *Chemosphere* 139: 422-427.

Niu Q, Chen J, Xia T, Li P, Zhou G, Xu C, Zhao Q, Dong L, Zhang S, Wang A. 2018. Excessive ER stress and the resulting autophagic flux dysfunction contribute to fluoride-induced neurotoxicity. *Environ Pollut* 233: 889-899.

Niu R, Chen H, Manthari RK, Sun Z, Wang J, Zhang J, Wang J. 2018. Effects of fluoride on synapse morphology and myelin damage in mouse hippocampus. *Chemosphere* 194: 628-633.

Nkpaa KW, Onyeso GI. 2018. Rutin attenuates neurobehavioral deficits, oxidative stress, neuro-inflammation and apoptosis in fluoride treated rats. *Neurosci Lett* 682: 92-99.

Paul V, Ekambaram P, Jayakumar AR. 1998. Effects of sodium fluoride on locomotor behavior and a few biochemical parameters in rats. *Environ Toxicol Pharmacol* 6: 187-191.

Pereira M, Dombrowski PA, Losso EM, Chioca LR, Da Cunha C, Andreatini R. 2011. Memory impairment induced by sodium fluoride is associated with changes in brain monoamine levels. *Neurotoxicol Res* 19: 55-62.

Pulungan ZS, Sofro ZM, Partadiredja G. 2016. Sodium fluoride does not affect the working memory and number of pyramidal cells in rat medial prefrontal cortex. *Anat Sci Int* 93(1): 128-138.

Raghu J, Raghuveer VC, Rao MC, Somayaji NS, Babu PB. 2013. The ameliorative effect of ascorbic acid and ginkgo biloba on learning and memory deficits associated with fluoride exposure. *Interdiscip Toxicol* 6: 217-221.

Raju S, Sivanesan SK, Gudemalla K. 2019. Cognitive enhancement effect of Ginkgo biloba extract on memory and learning impairments induced by fluoride neurotoxicity. *Int J Res Pharm Sci* 10(1): 129-134.

Reddy YP, Tiwari SK, Shaik AP, Alsaeed A, Sultana A, Reddy PK. 2014. Effect of sodium fluoride on neuroimmunological parameters, oxidative stress and antioxidative defenses. *Toxicol Mech Methods* 24: 31-36.

Reddy MM, Karnati PR. 2015. Protective effects of aqueous extract of fruit pulp of tamarindus indica on motor activity and metabolism of the gastrocnemius muscle of rats treated with fluoride. *Int J Toxicol Pharmacol Res* 7: 241-246.

Rumiantsev GI, Novikov SM, Mel'nikova NN, Levchenko NI, Kozeeva EE. 1988. [Experimental study of the biological effect of salts of hydrofluosilicic acid]. *Gig Sanit*: 80-82.

Sarkozi K, Horvath E, Vezer T, Papp A, Paulik E. 2015. Behavioral and general effects of subacute oral arsenic exposure in rats with and without fluoride. *Int J Environ Health Res* 25: 418-431.

Shah SD, Chinoy NJ. 2004. Adverse effects of fluoride and/or arsenic on the cerebral hemisphere of mice and recovery by some antidotes. *Fluoride* 37: 162-171.

Shalini B, Sharma JD. 2015. Beneficial effects of emblica officinalis on fluoride-induced toxicity on brain biochemical indexes and learning-memory in rats. *Toxicol Int* 22: 35-39.

Shan KR, Qi XL, Long YG, Nordberg A, Guan ZZ. 2004. Decreased nicotinic receptors in PC12 cells and rat brains influenced by fluoride toxicity: A mechanism relating to a damage at the level in post-transcription of the receptor genes. *Toxicology* 200: 169-177.

Sharma C, Suhalka P, Bhatnagar M. 2018. Curcumin and resveratrol rescue cortical-hippocampal system from chronic fluoride-induced neurodegeneration and enhance memory retrieval. *Int J Neurosci*: 1-15.

Shen X, Zhang Z, Xu X. 2004. [Effect of iodine and selenium on learning memory impairment induced by fluorosis and blood biochemical criterion of rats]. *Occupation and Health* 20(1): 6-8.

Sudhakar K, Nageshwar M, Pratap Reddy K. 2017. Seed extract of Abelmoschus moschatus medik reverses NAF-induced behavioral changes through neurodegeneration and oxidative stress in brain of rat. *Asian J Pharm Clin Res* 10: 165-171.

Sudhakar K, Nageshwar M, Reddy KP. 2018. Protective effect of okra, Abelmoschus moschatus seed extract on developing brain of rats during pre- and post-natal fluoride exposure. *Int J Pharm Sci Res* 9: 1519-1528.

Sudhakar K, Nageshwar M, Reddy KP. 2018. Abelmoschus moschatus extract reverses altered pain and neurohistology of a rat with developmental exposure of fluoride. *J Appl Pharm Sci* 8(6): 94-104.

Sudhakar K, Reddy KP. 2018. Protective effects of Abelmoschus moschatus seed extract on neurotransmitter system of developing brain of Wistar rats with gestational and post-natal exposure of sodium fluoride. *Int J Green Pharm* 12: S131-S139.

Sun ZR, Liu FZ, Wu LN, Lu Y, Yu DK. 2008. Effects of high fluoride drinking water on the cerebral function of mice. *Fluoride* 41: 148-151.

Sun Z, Zhang Y, Xue X, Niu R, Wang J. 2018. Maternal fluoride exposure during gestation and lactation decreased learning and memory ability, and glutamate receptor mRNA expressions of mouse pups. *Hum Exp Toxicol* 37: 87-93.

Trivedi MH, Verma RJ, Chinoy NJ. 2007. Amelioration by black tea of sodium fluoride-induced changes in protein content of cerebral hemisphere, cerebellum and medulla oblongata in brain region of mice. *Acta Poloniae Pharm* 64: 221-225.

Trivedi MH, Verma RJ, Chinoy NJ. 2009. Mitigation of sodium fluoride induced toxicity in mice brain by black tea infusion. *Fluoride* 42: 29-33.

Trivedi MH, Verma RJ, Sangai NP, Chinoy NJ. 2011. Black tea extract mitigation of NaF-induced lipid peroxidation in different regions of mice brains. *Fluoride* 44: 243-254.

Trivedi MH, Verma RJ, Sangai NP, Chinoy NJ. 2012. Mitigation by black tea extract of sodium fluoride induced histopathological changes in brain of mice. *Fluoride* 45: 13-26.

Tsunoda M, Aizawa Y, Nakano K, Liu Y, Horiuchi T, Itai K, Tsunoda H. 2005. Changes in fluoride levels in the liver, kidney, and brain and in neurotransmitters of mice after subacute administration of fluoride. *Fluoride* 38: 284-292.

Varner JA, Jensen KF, Horvath W, Isaacson RL. 1998. Chronic administration of aluminum-fluoride or sodium-fluoride to rats in drinking water: Alterations in neuronal and cerebrovascular integrity. *Brain Res* 784(1-2): 284-298.

Verma RJ, Trivedi MH, Chinoy NJ. 2007. Black tea amelioration of sodium fluoride-induced alterations of DNA, RNA, and protein contents in the cerebral hemisphere, cerebellum, and medulla oblongata regions of mouse brain. *Fluoride* 40: 7-12.

Wang JD, Ge YM, Ning HM, Wang SL. 2004. Effects of high fluoride and low iodine on biochemical indexes of the brain and learning-memory of offspring rats. *Fluoride* 37: 201-208.

Wang G, Li J, Zhu H, Zhu J. 2006. Effect of different doses of chronic exposure of fluoride on rat learning and memory behavior. *Studies of Trace Elements and Health* 23(2): 1-2.

Wang J, Zhang Y, Guo Z, Li R, Xue X, Sun Z, Niu R. 2018. Effects of perinatal fluoride exposure on the expressions of miR-124 and miR-132 in hippocampus of mouse pups. *Chemosphere* 197: 117-122.

Wei N, Dong Y, Wang Y, Guan Z. 2014. [Effects of chronic fluorosis on neurobehavioral development in offspring of rats and antagonistic effect of vitamin E]. *Chin J Endemiol* 33: 125-128.

Whitford GM, Whitford JL, Hobbs SH. 2009. Appetitive-based learning in rats: Lack of effect of chronic exposure to fluoride. *Neurotoxicol Teratol* 31: 210-215.

Wu CX, Gu XL, Ge YM, Zhang JH, Wang JD. 2006. Effects of high fluoride and arsenic on brain biochemical indexes and learning-memory in rats. *Fluoride* 39: 274-279.

Wu NP, Zhao ZL, Gao WH, Li XQ. 2008. Behavioral teratology in rats exposed to fluoride. *Fluoride* 41: 129-133.

Xu X, Shen X, Zhang Z. 2001. Effect of fluorosis on mice learning and memory behaviors and brain SOD activity and MDA content *China Public Health* 17(1): 8-10.

Yang L, Jin P, Wang X, Zhou Q, Lin X, Xi S. 2018. Fluoride activates microglia, secretes inflammatory factors and influences synaptic neuron plasticity in the hippocampus of rats. *Neurotox* 69: 108-120.

Yu Q, Shao D, Zhang R, Ouyang W, Zhang Z. 2019. Effects of drinking water fluorosis on L-type calcium channel of hippocampal neurons in mice. *Chemosphere* 220: 169-175.

Yuan J, Li Q, Niu R, Wang J. 2019. Fluoride exposure decreased learning ability and the expressions of the insulin receptor in male mouse hippocampus and olfactory bulb. *Chemosphere* 224: 71-76.

Zhang Z, Shen X, Xu X. 2001. [Effects of selenium on the damage of learning-memory ability of mice induced by fluoride]. *J Hyg Res* 30: 144-146.

Zhang Z, Xu X, Shen X, Xua XH. 2008. Effect of fluoride exposure on synaptic structure of brain areas related to learning-memory in mice. *Fluoride* 41: 139-143.

Zhang J, Zhu W, Zhang Z. 2009. [The effect of fluorine exposure of pregnant rats on the learning and memory capabilities of baby rats]. *Chinese Journal of Public Health* 25(11): 1347-1348.

Zhang J, Zhu WJ, Xu XH, Zhang ZG. 2011. Effect of fluoride on calcium ion concentration and expression of nuclear transcription factor kappa-B rho65 in rat hippocampus. *Exp Toxicol Pathol* 63: 407-411.

Zhang C, Ren C, Chen H, Geng R, Fan H, Zhao H, Guo K, Geng D. 2013. The analog of Ginkgo biloba extract 761 is a protective factor of cognitive impairment induced by chronic fluorosis. *Biol Trace Elem Res* 153: 229-236.

Zhang J, Zhang Z. 2013. Effects of chronic fluorosis on camkiiα, c-FOS, BAX, and BCL-2 channel signaling in the hippocampus of rats. *Fluoride* 46: 135-141.

Zhang KL, Lou DD, Guan ZZ. 2015. Activation of the AGE/RAGE system in the brains of rats and in SH-SY5Y cells exposed to high level of fluoride might connect to oxidative stress. *Neurotoxicol Teratol* 48: 49-55.

Zhao XL, Wu JH. 1998. Actions of sodium fluoride on acetylcholinesterase activities in rats. *Biomed Environ Sci* 11: 1-6.

Zhao Q, Niu Q, Chen J, Xia T, Zhou G, Li P, Dong L, Xu C, Tian Z, Luo C, Liu L, Zhang S, Wang A. 2019. Roles of mitochondrial fission inhibition in developmental fluoride neurotoxicity: Mechanisms of action in vitro and associations with cognition in rats and children. *Arch Toxicol* 93(3): 709-726.

Zheng X, Sun Y, Ke L, Ouyang W, Zhang Z. 2016. Molecular mechanism of brain impairment caused by drinking-acquired fluorosis and selenium intervention. *Environ Toxicol Pharmacol* 43: 134-139.

Zhu W, Zhang J, Zhang Z. 2011. Effects of fluoride on synaptic membrane fluidity and PSD-95 expression level in rat hippocampus. *Biol Trace Elem Res* 139: 197-203.

Zhu YL, Zheng YJ, LV XM, Ma Y, Zhang J. 2012. Effects of fluoride exposure on performance in water labyrinth and monoamine neurotransmitters of rats. *Journal of Xinjiang Medical University* 3: 014.

Zhu YP, Xi SH, Li MY, Ding TT, Liu N, Cao FY, Zeng Y, Liu XJ, Tong JW, Jiang SF. 2017. Fluoride and arsenic exposure affects spatial memory and activates the ERK/CREB signaling pathway in offspring rats. *Neurotox* 59: 56-64.

Studies Not Available in HAWC

Abdelaleem MM, El-Tahawy NFG, Abozaid SMM, Abdel-Hakim SA. 2018. Possible protective effect of curcumin on the thyroid gland changes induced by sodium fluoride in albino rats: Light and electron microscopic study. *Endocr Regul* 52: 59-68.

Abd-Elhakim YM, Mohammed AT, Ali HA. 2018. Impact of subchronic exposure to triclosan and/or fluoride on estrogenic activity in immature female rats: The expression pattern of calbindin-D9k and estrogen receptor alpha genes. *J Biochem Mol Toxicol* 32(2): 22027.

Abdumajidov OR. 2004. [Sex differences in lipid peroxidation and antioxidant defense of the brain tissue in intoxication with low doses of inorganic compounds]. *Uzbekiston Tibbiet Zhurnali*: 58-60.

Adebayo OL, Shallie PD, Salau BA, Ajani EO, Adenuga GA. 2013. Comparative study on the influence of fluoride on lipid peroxidation and antioxidants levels in the different brain regions of well-fed and protein undernourished rats. *J Trace Elem Med Biol* 27: 370-374.

Adedara IA, Ojuade TJD, Olabiyi BF, Idris UF, Onibiyo EM, Ajeigbe OF, Farombi EO. 2016. Taurine ameliorates renal oxidative damage and thyroid dysfunction in rats chronically exposed to fluoride. *Biol Trace Elem Res*: 1-8.

Ahmed SK, Kalleny NK, Attia AAEM, Elkateb LA. 2015. The possible protective role of chromium chloride against sodium fluoride-induced changes in the structure of the cerebellar cortex of the adult male albino rat. *Egypt J Histol* 38: 402-414.

Al Badawi MH, Mahmoud OM, Salem NA. 2016. Therapeutic potential of omega-3 against sodium fluoride toxicity on the cerebellar cortex of adult male albino rats: Histological and immunohistochemical study. *Egypt J Histol* 39: 170-178.

Alhayani A, Elshal EB, Aal IHA, Al-Shammeri E, Kabra H. 2013. Does vitamin E protect against sodium fluoride toxicity on the cerebellar cortex of albino rats? *Middle East J Sci Res* 16: 1019-1026.

Ameeramja J, Raghunath A, Perumal E. 2018. Tamarind seed coat extract restores fluoride-induced hematological and biochemical alterations in rats. *Environ Sci Pollut Res Int* 25(26): 26157-26166.

Antonyan OA. 1980. [Lipid per oxidation in fluorosis and the protective role of dietary factors]. *Zh Eksp Klin Med* 20: 381-388.

Ardelean I, Racoveanu N, Manescu S, Lupulescu A, Diaconescu M, Ghelerter L. 1964. Experimental investigations concerning the effect of fluorine on the thyroid gland. *Rum Med Rev* 8: 17-20.

Atmaca N, Atmaca HT, Kanici A, Anteplioglu T. 2014. Protective effect of resveratrol on sodium fluoride-induced oxidative stress, hepatotoxicity and neurotoxicity in rats. *Food Chem Toxicol* 70: 191-197.

Auskaps AM, Shaw JH. 1955. Hemoglobin concentration, thyroid weight and growth rate in rats during minimum fluoride ingestion. *J Nutr* 55: 611-621.

Bagmut I, Kolisnyk I, Titkova A, Petrenko T, Filipchenko S. 2018. Content of catecholamines in blood serum of rats under fluoride intoxication. *Georgian Med News* (280-281): 125-129.

Bakalyan PH, Antonyan OA. 1981. [Effect of fluorosis on glutathione peroxidase and glutathione reductase activities and sulfhydryl groups]. *Zh Eksp Klin Med* 21: 10-14.

Basha PM, Madhusudhan N. 2010. Pre and post natal exposure of fluoride induced oxidative macromolecular alterations in developing central nervous system of rat and amelioration by antioxidants. *Neurochem Res* 35: 1017-1028.

Basha PM, Madhusudhan N. 2011. Effect of maternal exposure of fluoride on oxidative stress markers and amelioration by selected antioxidants in developing central nervous system of rats. *Biologia* 66: 187-193.

Basha PM, Rai P, Begum S. 2011. Evaluation of fluoride-induced oxidative stress in rat brain: A multigeneration study. *Biol Trace Elem Res* 142: 623-637.

Basha PM, Sujitha NS. 2012. Combined influence of intermittent exercise and temperature stress on the modulation of fluoride toxicity. *Biol Trace Elem Res* 148: 69-75.

Basha PM, Saumya SM. 2013. Suppression of mitochondrial oxidative phosphorylation and TCA enzymes in discrete brain regions of mice exposed to high fluoride: Amelioration by panax ginseng (ginseng) and lagerstroemia speciosa (banaba) extracts. *Cell Mol Neurobiol* 33: 453-464.

Basha MP, Begum S, Madhusudhan N. 2014. Antioxidants in the management of fluoride induced neural oxidative stress in developing rats. *Int J Pharm Sci Res* 5: 201-206.

Benetato G, Giuran AM, Cirmaciu R, Cirje M, Petrescu A, Vacariu A. 1970. [Effect of fluorine in drinking water on the metabolism of Ca and Mg and on neuromuscular excitability: Experimental studies and clinical observations]. *Rev Roum Physiol* 7: 335-352.

Bharti VK, Srivastava RS. 2009. Fluoride-induced oxidative stress in rat's brain and its amelioration by buffalo (Bubalus bubalis) pineal proteins and melatonin. *Biol Trace Elem Res* 130: 131-140.

Bhatnagar M, Rao P, Saxena A, Bhatnagar R, Meena P, Barbar S, Chouhan A, Vimal S. 2006. Biochemical changes in brain and other tissues of young adult female mice from fluoride in their drinking water. *Fluoride* 39: 280-284.

Bilgili A, Akdogan M, Yildiz M, Eraslan G, Cetin N. 2004. The effects of fluoride on thyroid hormones in rabbits. *Indian Vet J* 81: 986-988.

Bobek S, Kahl S, Ewy Z. 1976. Effect of long-term fluoride administration on thyroid hormones level blood in rats. *Endocrinol Exp* 10: 289-295.

Bouaziz H, Ammar E, Ghorbel H, Ketata S, Jamoussi K, Ayadi F, Guermazi F, Zeghal N. 2004. Effect of fluoride ingested by lactating mice on the thyroid function and bone maturation of their suckling pups. *Fluoride* 37: 133-142.

Bouaziz H, Soussia L, Guermazi F, Zeghal N. 2005. Fluoride-induced thyroid proliferative changes and their reversal in female mice and their pups. *Fluoride* 38: 185-192.

Bouaziz HB, Amara I, Essefi M, Croute F, Zeghal N. 2010. Fluoride-induced brain damages in suckling mice. *Pestic Biochem Physiol* 96: 24-29.

Chauhan SS, Ojha S, Mahmood A. 2013. Effects of fluoride and ethanol administration on lipid peroxidation systems in rat brain. *Indian J Exp Biol* 51: 249-255.

Chen J, Chen X, Yang K, Xia T, Xie H. 2002. [Studies on DNA damage and apoptosis in rat brain induced by fluoride]. *Chin J Prev Med* 36: 222-224.

Chirumari K, Reddy PK. 2007. Dose-dependent effects of fluoride on neurochemical milieu in the hippocampus and neocortex of rat brain. *Fluoride* 40: 101-110.

Chouhan S, Yadav A, Kushwah P, Kaul RK, Flora SJS. 2011. Silymarin and quercetin abrogates fluoride induced oxidative stress and toxic effects in rats. *Mol Cell Toxicol* 7: 25-32.

Clay AB, Suttie JW. 1987. Effect of dietary fluoride on dairy cattle: Growth of young heifers. *J Dairy Sci* 70: 1241-1251.

Czechowicz K, Osada A, Slesak B. 1974. Histochemical studies on the effect of sodium fluoride on metabolism in Purkinje's cells. *Folia Histochem Cytochem* 12: 37-44.

Demole V, Lerch P. 1956. [Normality of fixation of radioactive iodine in the thyroid of rats during experimental fluorosis]. *Helv Physiol Pharmacol Acta* 14(4): 62-63.

Dhurvey V, Patil V, Thakare M. 2017. Effect of sodium fluoride on the structure and function of the thyroid and ovary in albino rats (rattus norvegicus). *Fluoride* 50: 235-246.

Domzalska E. 1966. [Influence of sodium fluoride on hypophysis, thyroid gland, parathyroid, and adrenal gland in the white rat]. *Czas Stomatol* 19: 839-844.

El-lethey HS, Kamel MM, Shaheed IB. 2011. Perinatal exposure to sodium fluoride with emphasis on territorial aggression, sexual behaviour and fertility in male rats. *Life Sci J* 8: 686-694.

Flora SJS, Mittal M, Mishra D. 2009. Co-exposure to arsenic and fluoride on oxidative stress, glutathione linked enzymes, biogenic amines and DNA damage in mouse brain. *J Neurol Sci* 285: 198-205.

Flora SJS, Mittal M, Pachauri V, Dwivedi N. 2012. A possible mechanism for combined arsenic and fluoride induced cellular and DNA damage in mice. *Metallomics* 4: 78-90.

Gabovich RD, Verzhikovskaia NV. 1958. [Effect of fluoride compounds on absorption of radioactive iodine by the thyroid gland in humans and in experimental conditions]. *Probl Endokrinol Gormonoter* 4: 49-54.

Galamini-Ligori M, Di Blasi F. 1961. [Action of sodium fluoride on the thyroid of hypophysectomized rats]. *Boll Soc Ital Biol Sper* 37: 1503-1506.

Galletti P, Held HR, Korrodi H, Wegmann T. 1956. [Investigations on the possible damaging effect of fluorine on the thyroid gland]. *Helv Med Acta* 23: 601-605.

Ge YM, Ning HM, Wang SL, Wang JD. 2005. DNA damage in thyroid gland cells of rats exposed to longterm intake of high fluoride and low iodine. *Fluoride* 38: 318-323.

Ge YM, Ning HM, Wang SL, Wang JD. 2005. Comet assay of DNA damage in brain cells of adult rats exposed to high fluoride and low iodine. *Fluoride* 38: 209-214.

Ge YM, Ning HM, Wang SL, Wang JD. 2005. Effects of high fluoride and low iodine on brain histopathology in offspring rats. *Fluoride* 38: 127-132.

Ge Y, Ning H, Feng C, Wang H, Yan X, Wang S, Wang J. 2006. Apoptosis in brain cells of offspring rats exposed to high fluoride and low iodine. *Fluoride* 39: 173-178.

Ge Y, Niu R, Zhang J, Wang J. 2011. Proteomic analysis of brain proteins of rats exposed to high fluoride and low iodine. *Arch Toxicol* 85: 27-33.

Ge YM, Ning HM, Gu XL, Yin M, Yang XF, Qi YH, Wang JD. 2013. Effects of high fluoride and low iodine on thyroid function in offspring rats. *J Integr Agric* 12: 502-508.

Guan ZZ. 1986. [Morphology of the brain of the offspring of rats with chronic fluorosis]. *Chin J Pathol* 15: 297-299.

Guan Z, Wang Y, Xiao K. 1997. [Influence of experimental fluorosis on phospholipid content and fatty acid composition in rat brain]. *Chin Med J* 77: 592-596.

Guan Z-Z, Wang Y-N, Xiao K-Q, Dai D-Y, Chen Y-H, Liu J-L, Sindelar P, Dallner G. 1998. Influence of chronic fluorosis on membrane lipids in rat brain. *Neurotoxicol Teratol* 20: 537-542.

Guan ZZ, Shan KR, Wang YN, Dallner G. 2006. [Changes of lipids and nicotinic receptors in rat brains and pheochromocytoma with fluorosis]. *Chin J Endemiol* 25: 121-124.

Gushchin SK. 1951. [Effect of sodium fluoride on iodine metabolism in rabbit tissue organs; on the etiology of endemic goiter]. *Gig Sanit* 2: 45-48.

Hamza RZ, Al-Harbi MS. 2014. Sodium fluoride induced neurotoxicity and possible antioxidant role of selenium and curcumin in male mice. *Biosci Biotechnol Res Asia* 11: 81-87.

Hamza RZ, El-Shenawy NS, Ismail HAA. 2015. Protective effects of blackberry and quercetin on sodium fluoride-induced oxidative stress and histological changes in the hepatic, renal, testis and brain tissue of male rat. *J Basic Clin Physiol Pharmacol* 26: 237-251.

Haojun Z, Yaoling W, Ke Z, Jin L, Junling W. 2012. Effects of NaF on the expression of intracellular Ca2+ fluxes and apoptosis and the antagonism of taurine in murine neuron. *Toxicol Mech Methods* 22: 305-308.

Hara K. 1980. Studies on fluorosis especially effects of fluoride on thyroid metabolism. *J Dent Health* 30: 42-57.

Harris NO, Hayes RL. 1955. A tracer study of the effect of acute and chronic exposure to sodium fluoride on the thyroid iodine metabolism of rats. *J Dent Res* 34: 470-477.

Hassan HA, Abdel-Aziz AF. 2010. Evaluation of free radical-scavenging and anti-oxidant properties of black berry against fluoride toxicity in rats. *Food Chem Toxicol* 48: 1999-2004.

Hoogstratten B, Leone NCLG, Shupe J, Greenwood DA, Lieberman J. 1965. Effect of fluorides on hematopoietic system, liver, and thyroid gland in cattle. *J Amer Med Assoc* 192: 26-32.

Inkielewicz I, Rogowska M, Krechniak J. 2006. Lipid peroxidation and antioxidant enzyme activity in rats exposed to fluoride and ethanol. *Fluoride* 39: 53-59.

Inkielewicz I, Czarnowski W. 2008. Oxidative stress parameters in rats exposed to fluoride and aspirin. *Fluoride* 41: 76-82.

Inkielewicz-Stepniak I, Czarnowski W. 2010. Oxidative stress parameters in rats exposed to fluoride and caffeine. *Food Chem Toxicol* 48: 1607-1611.

Jiang SF, Xi SH, Yao SQ, Tong JW, Zhang YS, Wang Q, Su J, Li MY. 2013. [Effects of fluoride, arsenic and co-exposure on expression of Bcl-2 and Bax in hippocampus and cerebral cortex of rats]. *Chin J Endemiol* 32: 365-369.

Jiang Y, Guo X, Sun Q, Shan Z, Teng W. 2016. Effects of excess fluoride and iodide on thyroid function and morphology. *Biol Trace Elem Res* 170: 382-389.

Jiang P, Li G, Zhou X, Wang C, Qiao Y, Liao D, Shi D. 2018. Chronic fluoride exposure induces neuronal apoptosis and impairs neurogenesis and synaptic plasticity: Role of GSK-3beta/beta-catenin pathway. *Chemosphere* 214: 430-435.

Jin TX, Guan ZZ, Zhang H. 2011. [The effect of fluoride on α subunit of calcium/calmodulin-dependent protein kinase-II mRNA and protein expression in central nervous system]. *Chin J Endemiol* 30: 247-250.

Jonderko G, Kita K, Pietrzak J, Primus-Slowinska B, Ruranska B, Zylka-Wloszczyk M, Straszecka J. 1983. [Effect of subchronic sodium fluoride poisoning on the thyroid gland of rabbits with normal and increased supply of iodine]. *Endokrynol Pol* 34: 195-203.

Kahl S, Bobek S. 1975. [Effect of fluoride administration on radiothyroxine turnower in rats]. *Endokrynol Pol* 26: 391-396.

Kahl S, Ewy Z. 1975. Effect of single and long term sodium fluoride administration on biosynthesis of the thyroid hormone in rats. *Fluoride* 8: 191-198.

Kapoor V, Prasad T, Paliwal VK. 2001. Blood biochemical constituents in calves following subclinical levels of fluoride toxicosis. *Fluoride* 34: 126-131.

Karawya FS, Zahran NM, Azzam EZ. 2015. Is water fluoridation a hidden cause of obesity? Histological study on thyroid follicular cells of albino rats. *Egypt J Histol* 38: 547-557.

Kaur T, Bijarnia RK, Nehru B. 2009. Effect of concurrent chronic exposure of fluoride and aluminum on rat brain. *Drug Chem Toxicol* 32: 215-221.

Kelimu A, Liu KT, Lian J, Hu HH, Zheng YJ, Wang TM. 2008. [Effects of vitamin C and E on the ultrastructure in liver, kidney and brain of fluorosis rats]. *Chin J Endemiol* 27: 378-381.

Kinawy AA. 2019. Synergistic oxidative impact of aluminum chloride and sodium fluoride exposure during early stages of brain development in the rat. *Environ Sci Pollut Res Int* 26(11): 10951-10960.

Knizhnikov VA. 1959. [Effect of potable water with high fluoride concentration on thyroid function]. *Gig Sanit* 24: 20-25.

Knizhnikov VA, Tsypin AB, Shcherbova EN, Bugryshev PF. 1963. [The effect of drinking water with an increased fluorine content on the bioelectrical activity of the brain and heart under experimental conditions]. *Gig Sanit* 28: 16-19.

Kondo T, Yoshida M, Kasahara K. 1976. [Acute fluorosis in female rats: Time of inhibition and recovery of cholinesterase in serum and salivary glands]. *Jpn J Dent Health* 26: 187-192.

Kowalewska M. 1974. [Biopotentials of the organ of hearing in chronic sodium fluoride poisoning]. *J Pol Otolaryngol* 28: 417-424.

Krechniak J, Inkielewicz I. 2005. Correlations between fluoride concentrations and free radical parameters in soft tissues of rats. *Fluoride* 38: 293-296.

Leonard BE. 1972. Effect of phentolamine on the increase in brain glycolysis following the intraventricular administration of dibutyryl-3,5-cyclic adenosine monophosphate and sodium fluoride to mice. *Biochem Pharmacol* 21: 115-117.

Li H, Cai Q, Wang D. 2012. [Effect of fluoride on the expression of rat thyroid peroxidase mRNA]. *Chin J Endemiol* 31: 515-517.

Li H, Cai Q, Wang D. 2012. [Effects of fluoride on rat thyroid morphology, thyroid peroxidase activity and the expression of thyroid peroxidase protein]. *Chin J Endemiol* 31: 271-274.

Liu YJ, Gao Q, Wu CX, Guan ZZ. 2010. [Changes of the c-Jun N-terminal kinase in the brains of rats with chronic fluorosis]. *Chin J Endemiol* 29: 608-612.

Liu YJ, Guan ZZ, Gao Q, Pei JJ. 2011. Increased level of apoptosis in rat brains and SH-SY5Y cells exposed to excessive fluoride-A mechanism connected with activating JNK phosphorylation. *Toxicol Lett* 204: 183-189.

Liu G, Zhang W, Jiang P, Li X, Liu C, Chai C. 2012. Role of nitric oxide and vascular endothelial growth factor in fluoride-induced goitrogenesis in rats. *Environ Toxicol Pharmacol* 34: 209-217.

Liu H, Hou C, Zeng Q, Zhao L, Cui Y, Yu L, Wang L, Zhao Y, Nie J, Zhang B, Wang A. 2016. Role of endoplasmic reticulum stress-induced apoptosis in rat thyroid toxicity caused by excess fluoride and/or iodide. *Environ Toxicol Pharmacol* 46: 277-285.

Lohakare J, Pattanaik AK. 2013. Effects of addition of fluorine in diets differing in protein content on urinary fluoride excretion, clinical chemistry and thyroid hormones in calves. *Brazilian J Anim Sci* 42: 751-758.

Long YG, Wang YN, Chen J, Jiang SF, Nordberg A, Guan ZZ. 2002. Chronic fluoride toxicity decreases the number of nicotinic acetylcholine receptors in rat brain. *Neurotoxicol Teratol* 24: 751-757.

Lou DD, Liu YF, Zhang KL, Yu YN, Guan ZZ. 2011. [Changes of reactive oxygen species level and mitochondria fission-fusion in cortical neurons of rats with chronic fluorosis]. *Chin J Endemiol* 30: 256-260.

Lou DD, Liu YF, Qin SL, Zhang KL, Yu YN, Guan ZZ. 2012. [Changed transcription level of mitochondrial fission and fusion gene loci in cortical neurons of rats with chronic fluorosis]. *Chin J Endemiol* 31: 125-129.

Lou DD, Zhang KL, Qin SL, Liu YF, Yu YN, Guan ZZ. 2012. [Alteration of mitochondrial distribution and gene expression of fission 1 protein in cortical neurons of rats with chronic fluorosis]. *Chin J Pathol* 41: 243-247.

Lou DD, Pan JG, Zhang KL, Qin SL, Liu YF, Yu YN, Guan ZZ. 2013. [Changed expression of mito-fusion 1 and mitochondrial fragmentation in the cortical neurons of rats with chronic fluorosis]. *Chin J Prev Med* 47: 170-174.

Lou DD, Zhang KL, Pan JG, Qin SL, Liu YF, Yu YN, Guan ZZ. 2013. [Influence of chronic fluorosis on the expression of mitochondrial fission protein dynamin-related 1 in the cortical neurons of rats]. *Chin J Prev Med* 47: 561-564.

Lou DD, Zhang KL, Qin SL, Liu YF, Liu YJ, Guan ZZ. 2013. [Effects of chronic fluorosis on 4.8 kb mitochondrial DNA in liver, kidney and brain of rats]. *Chin J Endemiol* 32: 121-124.

Lou DD, Guan ZZ, Pei JJ. 2014. Alterations of apoptosis and expressions of Bax and Bcl-2 in the cerebral cortices of rats with chronic fluorosis. *Fluoride* 47: 199-207.

Luo GY, Niu RY, Sun ZL, Zhang JH, Wang JM, Wang C, Wang JD. 2011. Reduction of CaMKII expression in the hippocampus of rats from ingestion of fluoride and/or lead. *Fluoride* 44: 63-69.

Ma T, Liu D, Song K. 1999. Cytochemical study of neuron enzyme at anterior horn of spinal cord in rats with experimental fluorosis. *J Chin Med Univ* 28: 81-82.

Ma TX, Yu HT, Song KQ. 2008. [Expression of c-fos and Caspase 8 in cerebral cortex of rats with experimental fluorosis]. *Chin J Endemiol* 27: 131-133.

Mach Z, Zygulska-Machowa H. 1959. O wplywie fluoru na przemiane J131 [Russian and English summ.]. *Endokrynol Pol* 10: 157-162.

Machida H. 1989. [A study on the rabbit thermoregulatory system effects of high dose sodium fluoride]. *Dent Sci Rep* 89: 607-626.

Madan J, Puri JP, Singh JK. 2009. Growth, feed efficiency and blood profile of buffalo calves consuming high levels of fluoride. *Trop Anim Health Prod* 41: 295-298.

Madhusudhan N, Basha PM, Begum S, Ahmed F. 2009. Fluoride-induced neuronal oxidative stress and its amelioration by antioxidants in developing rats. *Fluoride* 42: 179-187.

Madhusudhan N, Basha PM, Rai P, Ahmed F, Prasad GR. 2010. Effect of maternal fluoride exposure on developing CNS of rats: Protective role of Aloe vera, Curcuma longa and Ocimum sanctum. *Indian J Exp Biol* 48: 830-836.

Manocha SL, Warner H, Olkowski ZL. 1975. Cytochemical response of kidney, liver and nervous system of fluoride ions in drinking water. *Histochem J* 7: 343-355.

Mansour HH, Tawfik SS. 2012. Efficacy of lycopene against fluoride toxicity in rats. *Pharm Biol* 50: 707-711.

Mietkiewski K, Walczak M, Trojanowicz R. 1966. [Effect of sodium fluoride on the neurosecretory system in guinea pigs]. *Endokrynol Pol* 17: 121-131.

Mohamed NE. 2016. The role of calcium in ameliorating the oxidative stress of fluoride in rats. *Biol Trace Elem Res* 170: 128-144.

Muhlemann HR, Schneider R. 1956. [Mitotic activity of rat thyroid epithelium after administration of fluoridated drinking water]. *Schweiz Med Wochenschr* 86: 625-627.

Nabavi SF, Eslami S, Moghaddam AH, Nabavi SM. 2011. Protective effects of curcumin against fluoride-induced oxidative stress in the rat brain. *Neurophysiology* 43: 287-291.

Nabavi SF, Moghaddam AH, Nabavi SM, Eslami S. 2011. Protective effect of curcumin and quercetin on thyroid function in sodium fluoride intoxicated rats. *Fluoride* 44: 147-152.

Nabavi SF, Habtemariam S, Jafari M, Sureda A, Nabavi SM. 2012. Protective role of gallic acid on sodium fluoride induced oxidative stress in rat brain. *Bull Environ Contam Toxicol* 89: 73-77.

Nabavi SF, Nabavi SM, Latifi AM, Mirzaei M, Habtemariam S, Moghaddam AH. 2012. Mitigating role of quercetin against sodium fluoride-induced oxidative stress in the rat brain. *Pharm Biol* 50: 1380-1383.

Nabavi SM, Sureda A, Nabavi SF, Latifi AM, Moghaddam AH, Hellio C. 2012. Neuroprotective effects of silymarin on sodium fluoride-induced oxidative stress. *J Fluor Chem* 142: 79-82.

Nabavi SF, Nabavi SM, Habtemariam S, Moghaddam AH, Sureda A, Mirzaei M. 2013. Neuroprotective effects of methyl-3-O-methyl gallate against sodium fluoride-induced oxidative stress in the brain of rats. *Cell Mol Neurobiol* 33: 261-267.

Narayanaswamy M, Piler MB. 2010. Effect of maternal exposure of fluoride on biometals and oxidative stress parameters in developing CNS of rat. *Biol Trace Elem Res* 133: 71-82.

Narbutt B, Romer TE, Grabski J, Szymik N. 1971. [Influence of natrium fluoride on the structure of the rat thyroid]. *Endokrynol Pol* 22: 445-451.

Narbutt B, Romer TE, Grabski J, Szymik N. 1971. [The influence of sodium fluoride on the morphology of the thyroid gland in rats]. *Endokrynol Pol* 22: 361-365.

Niu RY, Sun ZL, Cheng ZT, Liu HT, Chen HC, Wang JD. 2008. Effects of fluoride and lead on N-methyl-D-aspartate receptor 1 expression in the hippocampus of offspring rat pups. *Fluoride* 41: 101-110.

Niu R, Wang J, Sun Z, Xue X, Yan X, Zhang J. 2015. Transcriptional regulatory dynamics of the hypothalamic-pituitary-testicular axis in male mice exposed to fluoride. *Environ Toxicol Pharmacol* 40: 557-562.

Niu R, Zhang Y, Liu S, Liu F, Sun Z, Wang J. 2015. Proteome alterations in cortex of mice exposed to fluoride and lead. *Biol Trace Elem Res* 164: 99-105.

Ogilvie AL. 1952. Histological findings in the kidney, liver, pancreas, adrenal and thyroid gland of the rat following sodium fluoride administration. *J Dent Res* 31: 598-598.

Okayasu I, Tsuchida M, Yanagisawa F. 1985. Hyperplastic nodules of thyroid parafollicular cells (C cells) in rats induced by prolonged low dose ingestion of NaF. *Fluoride* 18: 111-117.

Pal S, Sarkar C. 2014. Protective effect of resveratrol on fluoride induced alteration in protein and nucleic acid metabolism, DNA damage and biogenic amines in rat brain. *Environ Toxicol Pharmacol* 38: 684-699.

Pan Y, Lu P, Yin L, Chen K, He Y. 2015. Effect of fluoride on the proteomic profile of the hippocampus in rats. *Z Naturforsch C* 70: 151-157.

Phillips PH, Lamb AR. 1934. Histology of certain organs and teeth in chronic toxicosis due to fluorin. *Arch Path* 17: 169-176.

Portela ML. 1972. [Biochemical effects in the prolonged ingestion of fluorides in rats]. *Arch Latinoam Nutr* 22: 291-308.

Prestes DS, Filappi A, Schossler DR, Duarte FA, Dressler VL, Flores EMM, Cecim M. 2009. Functional and histological evaluations of thyroid of sheep submitted to sodium fluoride administration. *Arq Bras Med Vet Zootec* 61: 293-298.

Puentes F, Cremer HD. 1966. Experiments on fluoride-iodine antagonism in the thyroid gland. *Adv Fluorine Res* 4: 213-220.

Qian W, Miao K, Li T, Zhang Z. 2013. Effect of selenium on fluoride-induced changes in synaptic plasticity in rat hippocampus. *Biol Trace Elem Res* 155: 253-260.

Qing-Feng S, Ying-Peng X, Tian-Tong X. 2019. Matrix metalloproteinase-9 and p53 involved in chronic fluorosis induced blood-brain barrier damage and neurocyte changes. *Arch Med Sci* 15(2): 457-466.

Qiu YH, Kong DM, Yang Q, Zhao N. 2010. [Influence of high-fluoride on thyroid function and brain damage in rats]. *Chin J Endemiol* 29: 146-149.

Raghavendra M, Ravindra RK, Raghuveer YP, Narasimha JK, Uma MRV, Navakishor P. 2016. Alleviatory effects of hydroalcoholic extract of cauliflower (brassica oleracea var. botrytis) on thyroid function in fluoride intoxicated rats. *Fluoride* 49: 84-90.

Rakhov GM. 1964. [Effect of calcium and fluorine in drinking water on the iodine metabolism and status of the thyroid gland in iodine insufficiency in food]. *Gig Sanit* 29: 12-17.

Ranpariya VL, Parmar SK, Sheth NR, Chandrashekhar VM. 2011. Neuroprotective activity of matricaria recutita against fluoride-induced stress in rats. *Pharm Biol* 49: 696-701.

Reddy KP, Sailaja G, Krishnaiah C. 2009. Protective effects of selenium on fluoride induced alterations in certain enzymes in brain of mice. *J Environ Biol* 30: 859-864.

Rogalska A, Kuter K, Zelazko A, Glogowska-Gruszka A, Swietochowska E, Nowak P. 2017. Fluoride alteration of [3H]glucose uptake in Wistar rat brain and peripheral tissues. *Neurotoxicol Res* 31: 436-443.

Saka O, Hallac P, Urgancioğlu I. 1965. The effect of fluoride on the thyroid of the rat. *New Istanbul Contrib Clin Sci* 8: 87-90.

Samanta A, Chanda S, Bandyopadhyay B, Das N. 2016. Establishment of drug delivery system nanocapsulated with an antioxidant (+)-catechin hydrate and sodium meta borate chelator against sodium fluoride induced oxidative stress in rats. *J Trace Elem Med Biol* 33: 54-67.

Sarkar C, Das N, Pal S, Dinda B. 2014. Oxidative stress induced alteration of protein and nucleic acid metabolism in fluoride-intoxicated rat brain: Protection by 3α-hydroxy olean-12-en-27-oic acid isolated from neanotis wightiana. *Int J Pharm Sci Res* 5: 3047-3066.

Sarkar C, Pal S. 2014. Ameliorative effect of resveratrol against fluoride-induced alteration of thyroid function in male Wistar rats. *Biol Trace Elem Res* 162: 278-287.

Sarkar C, Pal S, Das N, Dinda B. 2014. Ameliorative effects of oleanolic acid on fluoride induced metabolic and oxidative dysfunctions in rat brain: Experimental and biochemical studies. *Food Chem Toxicol* 66: 224-236.

Seffner W, Teubener W, Runde H, Wiedner H, Vogt J, Otto G, Zschau E, Geinitz D, Franke J. 1990. Boron as an antidote to fluorosis? II. Studies on various organs of pigs. *Fluoride* 23: 68-79.

Selim AOA, El-Haleem MR, Ibrahim IH. 2012. Effect of sodium fluoride on the thyroid gland of growing male albino rats: Histological and biochemical study. *Egypt J Histol* 35: 470-482.

Shao Q, Wang Yn, Guan Z. 2000. [Influence of free radical inducer on the level of oxidative stress in brain of rats with fluorosis]. *Chin J Prev Med* 34: 330-332.

Sharma C, Suhalka P, Sukhwal P, Jaiswal N, Bhatnagar M. 2014. Curcumin attenuates neurotoxicity induced by fluoride: An in vivo evidence. *Pharmacogn Mag* 10: 61-65.

Shashi A. 1992. Studies on alterations in brain lipid metabolism following experimental fluorosis. *Fluoride* 25: 77-84.

Shashi A. 1993. Nucleic acid levels in thyroid gland in acute and chronic fluoride intoxication. *Fluoride* 26: 191-196.

Shashi A, Singh JP, Thapar SP. 1994. Effect of long-term administration of fluoride on levels of protein, free amino acids and RNA in rabbit brain. *Fluoride* 27: 155-159.

Shashi A. 2003. Histopathological investigation of fluoride-induced neurotoxicity in rabbits. *Fluoride* 36: 95-105.

Shashi A, Neetika S, Bhardwaj M. 2009. Neuronal DNA damage and apoptosis in brain of rat exposed to fluoride. *Asian J Microbiol Biotechnol Environ Sci* 11: 629-632.

Shayiq RM, Raza H, Kidwai AM. 1986. Fluoride and lipid peroxidation a comparative study in different rat tissues. *Bull Environ Contam Toxicol* 37: 70-76.

Shen X, Zhang Z, Xu X. 2004. [Influence of combined iodine and fluoride on phospholipid and fatty acid composition in brain cells of rats]. *J Hyg Res* 33: 158-161.

Shen QF, Li HN, Xu TT, Xia YP. 2012. [Damage of blood brain barrier of spinal cord in rats with chronic fluorosis]. *Chin Med J* 92: 2357-2361.

Shen Q, Tian R, Li H, Xu T, Xia Y. 2014. [White matter injury of spinal cord in rats with chronic fluorosis and recovery after defluoriation]. *Chin Med J* 94: 1189-1192.

Shivarajashankara YM, Shivashankara AR, Bhat PG, Rao SH. 2001. Effect of fluoride intoxication on lipid peroxidation and antioxidant systems in rats. *Fluoride* 34: 108-113.

Shivarajashankara YM, Shivashankara AR, Bhat PG, Rao SH. 2002. Brain lipid peroxidation and antioxidant systems of young rats in chronic fluoride intoxication. *Fluoride* 35: 197-203.

Shivarajashankara YM, Shivashankara AR, Bhat PG, Rao SM, Rao SH. 2002. Histological changes in the brain of young fluoride-intoxicated rats. *Fluoride* 35: 12-21.

Siebenhuner L, Miloni E, Burgi H. 1984. [Effects of fluoride on thyroid hormone biosynthesis: Studies in a highly sensitive test system]. *Klin Wochenschr* 62: 859-861.

Singh R, Srivastava AK, Gangwar NK. 2017. Clinico-pathological studies on the co-exposure of cypermethrin and fluoride in experimental rats with ameliorative action of Vitamin E. *Vet Pract* 18(2): 207-210.

Soni KK, Shrivastava VK. 2007. Sodium fluoride induced histopathological changes in thyroid gland of male mus musculus. *Biochem Cell Arch* 7: 317-320.

Stee EW. 1968. *Effect of sodium fluoride and AMOX (NF3O) on growth and thyroid function in the rat*. No. AMRL-TR-67-189. Wright-Patterson Air Force Base, OH: pp. 67.

Štolc V, Podoba J. 1960. Effect of fluoride on the biogenesis of thyroid hormones. *Nature* 188: 855-856.

Sugivama Y. 1967. [The effect of sodium fluoride administration on the parathyroid glands]. *Hirosaki Med J* 19: 520-529.

Sun Y, Ke L, Zheng X, Li T, Ouyang W, Zhang Z. 2016. Effects of different levels of calcium intake on brain cell apoptosis in fluorosis rat offspring and its molecular mechanism. *Biol Trace Elem Res*: 1-12.

Takata H. 1958. The effect of fluorine upon the uptake of I131 by the thyroid glands. *Folia Pharmacol Jpn* 54: 230-236.

Teng Y, Zhang J, Zhang Z, Feng J. 2017. The effect of chronic fluorosis on calcium ions and CaMKIIalpha, and c-fos expression in the rat hippocampus. *Biol Trace Elem Res*: 295-302.

Trabelsi M, Guermazi F, Zeghal N. 2001. Effect of fluoride on thyroid function and cerebellar development in mice. *Fluoride* 34: 165-173.

Tsuchida M, Okayasu I, Kohyama Y, Kurihara H, Tanaka H, Yanagisawa F, Date C, Hayashi M, Mui K, Asada M. 1986. Effects of long term, low dose ingestion of fluoride on the thyroid gland in rats. *Stud Environ Sci* 27: 307-312.

Vani ML, Reddy KP. 2000. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. *Fluoride* 33: 17-26.

Wang Y, Guan Z, Xiao K. 1997. [Changes of coenzyme Q content in brain tissues of rats with fluorosis]. *Chin J Prev Med* 31: 330-333.

Wang JD, Ge YM, Ning HM, Wang SL. 2004. Effects of high fluoride and low iodine on oxidative stress and antioxidant defense of the brain in offspring rats. *Fluoride* 37: 264-270.

Wang JL. 2007. [Effect of fluoride on the intracellular Ca2+ in neurons of mice]. *Chin J Endemiol* 26: 505-507.

Wang J, Niu R, Sun Z, Lv L, Smith GW. 2008. Effects of protein and calcium supplementation on bone metabolism and thyroid function in protein and calcium deficient rabbits exposed to fluoride. *Fluoride* 41: 283-291.

Wang H, Yang Z, Zhou B, Gao H, Yan X, Wang J. 2009. Fluoride-induced thyroid dysfunction in rats: Roles of dietary protein and calcium level. *Toxicol Ind Health* 25: 49-57.

Wang Y, Dong Y, Wei N, Guan Z. 2015. [Influence of chronic fluorosis on expression of quinone oxidoreductase-1 and heme oxygenase-1 in rat brains]. *Chin J Endemiol* 34: 250-253.

Wang C, Liang C, Ma J, Manthari RK, Niu R, Wang J, Wang J, Zhang J. 2018. Co-exposure to fluoride and sulfur dioxide on histological alteration and DNA damage in rat brain. *J Biochem Mol Toxicol* 32.

Wedzisz A, Cieciura J. 1988. Effect of small sodium fluoride feed supplements on the serum thyroid hormone content of rats. *Bromatol Chem Toksykol* 21: 174-175.

Wei N, Dong YT, Deng J, Wang Y, Qi XL, Yu WF, Xiao Y, Zhou JJ, Guan ZZ. 2018. Changed expressions of N-methyl-d-aspartate receptors in the brains of rats and primary neurons exposed to high level of fluoride. *J Trace Elem Med Biol* 45: 31-40.

Yan N, Liu Y, Liu S, Cao S, Wang F, Wang Z, Xi S. 2016. Fluoride-induced neuron apoptosis and expressions of inflammatory factors by activating microglia in rat brain. *Mol Neurobiol* 53: 4449-4460.

Yang H, Xing R, Liu S, Yu H, Li P. 2016. Gamma-Aminobutyric acid ameliorates fluoride-induced hypothyroidism in male Kunming mice. *Life Sci* 146: 1-7.

Yang M, Ren Z, Zhou B, Guan Z, Yu W. 2017. [Expression of endonuclease G in the brain tissue of rats with chronic fluorosis]. *Chin J Endemiol* 36: 327-332.

Yang H, Xing R, Liu S, Yu H, Li P. 2019. Analysis of the protective effects of gamma-aminobutyric acid during fluoride-induced hypothyroidism in male Kunming mice. *Pharm Biol* 57(1): 29-37.

Yuan SD, Xie QW, Lu FY. 1993. Changes of serotonin content and turnover rate in hypothalamus of female rat during fluorosis. *Fluoride* 26: 57-60.

Zhai JX, Guo ZY, Hu CL, Wang QN, Zhu QX. 2003. [Studies on fluoride concentration and cholinesterase activity in rat hippocampus]. *Chin J Ind Hyg Occup Dis* 21: 102-104.

Zhan CW, Huo DJ. 1988. Ultrastructural findings in liver, kidneys, thyroid-gland and cardiac-muscle of rabbits following sodium-fluoride administration. *Fluoride* 21: 32-38.

Zhan XA, Xu ZR, Li JX, Wang M. 2005. Effects of fluorosis on lipid peroxidation and antioxidant systems in young pigs. *Fluoride* 38: 157-161.

Zhan XA, Li JX, Wang M, Xu ZR. 2006. Effects of fluoride on growth and thyroid function in young pigs. *Fluoride* 39: 95-100.

Zhang WD, Zhang Y, Liu GY, Jiang P, Chai CY. 2008. [Effects of fluoride on ultrastructure of thyroids in rats]. *Chin J Endemiol* 27: 622-624.

Zhang KL, Lou DD, Liu YF, Qin SL, Guan ZZ. 2012. [Changes of P-glycoprotein and nuclear factor κB in the cerebral cortex of rat with chronic fluorosis]. *Chin J Endemiol* 31: 613-616.

Zhang KL, Lou DD, Guan ZZ. 2013. [Expression of receptor for advanced glycation endproducts and nuclear factor κB in brain hippocampus of rat with chronic fluorosis]. *Chin J Endemiol* 32: 625-628.

Zhang ZG, Wang XY, Nian WW, Liao QX, Zhang R, Ouyang W. 2017. Effects of calcium on drinking fluorosis-induced hippocampal syntaptic plasticity impairment in the offspring of rats. *Transl Neurosci* 8: 191-200.

Zhao WY, et al. 1988. [A preliminary stufy of the interaction of iodine and fluoride in experimental iodine goiter and fluorosis]. *Chin J Prev Med* 22: 146-148.

Zhao XL, Gao WH, Zhao ZL. 1994. [Effects of sodium fluoride on the activity of Ca2+Mg(2+)-ATPase in synaptic membrane in rat brain]. *Chin J Prev Med* 28: 264-266.

Zhao W, Zhu H, Yu Z, Aoki K, Misumi J, Zhang X. 1998. Long-term effects of various iodine and fluorine doses on the thyroid and fluorosis in mice. *Endocr Regul* 32: 63-70.

Zhavoronkov AA, Polyakova GA. 1973. Morphological and functional state of the hypothalamo-hypophyseal neurosecretory system in experimental fluorosis. *Bull Exp Biol Med* 75: 194-196.

Zhou B, Luo G, Wang C, Niu R, Wang J. 2014. Effects of fluoride on expression of cytokines in the hippocampus of adult rats. *Fluoride* 47: 191-198.

In Vitro Experimental Studies

As described in **Figure 4**, 60 in vitro experimental studies were included; however, data extraction was not conducted on in vitro studies. Therefore, in vitro experimental studies are not available in HAWC with the exception of in vitro studies that also reported in vivo non-human animal data that meet the relevant criteria for being made available in HAWC. The following lists of references are organized as studies that are available in HAWC (n = 6) followed by studies that are not available in HAWC (n = 54).

Studies Available in HAWC

Chen J, Niu Q, Xia T, Zhou G, Li P, Zhao Q, Xu C, Dong L, Zhang S, Wang A. 2018. ERK1/2-mediated disruption of BDNF-TrkB signaling causes synaptic impairment contributing to fluoride-induced developmental neurotoxicity. *Toxicology* 410: 222-230.

Niu Q, Chen J, Xia T, Li P, Zhou G, Xu C, Zhao Q, Dong L, Zhang S, Wang A. 2018. Excessive ER stress and the resulting autophagic flux dysfunction contribute to fluoride-induced neurotoxicity. *Environ Pollut* 233: 889-899.

Shan KR, Qi XL, Long YG, Nordberg A, Guan ZZ. 2004. Decreased nicotinic receptors in PC12 cells and rat brains influenced by fluoride toxicity: A mechanism relating to a damage at the level in post-transcription of the receptor genes. *Toxicology* 200: 169-177.

Zhang KL, Lou DD, Guan ZZ. 2015. Activation of the AGE/RAGE system in the brains of rats and in SH-SY5Y cells exposed to high level of fluoride might connect to oxidative stress. *Neurotoxicol Teratol* 48: 49-55.

Zhao XL, Wu JH. 1998. Actions of sodium fluoride on acetylcholinesterase activities in rats. *Biomed Environ Sci* 11: 1-6.

Zhao Q, Niu Q, Chen J, Xia T, Zhou G, Li P, Dong L, Xu C, Tian Z, Luo C, Liu L, Zhang S, Wang A. 2019. Roles of mitochondrial fission inhibition in developmental fluoride neurotoxicity: Mechanisms of action in vitro and associations with cognition in rats and children. *Arch Toxicol* 93(3): 709-726.

Studies Not Available in HAWC

Ardelean I, Racoveanu N, Manescu S, Lupulescu A, Diaconescu M, Ghelerter L. 1964. Experimental investigations concerning the effect of fluorine on the thyroid gland. *Rum Med Rev* 8: 17-20.

Chen J, Chen X, Yang K. 2000. [Effects of selenium and zinc on the DNA damage caused by fluoride in pallium neural cells of rats]. *J Hyg Res* 29: 216-217.

Chen L, Ning H, Yin Z, Song X, Feng Y, Qin H, Li Y, Wang J, Ge Y, Wang W. 2017. The effects of fluoride on neuronal function occurs via cytoskeleton damage and decreased signal transmission. *Chemosphere* 185: 589-594.

Chen R, Zhao LD, Liu H, Li HH, Ren C, Zhang P, Guo KT, Zhang HX, Geng DQ, Zhang CY. 2017. Fluoride induces neuroinflammation and alters Wnt signaling pathway in BV2 microglial cells. *Inflammation* 40: 1123-1130.

Cheng TJ, Chen TM, Chen CH, Lai YK. 1998. Induction of stress response and differential expression of 70 kDa stress proteins by sodium fluoride in HeLa and rat brain tumor 9L cells. *J Cell Biochem* 69: 221-231.

Deng MF, Zhu D, Liu YP, He WW, Gui CZ, Guan ZZ. 2018. Attenuation by 7-nitroindazole of fluoride-induced toxicity in SH-SY5Y cells exposed to high fluoride: Effects on nitric oxide, nitric oxide synthetase activity, nNOS, and apoptosis. *Fluoride* 51(4): 328-339.

Flores-Mendez M, Ramirez D, Alamillo N, Hernandez-Kelly LC, Del Razo LM, Ortega A. 2014. Fluoride exposure regulates the elongation phase of protein synthesis in cultured Bergmann glia cells. *Toxicol Lett* 229: 126-133.

Gao Q, Liu YH, Guan ZZ. 2008. Oxidative stress might be a mechanism connected with the decreased alpha 7 nicotinic receptor influenced by high-concentration of fluoride in SH-SY5Y neuroblastoma cells. *Toxicol In Vitro* 22: 837-843.

Goschorska M, Gutowska I, Baranowska-Bosiacka I, Piotrowska K, Metryka E, Safranow K, Chlubek D. 2018. Influence of acetylcholinesterase inhibitors used in Alzheimer's Disease treatment on the activity of antioxidant enzymes and the concentration of glutathione in THP-1 macrophages under fluoride-induced oxidative stress. *Int J Environ Res Pub Health* 16(1).

Guan ZZ, Shan KR, Xiu J, Long YG. 2005. [Fluorosis on expression of nicotinic acetylcholine receptors in protein and gene levels in human SH-SY5Y neuroblastoma cells]. *Chin J Prev Med* 39: 26-29.

Guan ZZ, Shan KR, Wang YN, Dallner G. 2006. [Changes of lipids and nicotinic receptors in rat brains and pheochromocytoma with fluorosis]. *Chin J Endemiol* 25: 121-124.

Haojun Z, Yaoling W, Ke Z, Jin L, Junling W. 2012. Effects of NaF on the expression of intracellular Ca2+ fluxes and apoptosis and the antagonism of taurine in murine neuron. *Toxicol Mech Methods* 22: 305-308.

Hong-Liang L, Qiang Z, Yu-Shan C, Lei Z, Gang F, Chang-Chun H, Liang Z, Aiguo W. 2014. Fluoride-induced thyroid cell apoptosis. *Fluoride* 47: 161-169.

Inkielewicz-Stepniak I, Radomski MW, Wozniak M. 2012. Fisetin prevents fluoride- and dexamethasone-induced oxidative damage in osteoblast and hippocampal cells. *Food Chem Toxicol* 50: 583-589.

Jin TX, Guan ZZ, Zhang H. 2011. [The effect of fluoride on α subunit of calcium/calmodulin-dependent protein kinase-II mRNA and protein expression in central nervous system]. *Chin J Endemiol* 30: 247-250.

Kariya T, Kotani M, Field JB. 1974. Effects of sodium fluoride and other metabolic inhibitors on basal and TSH stimulated cyclic AMP and thyroid metabolism. *Metab Clin Exper* 23: 967-973.

Ke L, Zheng X, Sun Y, Ouyang W, Zhang Z. 2016. Effects of sodium fluoride on lipid peroxidation and PARP, XBP-1 expression in PC12 cell. *Biol Trace Elem Res* 173: 161-167.

Lee J, Han YE, Favorov O, Tommerdahl M, Whitsel B, Lee CJ. 2016. Fluoride induces a volume reduction in CA1 hippocampal slices via MAP kinase pathway through volume regulated anion channels. *Exp Neurobiol* 25: 72-78.

Levesque L, Mizzen CA, McLachlan DR, Fraser PE. 2000. Ligand specific effects on aluminum incorporation and toxicity in neurons and astrocytes. *Brain Res* 877: 191-202.

Li H, Gao MT, Xu KY, Wang CY. 2007. Effect of sodium fluoride on the primary porcine thyroid cells and thyroid peroxidase activity. *J Clin Rehabil Tissue Eng Res* 11: 7425-7428.

Li H, Gao MT, Xu KY, Cui MY, Dai X. 2008. [Effect of fluoride on thyroid functioning in primary porcine thyrocyte]. *Chin J Endemiol* 27: 38-40.

Li H, Huang H, Xu Y, Gao Y, Liu Z. 2010. [Toxic effects of fluoride on rat cerebral cortex astrocytes in vitro]. *J Hyg Res* 39: 86-88.

Liu YJ, Guan ZZ, Gao Q, Pei JJ. 2011. Increased level of apoptosis in rat brains and SH-SY5Y cells exposed to excessive fluoride-A mechanism connected with activating JNK phosphorylation. *Toxicol Lett* 204: 183-189.

Liu H, Zeng Q, Cui Y, Yu L, Zhao L, Hou C, Zhang S, Zhang L, Fu G, Liu Y, Jiang C, Chen X, Wang A. 2014. The effects and underlying mechanism of excessive iodide on excessive fluoride-induced thyroid cytotoxicity. *Environ Toxicol Pharmacol* 38: 332-340.

Liu HL, Zeng Q, Cui YS, Zhao L, Zhang L, Fu G, Hou CC, Zhang S, Yu LY, Jiang CY, Wang ZL, Chen XM, Wang AG. 2014. The role of the IRE1 pathway in excessive iodide- and/or fluoride-induced apoptosis in Nthy-ori 3-1 cells in vitro. *Toxicol Lett* 224: 341-348.

Liu Y, Gao Q, Tang Z, Zhang X, Guan Z. 2015. [The expression and correlation between neural nicotinic acetylcholine receptor subunit α3 and mitogen-activated protein kinase cell signaling transduction pathway in human neuroblastoma cell line SH-SY5Y overexposed to fluoride]. *Chin J Endemiol* 34: 553-558.

Madaoui S, Rappaport L, Nunez J. 1974. Prostaglandins and in vitro TSH-dependent iodide binding by rat thyroid glands. *Biochimie* 56: 109-113.

Nakagawa-Yagi Y, Saito Y, Kitoh N, Ogane N, Fujisawa E, Nakamura H. 1993. Fluoride causes suppression of neurite outgrowth in human neuroblastoma via an influx of extracellular calcium. *Biochem Biophys Res Commun* 191: 727-736.

Ong J, Kerr DIB. 1995. Interactions of N-ethylmaleimide and aluminium fluoride with GABA(B) receptor function in rat neocortical slices. *Eur J Pharmacol* 287: 197-200.

Pastan I, Macchia V, Katzen R. 1968. Effect of fluoride on the metabolic activity of thyroid slices. *Endocrinology* 83: 157-160.

Rubakhova VM. 1977. [Effect of serotonin and sodium fluoride on visceral nerve conductors]. *Vyestsi Akademii Navuk BSSR Syeryya Biyalahichnykh Navuk* 1: 117-119.

Shayiq RM, Raza H, Kidwai AM. 1986. Fluoride and lipid peroxidation a comparative study in different rat tissues. *Bull Environ Contam Toxicol* 37: 70-76.

Shuhua X, Ziyou L, Ling Y, Fei W, Sun G. 2012. A role of fluoride on free radical generation and oxidative stress in BV-2 microglia cells. *Mediators Inflamm* 2012: 1-8.

Singh P, Das TK. 2019. Ultrastructural localization of 4-hydroxynonenal adducts in fluoride-exposed cells: Protective role of dietary antioxidants. *Fluoride* 52(1): 49-58.

Taytor P. 1972. Compirison of the effects of various agents on thyroidal adenyl cyclase activity with their effects on thyroid hormone release. *J Endocrinol* 54: 137-145.

Tu W, Zhang Q, Liu Y, Han LY, Wang Q, Chen PP, Zhang S, Wang AG, Zhou X. 2018. Fluoride induces apoptosis via inhibiting SIRT1 activity to activate mitochondrial p53 pathway in human neuroblastoma SH-SY5Y cells. *Toxicol Appl Pharmacol* 347: 60-69.

van der Voet GB, Schijns O, de Wolff FA. 1999. Fluoride enhances the effect of aluminium chloride on interconnections between aggregates of hippocampal neurons. *Arch Physiol Biochem* 107: 15-21.

Wang JL. 2007. [Effect of fluoride on the intracellular Ca2+ in neurons of mice]. *Chin J Endemiol* 26: 505-507.

Wang J, Gao Y, Cheng X, Yang J, Zhao Y, Xu H, Zhu Y, Yan Z, Manthari RK, Mehdi OM, Wang J. 2019. GSTO1 acts as a mediator in sodium fluoride-induced alterations of learning and memory related factors expressions in the hippocampus cell line. *Chemosphere* 226: 201-209.

Wei N, Dong YT, Deng J, Wang Y, Qi XL, Yu WF, Xiao Y, Zhou JJ, Guan ZZ. 2018. Changed expressions of N-methyl-d-aspartate receptors in the brains of rats and primary neurons exposed to high level of fluoride. *J Trace Elem Med Biol* 45: 31-40.

Willems CB-V, Sande J, Dumont JE. 1972. Inhibition of thyroid secretion by sodium fluoride in vitro. *Biochim Biophys Acta* 264: 197-204.

Woodward JJ, Harms J. 1992. Potentiation of N-methyl-D-aspartate-stimulated dopamine release from rat brain slices by aluminum fluoride and carbachol. *J Neurochem* 58: 1547-1554.

Wu J, Cheng M, Liu Q, Yang J, Wu S, Lu X, Jin C, Ma H, Cai Y. 2015. Protective role of tert-butylhydroquinone against sodium fluoride-induced oxidative stress and apoptosis in PC12 cells. *Cell Mol Neurobiol* 35: 1017-1025.

Xia T, Zhang M, He WH, He P, Wang AG. 2007. [Effects of fluoride on neural cell adhesion molecules mRNA and protein expression levels in primary rat hippocampal neurons]. *Chin J Prev Med* 41: 475-478.

Xu B, Xu Z, Xia T, He P, Gao P, He W, Zhang M, Guo L, Niu Q, Wang A. 2011. Effects of the Fas/Fas-L pathway on fluoride-induced apoptosis in SH-SY5Y cells. *Environ Toxicol* 26: 86-92.

Xu Z, Xu B, Xia T, He W, Gao P, Guo L, Wang Z, Niu Q, Wang A. 2013. Relationship between intracellular Ca2+ and ROS during fluoride-induced injury in SH-SY5Y cells. *Environ Toxicol* 28: 307-312.

Yamashita K, Field JB. 1972. Elevation of cyclic guanosine 3,5; monophosphate levels in dog thyroid slices caused by acetylcholine and sodium fluoride. *J Biol Chem* 247: 7062-7066.

Yan L, Liu S, Wang C, Wang F, Song Y, Yan N, Xi S, Liu Z, Sun G. 2013. JNK and NADPH oxidase involved in fluoride-induced oxidative stress in BV-2 microglia cells. *Mediators Inflamm* 2013: 895-975.

Zhang M, Wang A, He W, He P, Xu B, Xia T, Chen X, Yang K. 2007. Effects of fluoride on the expression of NCAM, oxidative stress, and apoptosis in primary cultured hippocampal neurons. *Toxicology* 236: 208-216.

Zhang M, Wang A, Xia T, He P. 2008. Effects of fluoride on DNA damage, S-phase cell-cycle arrest and the expression of NF-kappaB in primary cultured rat hippocampal neurons. *Toxicol Lett* 179: 1-5.

Zhang S, Zheng X, Sun Y, Wang Y, Zhang Z. 2015. Alterations in oxidative stress and apoptosis in cultured PC12 cells exposed to fluoride. *Fluoride* 48: 213-222.

Zhang CY, Chen R, Wang F, Ren C, Zhang P, Li Q, Li HH, Guo KT, Geng DQ, Liu CF. 2016. EGb-761 attenuates the anti-proliferative activity of fluoride via DDK1 in PC-12 cells. *Neurochem Res* 42(2): 606-614.

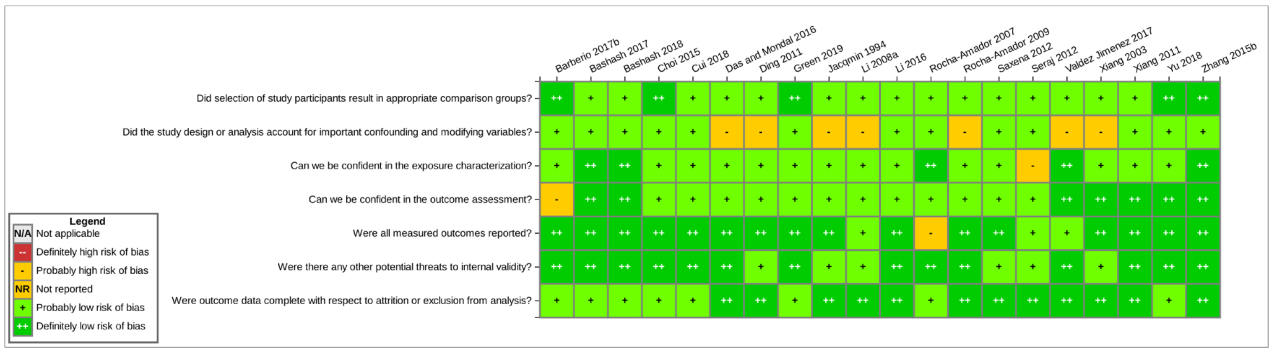
Zhao XL, Gao WH, Zhao ZL. 1994. [Effects of sodium fluoride on the activity of Ca2+Mg(2+)-ATPase in synaptic membrane in rat brain]. *Chin J Prev Med* 28: 264-266.

Zhao L, Xiao Y, Deng CM, Tan LC, Guan ZZ. 2016. Protective effect of lovastatin on neurotoxicity of excessive fluoride in primary hippocampal neurons. *Fluoride* 49: 36-46.

Appendix 3. Risk-of-bias Figures

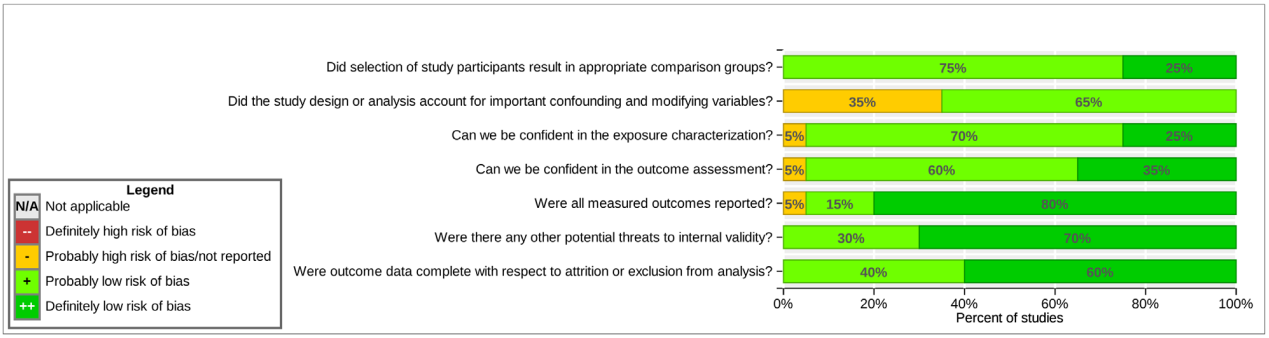
Studies in Humans

Figure A3-1. Risk-of-bias Heatmap for Lower Risk-of-bias Human Neurodevelopmental or Cognitive Studies Following Fluoride Exposure



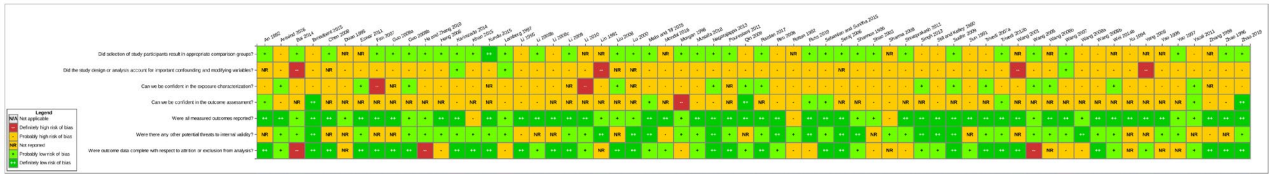
Interactive figure and additional study details in HAWC here.

Figure A3-2. Risk-of-bias Bar Chart for Lower Risk-of-bias Human Neurodevelopmental or Cognitive Studies Following Fluoride Exposure



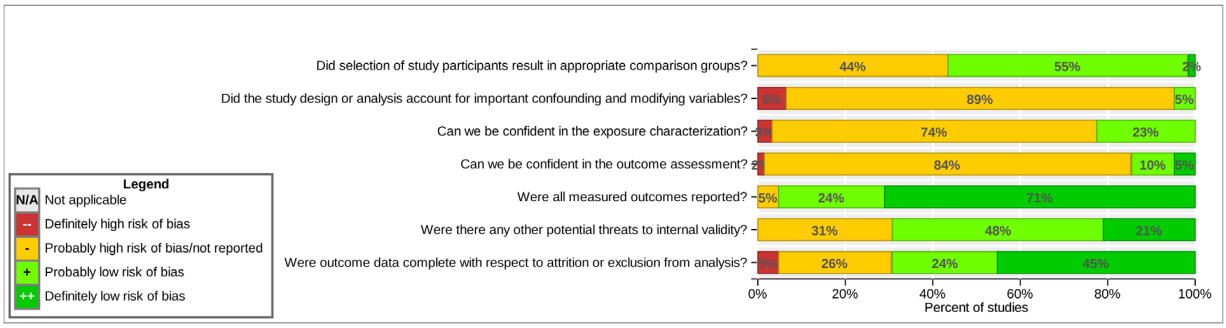
Interactive figure and additional study details in HAWC here.

Figure A3-3. Risk-of-bias Heatmap for Higher Risk-of-bias Human Neurodevelopmental or Cognitive Studies Following Fluoride Exposure



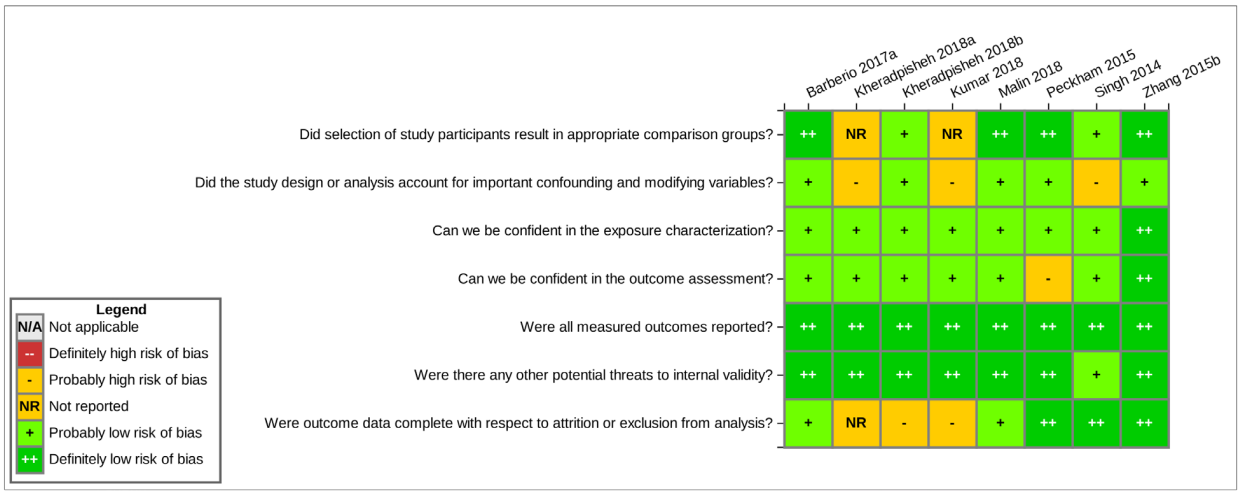
Interactive figure and additional study details in HAWC here.

Figure A3-4. Risk-of-bias Bar Chart for Higher Risk-of-bias Human Neurodevelopmental or Cognitive Studies Following Fluoride Exposure



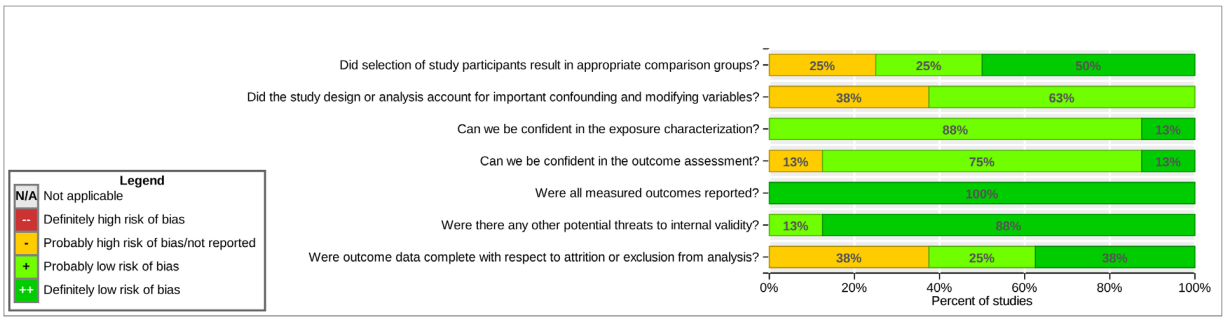
Interactive figure and additional study details in HAWC here.

Figure A3-5. Risk-of-bias Heatmap for Lower Risk-of-bias Human Mechanistic Studies Following Fluoride Exposure



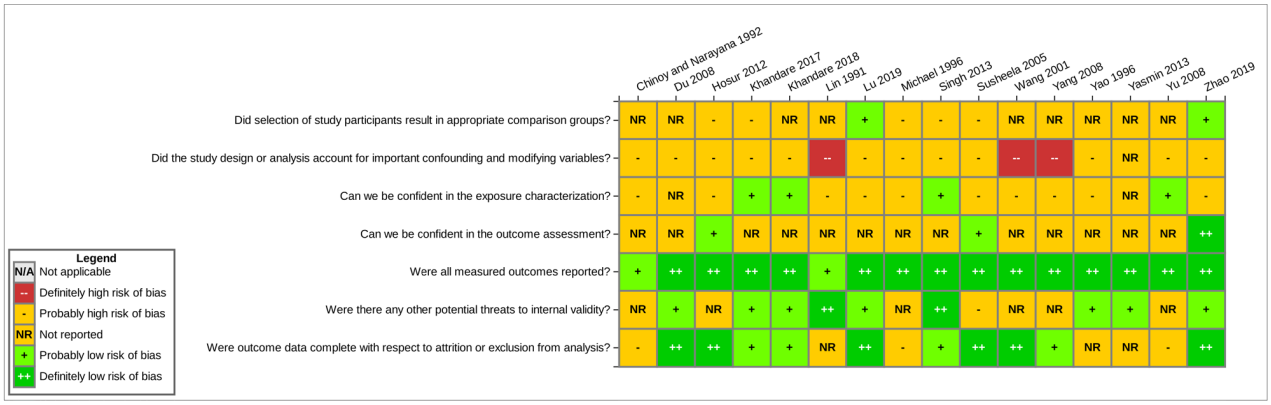
Interactive figure and additional study details in HAWC here.

Figure A3-6. Risk-of-bias Bar Chart for Lower Risk-of-bias Human Mechanistic Studies Following Fluoride Exposure



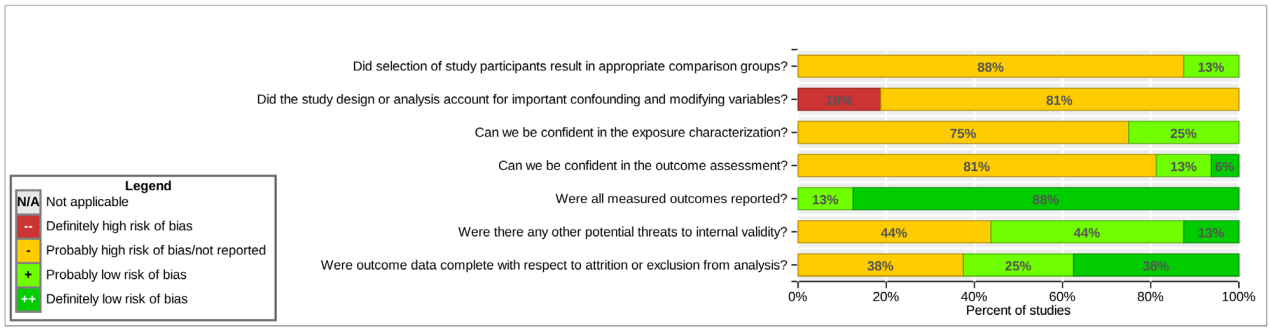
Interactive figure and additional study details in HAWC here.

Figure A3-7. Risk-of-bias Heatmap for Higher Risk-of-bias Human Mechanistic Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

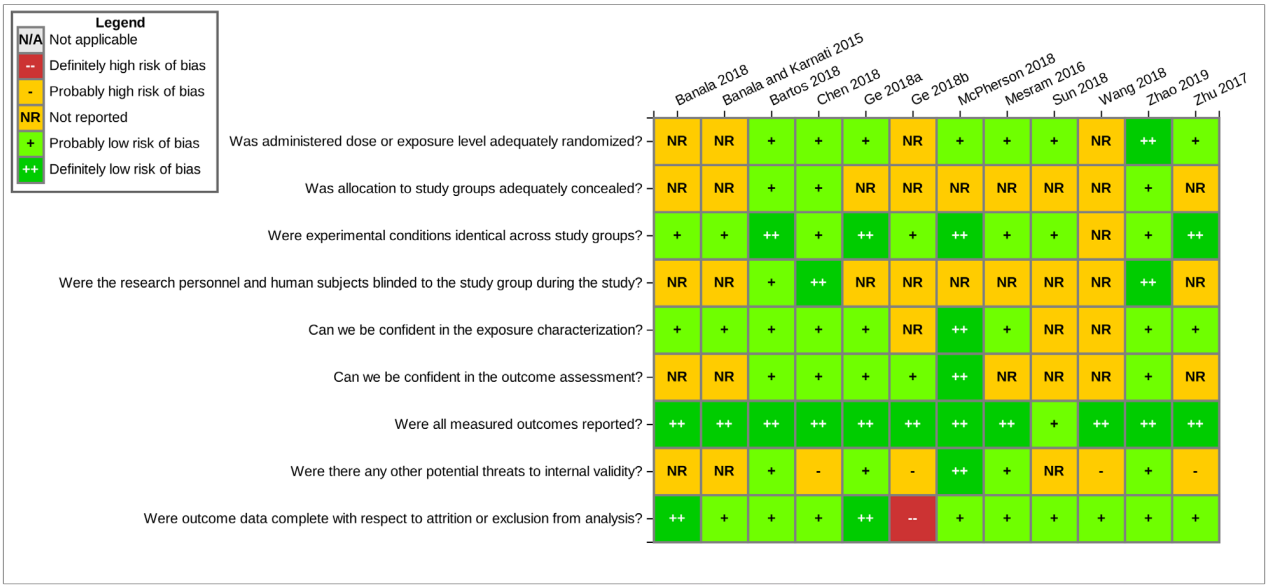
Figure A3-8. Risk-of-bias Bar Chart for Higher Risk-of-bias Human Mechanistic Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

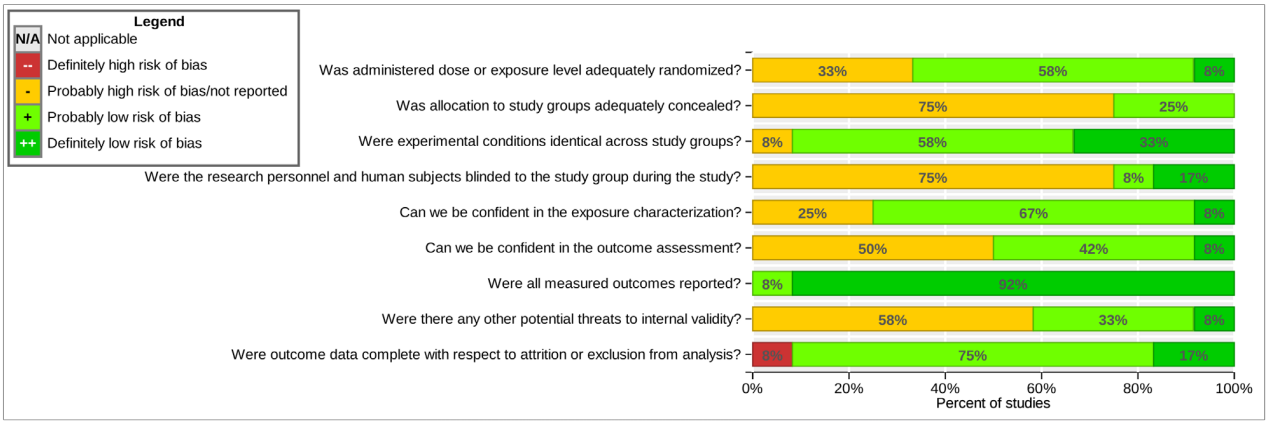
Studies in Non-human Animals

Figure A3-9. Risk-of-bias Heatmap for New Developmental Animal Learning and Memory Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

Figure A3-10. Risk-of-bias Bar Chart for New Developmental Animal Learning and Memory Studies Following Fluoride Exposure

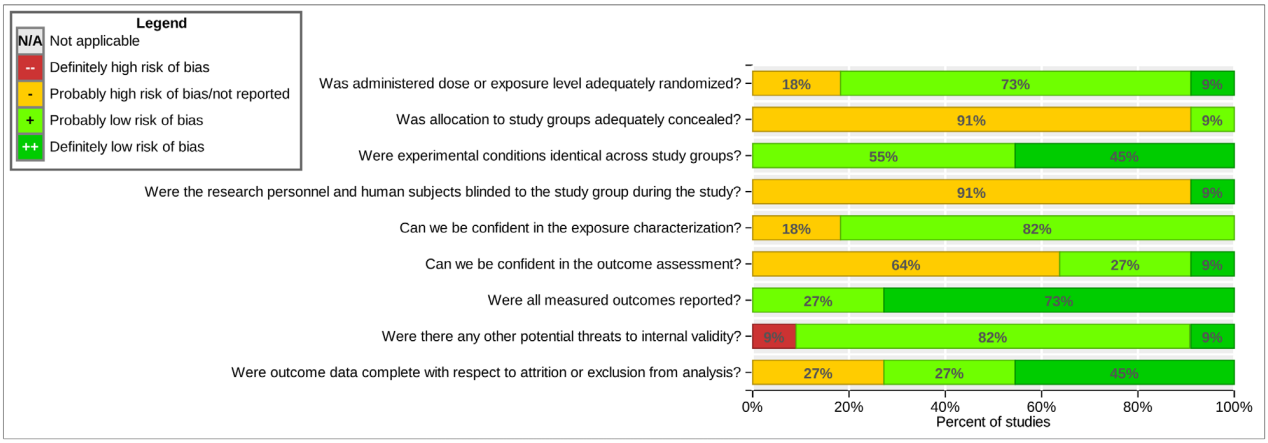


Interactive figure and additional study details in HAWC here.

Figure A3-11. Risk-of-bias Heatmap for New Adult Animal Learning and Memory Studies Following Fluoride Exposure

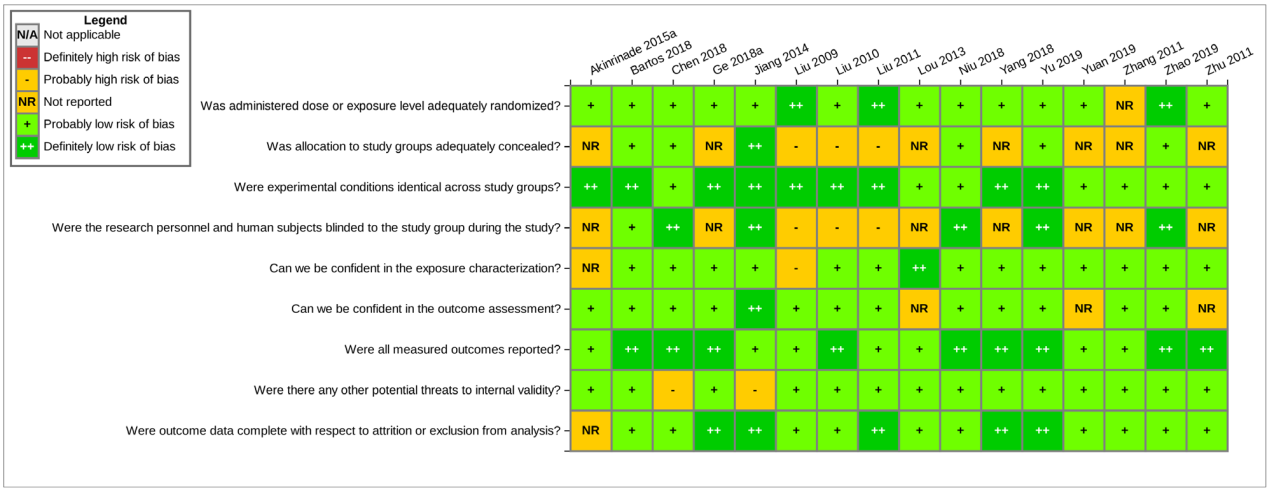
Interactive figure and additional study details in HAWC here.

Figure A3-12. Risk-of-bias Bar Chart for New Adult Animal Learning and Memory Studies Following Fluoride Exposure



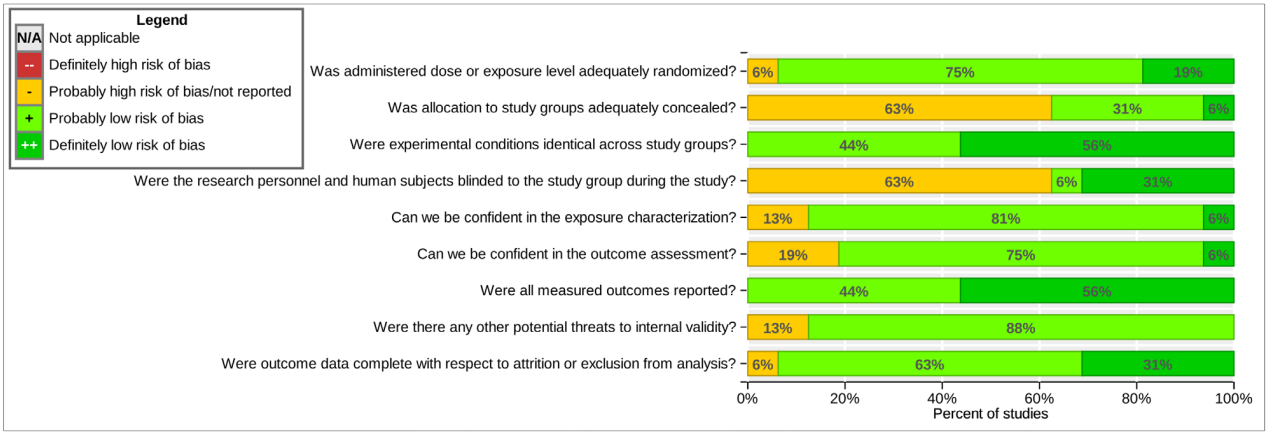
Interactive figure and additional study details in HAWC here.

Figure A3-13. Risk-of-bias Heatmap for Lower Risk-of-bias Animal Biochemical Studies Following Fluoride Exposure



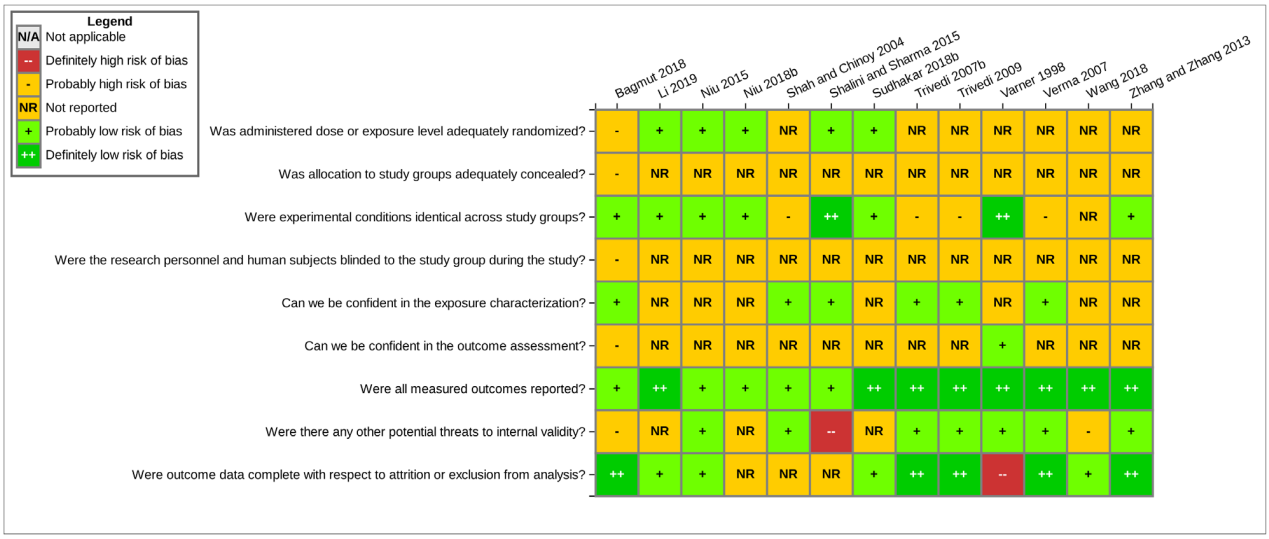
Interactive figure and additional study details in HAWC here.

Figure A3-14. Risk-of-bias Bar Chart for Lower Risk-of-bias Animal Biochemical Studies Following Fluoride Exposure



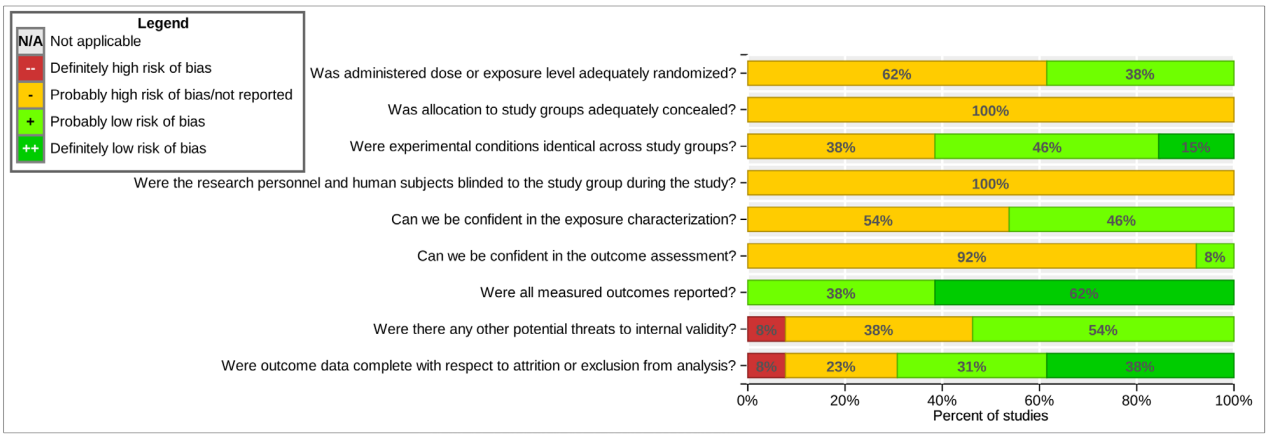
Interactive figure and additional study details in HAWC here.

Figure A3-15. Risk-of-bias Heatmap for Higher Risk-of-bias Animal Biochemical Studies Following Fluoride Exposure



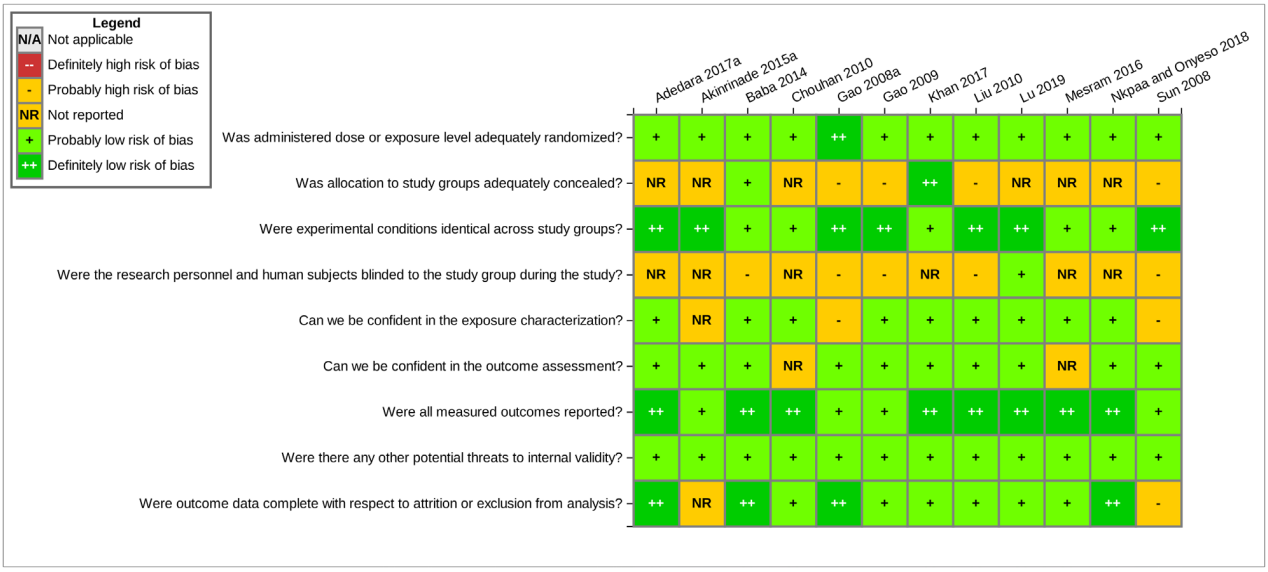
Interactive figure and additional study details in HAWC here.

Figure A3-16. Risk-of-bias Bar Chart for Higher Risk-of-bias Animal Biochemical Studies Following Fluoride Exposure



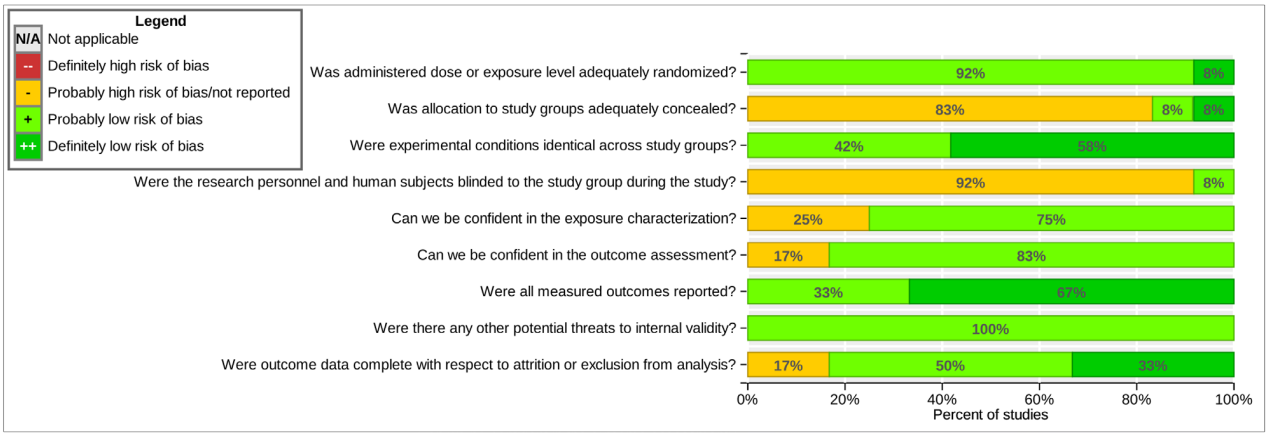
Interactive figure and additional study details in HAWC here.

Figure A3-17. Risk-of-bias Heatmap for Lower Risk-of-bias Animal Neurotransmission Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

Figure A3-18. Risk-of-bias Bar Chart for Lower Risk-of-bias Animal Neurotransmission Studies Following Fluoride Exposure

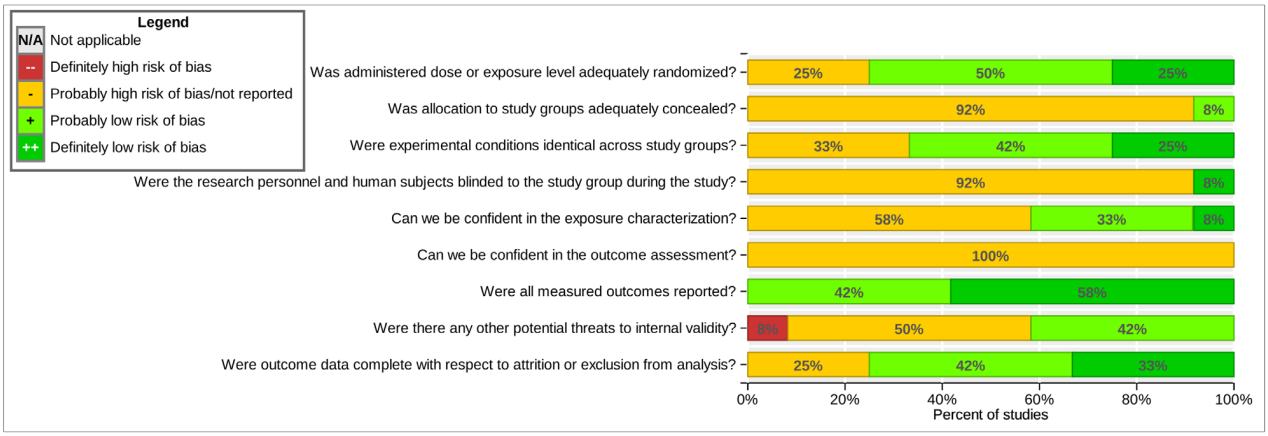


Interactive figure and additional study details in HAWC here.

Figure A3-19. Risk-of-bias Heatmap for Higher Risk-of-bias Animal Neurotransmission Studies Following Fluoride Exposure

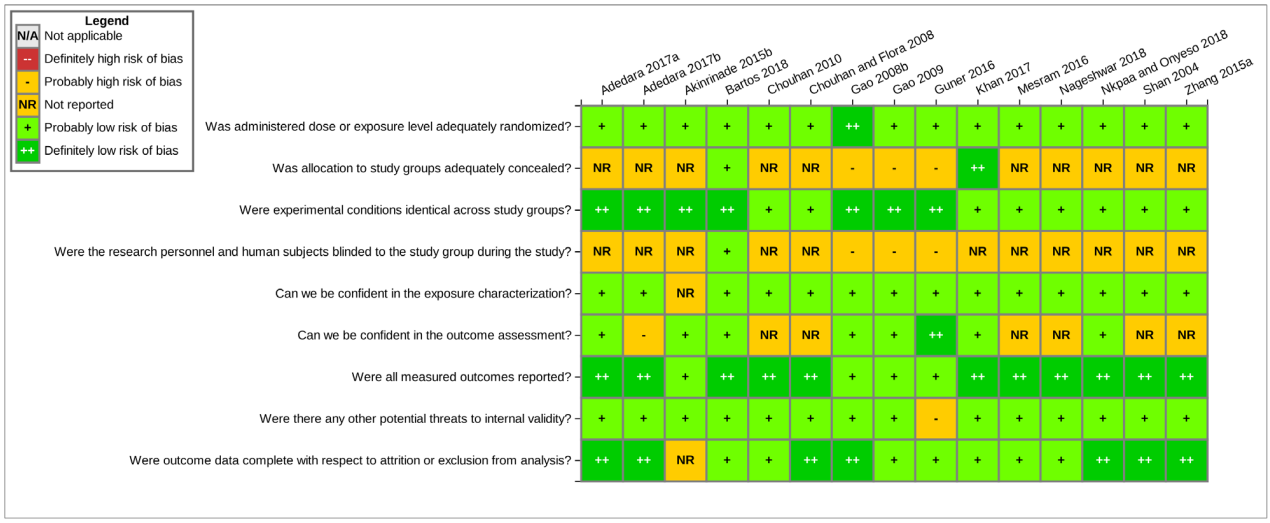
Interactive figure and additional study details in HAWC here.

Figure A3-20. Risk-of-bias Bar Chart for Higher Risk-of-bias Animal Neurotransmission Studies Following Fluoride Exposure



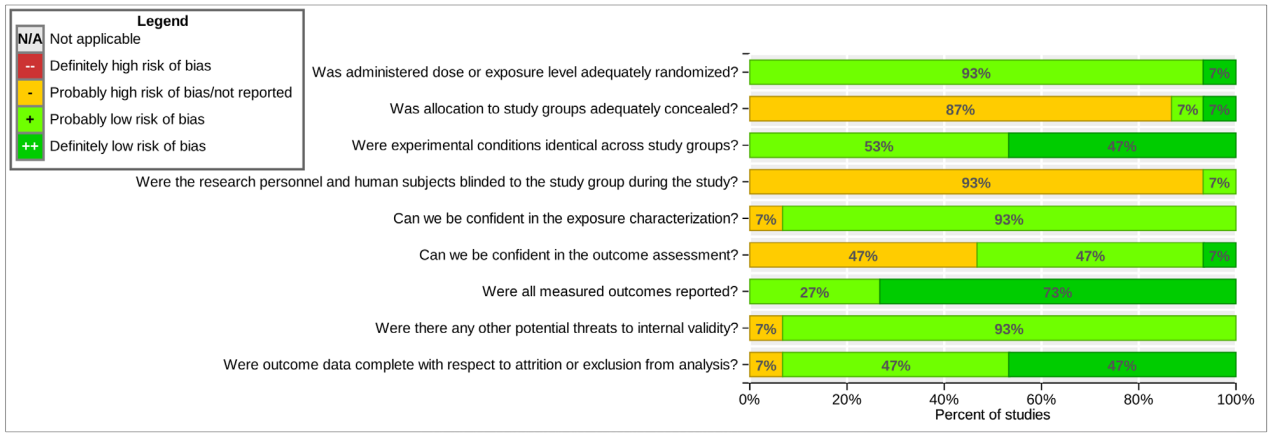
Interactive figure and additional study details in HAWC here.

Figure A3-21. Risk-of-bias Heatmap for Lower Risk-of-bias Animal Oxidative Stress Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

Figure A3-22. Risk-of-bias Bar Chart for Lower Risk-of-bias Animal Oxidative Stress Studies Following Fluoride Exposure

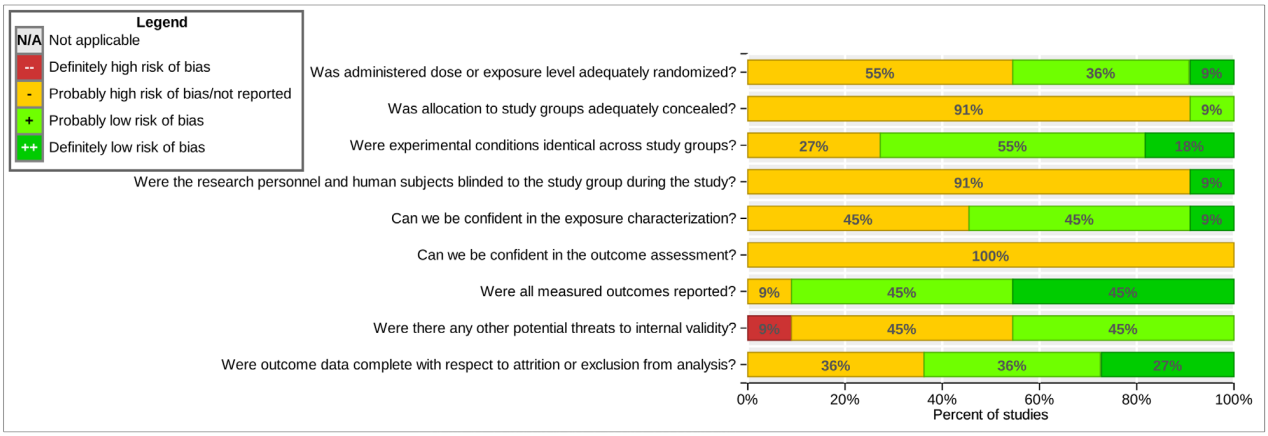


Interactive figure and additional study details in HAWC here.

Figure A3-23. Risk-of-bias Heatmap for Higher Risk-of-bias Animal Oxidative Stress Studies Following Fluoride Exposure

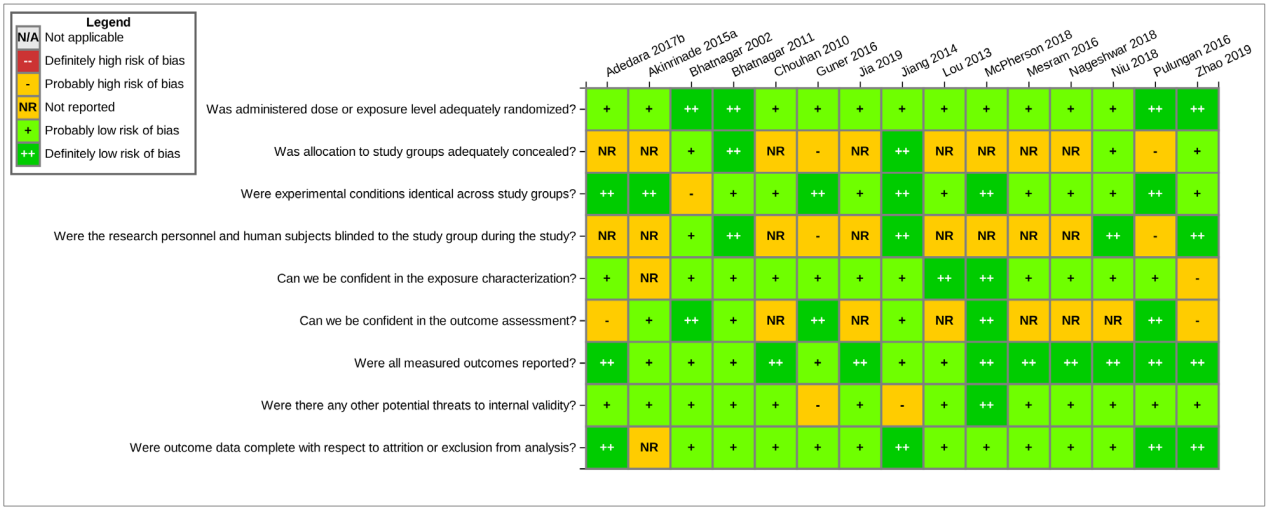
Interactive figure and additional study details in HAWC here.

Figure A3-24. Risk-of-bias Bar Chart for Higher Risk-of-bias Animal Oxidative Stress Studies Following Fluoride Exposure



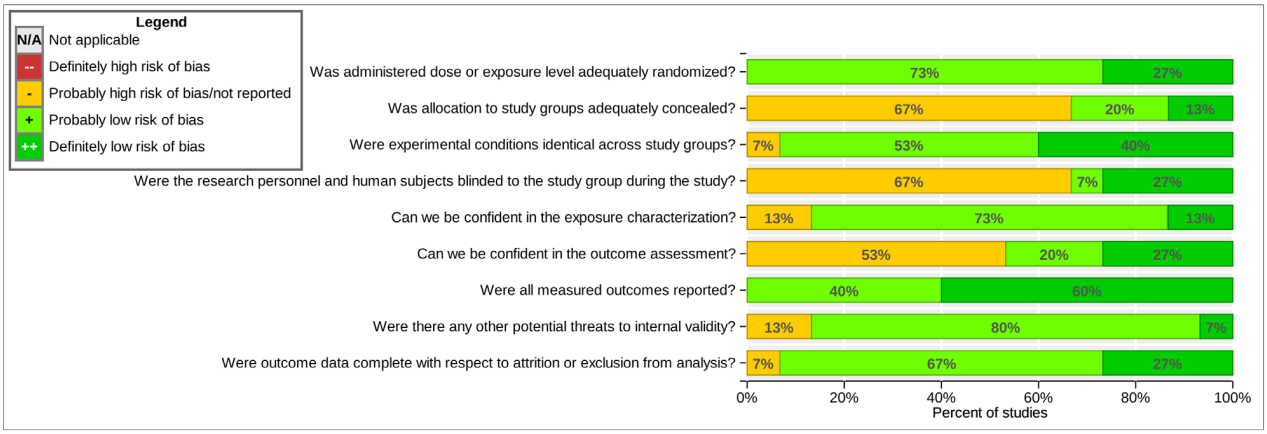
Interactive figure and additional study details in HAWC here.

Figure A3-25. Risk-of-bias Heatmap for Lower Risk-of-bias Animal Histopathology Studies Following Fluoride Exposure



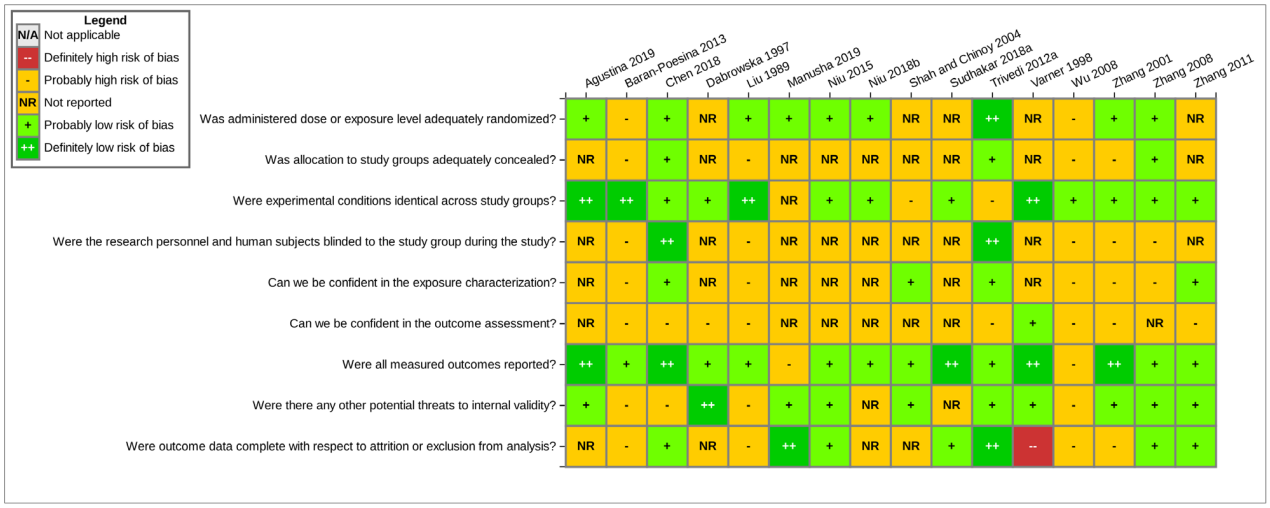
Interactive figure and additional study details in HAWC here.

Figure A3-26. Risk-of-bias Bar Chart for Lower Risk-of-bias Animal Histopathology Studies Following Fluoride Exposure



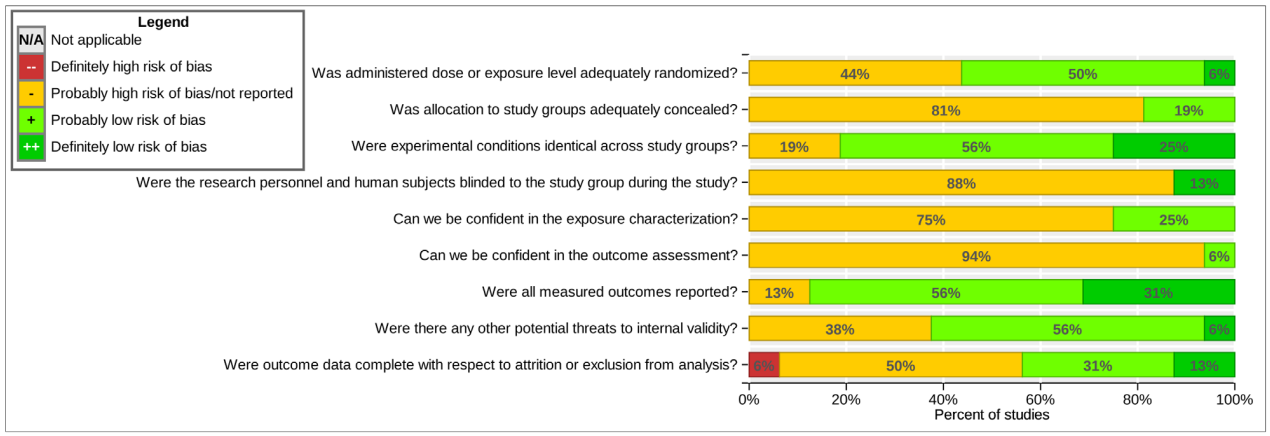
Interactive figure and additional study details in HAWC here.

Figure A3-27. Risk-of-bias Heatmap for Higher Risk-of-bias Animal Histopathology Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.

Figure A3-28. Risk-of-bias Bar Chart for Higher Risk-of-bias Animal Histopathology Studies Following Fluoride Exposure



Interactive figure and additional study details in HAWC here.