

HEALTH EFFECTS OF WATER FLUORIDATION

An evidence review 2015



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Table of contents

Table of contents	3
Foreword	6
Acknowledgements	7
List of tables and figures.....	8
List of abbreviations	9
Glossary of terms.....	10
Executive summary.....	13
Purpose.....	13
Review question	13
Methods	13
Findings.....	14
Summary on musculoskeletal effects.....	14
Summary on IQ and neurological manifestations.....	15
Summary on cancer	16
Summary on cardiovascular disease	17
Summary on other potential health effects	17
Conclusion	18
Introduction.....	19
Purpose of the review	19
Research question	19
Methods	20
Search approach.....	20
Search results	22
Critical appraisal and data extraction.....	22
Review limitations	24
Background.....	25
Fluoride.....	25
Community water fluoridation (CWF).....	26
Policy considerations.....	26
Findings.....	28
Musculoskeletal effects.....	28
Musculoskeletal effects and water fluoridation, up to 2006	28
Musculoskeletal effects and water fluoridation in non-endemic fluoride or CWF areas, 2006–2014	30

Skeletal fluorosis in areas with high levels of natural fluoridation in the water (fluoride-endemic or above a threshold of 1.5 ppm)	34
International reports and expert bodies' conclusions on musculoskeletal effects.....	39
Summary on musculoskeletal effects.....	41
Intelligence quotient (IQ) and other neurological conditions	43
Intelligence quotient and water fluoridation, up to 2006.....	43
Intelligence quotient (IQ) and water fluoridation in non-endemic or CWF-fluoride areas, 2006–2014	44
Intelligence quotient (IQ) and water fluoridation in fluoride-endemic areas (above a threshold of 1.5 ppm), 2006–2014	45
Neurological manifestations and water fluoridation in fluoride-endemic areas (above a threshold of 1.5 ppm)	53
International reports and expert bodies' conclusions on IQ and neurological manifestations	57
Summary on IQ and neurological manifestations	59
Cancer	60
Bone cancer and osteosarcoma	60
Bone cancer and osteosarcoma in non-endemic fluoride areas.....	61
Bone cancer and osteosarcoma in endemic areas (above a threshold of 1.5 ppm)	67
All-cause cancer in fluoride-endemic and non-endemic areas	68
International reports and expert bodies' conclusions on cancer	70
Summary on cancer	71
Cardiovascular disease	73
Cardiovascular disease in fluoride-endemic areas (above a threshold of 1.5 ppm)	73
International reports and expert bodies' conclusions on cardiovascular disease	79
Summary on cardiovascular disease	79
Other potential health effects	80
Kidney disorders and fluoride	80
Endocrine disorders and fluoride	83
Immune system disorders and fluoride (above a threshold of 1.5 ppm).....	85
Birth defects and fluoride.....	87
All-cause mortality and fluoride	89
International reports and expert bodies' conclusions on other potential health effects	90
Summary on other potential health effects	91
Overall conclusion	92
Non-endemic or CWF areas.....	92
Fluoride-endemic areas.....	92
Addendum: Essays on other fluoride-related topics.....	93

References.....	109
Appendix 1 Flow chart of searches and screening process.....	122
Appendix 2 Search strategy used to find articles	123
Appendix 3 Extraction form.....	125
Appendix 4 Quality assessment tool for reviews	126

Foreword

In recent years public concern about water fluoridation has intensified. There has been a lot of debate on whether or not it is linked to a number of health effects.

Community water fluoridation at a level of 1 ppm began in Ireland in 1964 as a measure to prevent dental caries. A major review of Ireland's water fluoridation policy in 2002 showed an increasing occurrence of dental fluorosis. As a result, in 2007, the fluoride level in drinking water in Ireland was lowered to a range of 0.6 to 0.8 ppm, with a target of 0.7 ppm. This remains the target and range applied in Ireland today.



It is good practice to regularly monitor and evaluate emerging evidence with regard to possible newly identified health effects. Accordingly, in 2014 the Department of Health asked the Health Research Board to assess the existing evidence base to determine 'what is the impact on the systemic health of the population for those exposed to artificially fluoridated water between 0.4 and 1.5 ppm?'

The Health Research Board sought to answer this question using a systematic review process. This report contains a detailed analysis of the evidence available in the peer reviewed literature. The topics addressed were musculoskeletal effects, IQ and neurological manifestations, cancer, cardiovascular disease and other potential health effects.

The HRB presents its findings in this report.

A handwritten signature in dark ink, appearing to read 'Graham Love'. The script is fluid and cursive.

Dr Graham Love
Chief Executive

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List of tables and figures

Table 1: Hierarchy of evidence for aetiology/harms	24
Table 2: Musculoskeletal effects of water fluoridation up to 2006: results of two index reports	29
Table 3: Original studies of effects of lower levels of water fluoridation (artificial and natural) on musculoskeletal effects, 2006–2014	30
Table 4: Original studies of effects of natural water fluoridation on skeletal fluorosis in fluoride-endemic areas (above a threshold of 1.5 ppm), 2006–2014	34
Table 5: Water fluoridation and IQ up to 2006: results of the York review	43
Table 6: Original studies in non-endemic-fluoride areas examining effects of community water fluoridation and IQ, 2006–2014	44
Table 7: Reviews studies in fluoride-endemic areas (above a threshold of 1.5 ppm) examining effects of water fluoridation and IQ, 2006–2014	45
Table 8: Original studies in fluoride-endemic areas (above a threshold of 1.5 ppm), examining effects of water fluoridation and IQ, 2006–2014	49
Table 9: Original studies in fluoride-endemic areas (above a threshold of 1.5 ppm) on the effects of water fluoridation and neurological manifestations, 2006–2014	55
Table 10: Review studies in fluoride-endemic-areas (above a threshold of 1.5 ppm) examining natural water fluoridation and neurological manifestations 2006–2014	57
Table 11: Bone cancer, osteosarcoma and water fluoridation: results of two index reports	60
Table 12: Original studies carried out in non-endemic-and endemic-fluoride areas between 2006 and 2014, which examined bone cancer and osteosarcoma and its association with water fluoridation.	61
Table 13: Other cancers and water fluoridation up to 2006: results of two index reports	68
Table 14: Original studies in non-endemic fluoride areas examining other (bladder and all) cancers and fluoride, 2006–2014	69
Table 15: Cardiovascular disease and water fluoridation up to 2006: results index report	73
Table 16: Original studies in fluoride-endemic areas (above a threshold of 1.5 ppm) examining cardiovascular disease and water fluoridation, 2006–2014	74
Table 17: Other potential health effects and water fluoridation: kidney disorders up to 2006, results of the NHMRC review	80
Table 18: Additional review papers examining other potential health effects and water fluoridation: kidney disorders: 2006–2014	80
Table 19: Additional original studies examining other potential health effects: kidney disorders and water fluoridation, 2006–2014	82
Table 20: Other potential health effects and water fluoridation: endocrine disorders up to 2006, results of index report	83
Table 21: Additional original studies examining other potential health effects and water fluoridation: endocrine disorders, 2006–2014	84
Table 22: Additional original studies examining other potential health effects and water fluoridation: immune system disorders (above a threshold of 1.5 ppm), 2006–2014	86
Table 23: Other potential health effects and water fluoridation, birth defects up to 2006: results of index reports	87
Table 24: Additional original studies examining other potential health effects and water fluoridation: birth defects, 2006–2014	88
Table 25: Other potential health effects and water fluoridation: all-cause mortality up to 2006, results of index reports	89
Table 26: Additional original studies examining other potential health effects and water fluoridation: all-cause mortality, 2006–2014	89
Figure 1: Map of documented occurrences of high or endemic fluoride in groundwater (>1.5 mg/)	25

List of abbreviations

BMD	bone mass density
BMI	body mass index
CAU	census area unit
CD	cluster differentiation
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CKD	chronic kidney disease
CRT-RC	Combined Raven's Test-Rural edition in China
CVD	cardiovascular disease
CWF	community water fluoridation
DALYs	disability-adjusted life years
DBP	diastolic blood pressure
DoH	Department of Health
DT	deceleration time
DXA	dual-energy x-ray absorptiometry
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
ET	endothelins
FSAI	Food Safety Authority of Ireland
GB	Great Britain
GWRs	groundwater resources
HES	hospital episode statistics
HRB	Health Research Board
IMD	Index of Multiple Deprivation
IQ	intelligence quotient
IVRT	isovolumic relaxation time
LSOA	lower super output area
MCLG	maximum contaminant level goal
Mg/L	milligrams per litre
MPI	myocardial performance index
NCRI	National Cancer Registry of Ireland
NFIS	National Fluoridation Information Service
NHMRC	National Health and Medical Research Council
NRC	National Research Council
ONS	Office of National Statistics
OR	odds ratio
PHE	Public Health England
PPM	parts per million
PYAR	person-years at risk
QAT	quality appraisal tool
RBC	red blood cell
SBP	systolic blood pressure
SCHER	Scientific Committee on Health and Environmental Risks
TH	thyroid hormone
TILDA	The Irish Longitudinal Study on Ageing
TREG	T regulatory cells
TSH	thyroid-stimulating hormone
WSZs	water supply zones

Glossary of terms

Fluorine is a chemical element with symbol F and atomic number 9. Fluoride is the negative ion of the element fluorine.

Community water fluoridation (CWF): water that is artificially fluoridated with a precise low dose of fluoride as a public health prevention measure to protect teeth from developing caries or cavities. In Ireland, statutory regulations for Fluoridation of Water Supplies stipulate that fluoride may be added to public water supplies, typically in the form of hydrofluorosilicic acid. The 2000 Forum on Fluoridation recommended the fluoride level in drinking water to be within a range of 0.6 to 0.8 ppm, with a target of 0.7 ppm.

ppm or mg/L: The units of measurement for fluoride in water is parts per million (ppm) or milligrams per litre (mg/L). 1 ppm equals 1 mg/L.

Endemic: In the context of this report endemic refers to areas **in the world** with high levels of naturally occurring fluoride in water **leading to fluorosis**.

Incidence is a term used to describe the number of new cases of disease or events that develop among a population during a specified time interval.

Prevalence is a term used to describe the proportion of people in a population who have a disease or condition at a specific point in time or during a specific period of time.

An **ecological study** is a descriptive epidemiological study carried out using aggregated population-based data to describe a disease (outcome) in relation to a factor of interest (exposure) and is used to formulate a theory. Both factors are correlated to determine their linear association, which is expressed as a proportion (r). This study type is vulnerable to 'ecological fallacy', as we do not know whether the individuals who were exposed were the same individuals who suffered the disease.

A **cross-sectional study or prevalence survey** is a descriptive epidemiological study in which the presence or absence of both the exposure and outcome is assessed at the same point in time. They are often used to assess the prevalence of acute or chronic conditions; to inform health planning and evaluation; or to formulate a theory. It can be difficult to control for factors that may be related to the exposure and outcome in cross-sectional studies, so they cannot be used to determine causality.

A **case-control** study is an analytic observational epidemiological study which studies self-selected subjects (cases) with an outcome (disease) back to exposure (cause), and their exposures are compared to self-selected controls that do not have the disease to determine the odds that the exposure may have caused the disease. This type of study can be used to identify exposures that cause rare diseases. The main drawback in case-control studies is their potential for recall bias.

A **cohort study** is a form of longitudinal (analytic observational) epidemiological study in which a group of subjects, called a cohort, is followed over a period of time, and data relating to predetermined exposures and outcomes are collected over time. The incidence of the outcomes of interest is calculated in the exposed people and compared to the incidence in the non-exposed people. This comparison of incidence is known as a relative risk. The data for the cohort can be collected either by following the participants into the future (prospective

study) or by asking them about their past (retrospective study). However, retrospective cohort studies are limited by recall bias.

A **systematic review** is a literature review focused on a research question that tries to identify, appraise, select and synthesise all high-quality research evidence relevant to that question. Systematic reviews provide an overview of the effects of exposures or interventions with respect to health and, where possible, an estimate of the size of any benefits or harms of these exposures or interventions. Each review covers a specific and well-defined area of health, and evidence from studies (preferably clinical trials or prospective cohort studies) is included or excluded on the basis of explicit quality criteria. Data in reviews are often combined statistically to increase the power of the findings of numerous studies, which on their own may be too small to produce reliable results

In research, **meta-analysis** comprises statistical methods to contrast and combine results from different studies in the hope of identifying patterns among study results, sources of disagreement among those results, or other interesting relationships that may come to light in the context of multiple studies. Meta-analysis can be thought of as 'conducting research about previous research'. In its simplest form, meta-analysis is done by identifying a common statistical measure that is shared between studies, such as effect size or p-value, and calculating a weighted average of that common measure. This weighting is usually related to the sample sizes of the individual studies, although it can also include other factors, such as study quality.

In statistics, **study heterogeneity** is a problem that can arise when attempting to undertake a meta-analysis. Ideally, the studies whose results are being combined in the meta-analysis should all be undertaken in the same way and to the same experimental protocols: study heterogeneity is a term used to indicate that this ideal is not fully met.

Publication bias is a bias with regard to what research results are published compared to results that are not published. One problematic and much-discussed bias is the tendency of researchers, editors, and pharmaceutical companies to handle the reporting of experimental results that are positive (i.e., showing a statistically significant finding) differently from results that are negative (i.e., supporting the null hypothesis) or inconclusive, leading to a misleading bias in the overall published literature.

Chance is sampling variability which can give rise to a particular result. It is the luck of the draw. It is an unsystematic over- or underestimation of the cause-effect relationship. The p-value measures the probability or likelihood that observed result occurred by chance alone.

Bias is a systematic overestimation or underestimation of the association in research. There are many types of bias such as selection, recall, and interviewer. Bias is minimised through good study design and implementation.

Confounding is when a factor has an association with the exposure and can independently cause the outcome or disease. It can over or under estimate an effect of interest or association. A **confounding variable** (also confounding factor or confounder) is a variable that has a relationship with both the exposure and outcome variable.

A **confidence interval** is the range of values (for example, proportions) in which the true value is likely to be found with a degree of certainty (by convention 95 per cent degree), that is, the range of values will include the true value 95 per cent of the time.

Blinding is a method used in research to ensure that the people involved in a research study – participants, clinicians, or researchers – do not know which participants are assigned to each study group, or which experienced the exposure or outcome of interest. Blinding is used to make sure that knowing the type of exposure, treatment, or diagnosis does not affect a participant's response to the treatment, a healthcare provider's behaviour, or an interviewer's approach to data collection.

Logistic regression is a statistical technique used in research designs that require analysing the relationship of an outcome or dependent variable to one or more predictors or independent variables when the dependent variable is either (a) dichotomous, having only two categories, for example, whether one uses illicit drugs (no or yes); (b) unordered polytomous, which is a nominal scale variable with three or more categories, for example, eye colour (blue, brown, grey or green); or (c) ordered polytomous, which is an ordinal scale variable with three or more categories, for example, highest level of education completed (e.g., none or primary school incomplete, primary school, secondary school, third-level diploma, third-level primary degree, third-level masters, third-level doctorate).

Executive summary

Purpose

One of the Department of Health's (DoH) main public health interventions to prevent dental caries is the fluoridation of public piped water supplies in the Republic of Ireland at levels of 0.6 to 0.8 ppm. Although approximately 400 million people around the world live in areas served by optimally fluoridated water supplies, it is considered appropriate to continuously monitor and evaluate the evidence to ensure that no new adverse health issues emerge. Optimally fluoridated water refers to water that is fluoridated either naturally or artificially at levels between 0.4 and 1.5 ppm. In recent years, media attention highlighting public concerns about water fluoridation has intensified. This review will be used to inform the DoH with regard to any impact, positive and/or negative, on the general health of those exposed to community water fluoridation (CWF) at its current levels. This evidence review will be a core component of definitive intelligence to inform the DoH and, thus inform future policy.

Review question

The DoH asked the following question: What is the impact, positive and/or negative, on the systemic health of the population (excluding dental health) for those exposed to artificially fluoridated water between 0.4 and 1.5 ppm?

The Department of Health's question did not cover the health effects of naturally occurring fluoridated water. However, two highly-regarded existing systematic reviews included areas with naturally occurring fluoride. Also, many of the health concerns raised by the public come from studies that were completed in areas with high levels of naturally occurring fluoride in their drinking water (>1.5ppm). Therefore, the HRB, in consultation with the DoH, decided to include these areas along with CWF areas.

The health outcomes identified by the formal systematic search, which are covered in the Findings chapter, relate to: musculoskeletal effects, IQ and neurological manifestations, cancer, cardiovascular disease and other potential health effects.

Methods

The strategy for the selection of the papers to include in the review is crucial, as this lays the foundation for the report findings. We identified two systematic reviews that answered a similar question to serve as index reports:

1. A Systematic Review of Water Fluoridation by McDonagh, Whiting and Bradley *et al.* (2000) commonly known as the York review.
2. A Systematic Review of the Efficacy and Safety of Fluoridation by the National Health and Medical Research Council (NHMRC) in Australia (2007).

These two reviews were chosen because they are formal systematic reviews which present a detailed methodology. The detailed methodology included a repeatable search strategy, an assessment of the level of evidence that each study provided, and a quality assessment of each primary study included in the systematic review. The two studies followed a very similar set of methods, and the NHMRC study in Australia was designed to update the York review. Both studies were peer reviewed and are highly respected and widely cited. The Australian review searched the available literature until the end of 2006 and we updated their search from that point to mid-2014. In order to cover the eventuality that something from 2006 might have been missed, we conducted our search from January 2006 to mid-2014. We searched MEDLINE, Embase and Cochrane Systematic Review databases; the journal *Fluoride* is in Embase but not MEDLINE. As well as using the NHMRC terms we added a number of study design terms. For additional coverage, and in order to ensure that the search was as

comprehensive as possible, we searched CINAHL and PsycINFO databases from 1999 to mid-2014. We also searched the TRIP database using a refined search string. In order to ensure that we obtained all relevant articles in our Embase search, we also searched the topic-specific journal *Fluoride*.

We found 3,537 articles. After the title and abstract screening, we were left with 108 papers that were relevant to our broader review question. We identified a further 32 articles from reference chasing. We obtained 140 full-text articles. After reading the 140 articles, 48 papers were included to answer the question. After completing the draft report, another original study was published linking water fluoridation and hypothyroidism. In light of the possible clinical importance of this paper we decided to include it, even though it was outside the timeline of the search.

Reviews similar to ours have been conducted in a number of other countries and these constitute the grey literature. We refer to their conclusions in the Findings chapter of this HRB review.

We assigned a level of evidence using the hierarchy of evidence used in the NHMRC review and we assessed the quality of each primary study and review included. We extracted the key findings from the study using an adapted version of the NHMRC extraction sheet.

There are a number of topical issues in relation to water fluoridation which are of public concern but which were not addressed by the literature returned from our search. These are covered in the addendum to this document and include tea, infant formula, depression, Alzheimer's disease, neurodevelopmental issues, and arthritis.

Findings

Summary on musculoskeletal effects

Bone fracture, bone mass density and skeletal fluorosis are the musculoskeletal effects associated with fluoride covered in the literature.

Bone fracture incidence and bone mass density in non-endemic or CWF areas

The literature was reviewed to determine if artificial water fluoridation increases the risk of bone fracture. Over the years, a number of community-level studies compared rates of fracture, specific to age and gender, between fluoridated water and non-fluoridated water areas. Some of these studies indicated that exposure to fluoridated water increased the risk of fracture; some studies indicated that water fluoridation reduced the risk of fracture; and several studies found no effect. However, more than half of these studies were designed using an ecological approach, which means that data for individuals were based on community-level data and lacked actual exposure and outcome data specific to each individual. Focusing on the more recent original studies (2006 to mid-2014) identified by the HRB search, five studies covering the topic were identified. Two studies revealed that there was no evidence of a difference in the rate of hip fractures between fluoridated and non-fluoridated areas; one study theorised a weak relationship between fluoride exposure, accumulated fluoride, and the physical characteristics of bone; one American study suggests that fluoride exposure at the typical CWF levels for most US adolescents residing in fluoridated areas do not have statistically significant effects on bone mineral measures, and one Irish study concluded that there was no statistically significant relationship between bone health and the proportion of households with and without a fluoridated water supply. However, these studies were mainly ecological studies and cannot prove or disprove a causal link. A summary of the existing literature indicates that the relationship between fluoride in drinking water and bone health is inconsistent with no definitive proof of protective or harmful effects.

Skeletal fluorosis and bone mass density in fluoride-endemic areas

Studies have shown that fluoride ingestion at elevated levels (above a threshold of 1.5 ppm) appears to affect skeletal tissues (skeletal fluorosis) and these effects are more severe as exposure to naturally occurring fluoride increases. Very mild skeletal fluorosis is characterised by slight increases in bone mass density. The most severe form of this condition, 'crippling skeletal fluorosis', involves bone deformities, calcification of ligaments, pain, and immobility. In 1993, the United States National Research Council (NRC) reported that few cases of this condition had been reported in the United States and that it was not considered a public health concern. Having examined the evidence available on skeletal fluorosis, it is clear that people consuming water containing levels of fluoride greater than the World Health Organization (WHO) permissible limits (>1.5 ppm) may be at risk of developing skeletal fluorosis. Outside of the endemic fluorosis belt, including countries with CWF, skeletal fluorosis is only seen in workers in the aluminium industry, fluorspar processing and superphosphate manufacturing. The studies published since 2006 that investigate skeletal fluorosis (4) or genu valgum (2) are mainly cross-sectional prevalence studies in fluoride-endemic areas, which do not attempt to examine risk factors or infer causality. The quality of the implementation of these studies is low to moderate, as generally they do not provide a rationale for the sample size or confidence intervals around the main outcome measure. In addition, they often contain investigator bias. A summary of the existing literature indicates that skeletal fluorosis is a health problem in areas with fluoride levels in drinking water greater than the WHO permissible limits (above a threshold of 1.5 ppm), but not in areas with CWF, such as Ireland.

Summary on IQ and neurological manifestations

Non-endemic or CWF areas

There was only one study carried out in a non-endemic or CWF area (like Ireland) that examined fluoride and IQ. This was a prospective cohort study (whose design is appropriate to infer causality) in New Zealand. The study concluded that there was no evidence of a detrimental effect on IQ as a result of exposure to CWF.

Fluoride-endemic areas

There were six primary cross-sectional studies and six reviews published between 2006 and mid-2014 which examined the association between fluoride and neurological or IQ effects in endemic areas. In some of the review papers the lower and higher range of fluoride levels is not clearly specified and in other reviews the lower and higher range of fluoride levels overlap. In the six primary studies, where the fluoride levels were clearly specified, the normal level of fluoride was less than 1.5 ppm and the higher levels of fluoride were greater than or equal to 1.5 ppm, and could be as high as 10.3 ppm. These studies suggest, but do not prove, that children living in areas with naturally occurring high fluoride in the water (higher than the levels in CWF of 0.4–1 ppm) have a lower IQ compared with children drinking water with naturally occurring levels of fluoridation similar to CWF levels in Ireland. It is important to state that the six primary studies and the six reviews published cannot prove a causal link. Apart from fluoride, there are other chemicals (arsenic or lead) and mineral deficiencies (iodine or iron) that could cause neurological or IQ effects, and these factors have not been comprehensively evaluated or controlled for using an appropriate study design, namely a prospective cohort study. The participants in these studies have very different socioeconomic profiles and nutritional status compared with children receiving CWF in developed countries; lower socioeconomic status and nutritional status may also lower IQ. Overall, the studies are of a low quality and of a design unsuited to prove or disprove theories. A summary of the existing literature indicates that lower IQ as a result of exposure to fluoride in drinking water is potentially problematic in areas with high levels of naturally occurring fluoride (above a threshold of 1.5 ppm), but such experiences were not reported in areas with CWF, such as Ireland.

Summary on cancer

Non-endemic or CWF areas

Concerns have been expressed about the possible carcinogenic effect of fluoride in drinking water, particularly in relation to osteosarcoma, a rare primary cancer of the bone. A number of studies have examined the association between water fluoridation and osteosarcoma, and there have also been studies that examined water fluoridation and general cancer incidence and mortality.

Between 2006 and mid-2014 a possible link between fluoride and osteosarcoma incidence has been investigated in five ecological studies and two case-control studies in non-endemic areas, and one case-control study in an endemic area. Most osteosarcomas occur in children and young adults. Teenagers are the most commonly affected age group, but osteosarcoma can occur at any age. Approximately 15 cases of osteosarcoma are diagnosed each year in Ireland, with incidence rates being slightly higher in males than in females. A number of authors report that there is biological plausibility for linking fluoride to osteosarcoma, as fluoride accumulates in bones and changes the properties of bone. Much of the concern about fluoride and its possible link to osteosarcoma arises from the findings of a paper by Bassin *et al.* published in 2006, which concluded that their exploratory analysis found a statistically significant association between osteosarcoma and fluoride in drinking water in young males, but not in young females. They also acknowledged that there were limitations to their study design. While the study design employed has potential to infer causality, the conduct of the study has flaws and these have led to its findings being disputed. Added to this is the fact that Bassin's PhD supervisor, Chester W Douglass, the lead investigator on the complete study, published a statement in the same edition of the journal as the article, urging caution in interpreting the results. Douglass explained that preliminary results on the full study cohort did not support Bassin's findings. Subsequently, there was an allegation of fraud and suppression of Bassin's results made against Douglass. The allegations were investigated by Harvard University, and Douglass was cleared of any wrongdoing. Douglass is an author on a further study, by Kim *et al.*, which examined the same cohort of study subjects in relation to fluoride in drinking water and osteosarcoma. The researchers measured fluoride concentration in samples of normal bone adjacent to the person's tumour and found no difference in bone fluoride levels between people with osteosarcoma and people in the control group who had other malignant bone tumours. The study authors concluded that there was no statistically significant association between bone fluoride levels and osteosarcoma risk detected in that study's subjects; however, that study also had flaws in its implementation. There has been no formal publication detailing the findings for the complete cohort.

The existence of biological plausibility in relation to fluoride and bone cancer (mentioned earlier) renders the 2014 Levy *et al.* paper important to this discussion, although it does not examine osteosarcoma specifically. In the Levy *et al.* study, data were collected from a birth cohort in the Iowa fluoride study since the early 1990s. The researchers aimed to quantify fluoride intake from all sources and estimate the exact influence of fluoride intake on bone density and mineral content. The study subjects had complete accelerometry data (a technique used to study bone movement) and bone scans at age 15 years, the age at which many osteosarcomas are diagnosed. Data were analysed and adjusted for a large number of variables. The findings suggest that fluoride exposures at the typical levels for most US adolescents in fluoridated areas do not have statistically significant effects on bone mineral measures. The Levy *et al.* study is a prospective cohort, a design in which far fewer opportunities exist for bias compared with studies of weaker design, such as case-control studies, cross-sectional surveys or ecological studies. The study has extremely important strengths in that data were from a cohort that was followed longitudinally, and measures of fluoride intake were calculated for each year in individuals rather than relying on population data or long-term recall exposure. However, it is important to point out that a large number of the original cohort were lost to follow-up.

A summary of the existing literature indicates that the effects of fluoride in drinking water on osteosarcoma incidence are mixed and to date, no link has been proven. Therefore it is difficult to draw a definitive conclusion.

Fifteen studies were included in the York review on cancer incidence or mortality and an additional three studies were included in the Australian review. The current authors found only one more recent paper, the Public Health England study, which investigated this possible association. Both of the index reviews found mixed evidence in relation to fluoride in the drinking water and all-cancer incidence or mortality. The Public Health England study found no evidence of any association between fluoridation status and all-cancer incidence, with the exception of bladder cancer. The Public Health England study concluded that the analysis suggested the rate of bladder cancer may be lower in fluoridated areas than in non-fluoridated areas. Researchers have advanced hypotheses linking fluoride and all-cause cancer incidence or mortality, but there is a dearth of good quality longitudinal research available to affirm or rule out these suggested links.

Summary on cardiovascular disease

Non-endemic or CWF areas

Only one of the index reports examined a study concerned with the possible effect of drinking fluoridated water on the associated risk of cardiovascular disease in non-endemic areas similar to Ireland. The ecological study included in the index report was conducted in Finland, and the results suggested a slight protective effect of fluoride with respect to coronary heart disease. The HRB authors did not find any more recent studies in CWF areas on this topic.

Fluoride-endemic areas

The six studies the HRB authors found and reviewed were all completed in areas where fluoride is naturally occurring in groundwater and generally at higher levels than those found in water with CWF (0.4–1 ppm), like Ireland. The higher levels of naturally occurring fluoride varied across the six studies, with moderate and higher levels being defined as greater than 2 ppm with no upper limit. Two case-control studies in Turkey found that high levels of natural water fluoridation decreased aortic elasticity and contributed to cardiac dysfunction; however, it is difficult to make definitive statements based on these studies, as their execution is judged to be of low quality. Three studies, two in Iran and one in China, examined water fluoridation and its link with hypertension. Two of these were ecological studies and one was a cross-sectional study; two found a higher prevalence of hypertension with increased fluoride level, and one found the opposite. However, due to the study design employed in all three studies, it can only be suggested that a relationship may exist between fluoride and blood pressure levels. The sixth study, a cross-sectional study in China, reported a positive correlation between atherosclerosis prevalence and water fluoride concentration; however, it is a suggested correlation, not a proven cause and effect. A summary of the existing literature indicates that the evidence is inconsistent and lacking in methodological rigour.

Summary on other potential health effects

In relation to a possible link between exposure to water fluoridation and a number of other health effects, the literature search did not provide enough evidence on any particular outcome to make an evidence-based statement. These health effects relate to kidney disorders (two primary studies); hypothyroidism (one primary studies); immune system disorders (one primary study); birth defects (one primary study); and all-cause mortality (one primary study).

On the topic of hypothyroidism there was one primary study. Peckham *et al.*, in an ecological study, found a statistically significant association between water fluoride levels of greater than 0.3 ppm and the prevalence of hypothyroidism in GP practices.

In summary the findings of the ecological study-by-Peckham et al suggest that fluoride in water may be linked to the development of hypothyroidism. The published studies examining other possible negative health effects (renal stones, Downs syndrome and all-cause mortality) provide no evidence of harmful outcomes in CWF areas.

Conclusion

Non-endemic or CWF areas

In summary the literature found no strong evidence that CWF is definitively associated with negative health effects. However, the evidence base examining the association between health effects and community water fluoridation is scarce. It is mainly based on ecological studies and a small number of prospective cohort studies. Ecological studies are not adequate to infer causality.

Having examined the evidence, and given the paucity of studies of appropriate design, further research would be required in order to provide definitive proof, especially in relation to bone health (osteosarcoma and bone density) and thyroid disease (hypothyroidism).

Fluoride-endemic areas

In geographical areas where there is a naturally occurring high level of fluoride in drinking water (> 1.5 ppm), the health concerns have a somewhat different emphasis; these areas do not include CWF areas like Ireland. There are strong suggestions that high levels of naturally occurring fluoride in water may be associated with negative health effects, in particular, skeletal fluorosis and lowering of IQ. In addition, there are some indications that high levels of naturally occurring fluoride in water may also be associated with cardiovascular disease. However, the evidence base examining the association between health effects and high fluoride exposure emanates from low quality studies of inappropriate study design.

Introduction

This report presents the findings of a review of the evidence carried out by a team at the Evidence Centre of the Health Research Board (HRB) on the health impacts of community water fluoridation (CWF).

Purpose of the review

One of the Department of Health's (DoH) main public health interventions to prevent dental caries is the fluoridation of public piped water supplies in the Republic of Ireland at levels of 0.6 to 0.8 ppm. Although approximately 400 million people around the world live in areas served by optimally fluoridated water supplies,¹ it is considered appropriate to continuously monitor and evaluate the evidence so as to ensure that no new adverse safety issues emerge. In recent years, media attention highlighting opposition to CWF has intensified, particularly via the internet and social media. This review will be used to inform the DoH with regard to any impact, positive and/or negative, on the health of those exposed to water fluoridation at its current levels. The evidence review will be a core component of definitive intelligence to inform the DoH and thus inform future policy.

Research question

The aim of this review is to provide the DoH with the best available evidence on the impact of CWF on the systemic health of the population. In order to meet this aim, the DoH asked the following question:

What is the impact, positive and/or negative, on the systemic health of the population (including oral health, but excluding dental health) for those exposed to artificially fluoridated water between 0.4 and 1.5 ppm?

- Systemic health includes such conditions as higher incidence of bone-related disorders, higher incidence of associated cancers and higher incidence of other associated health conditions focusing on the health of populations consuming fluoridated water in Europe or other countries with temperate climates; similar demographic as well as socioeconomic conditions are of particular interest.

Methods

Search approach

In order to comprehensively answer the research question, the ideal approach for this work would be to undertake a systematic review of all the literature. However, given the limited timeframe available (six months) and the considerable body of literature that exists on the topic of CWF it was decided, in consultation with the DoH, to focus resources on identifying systematic review papers that would serve as index reports in the area of fluoridated water and related health effects. The current review would collect evidence published since the most recent index report and add these data to the body of evidence already reported. This section outlines the approach taken to carrying out this current review.

The question posed by the DoH and addressed in this report relates only to potential health effects that may be associated with CWF; it does not deal with dental benefits or dental fluorosis that may be associated with this intervention. In addition, the Department of Health's question did not cover the health effects of naturally occurring fluoridated water. However, two highly-regarded existing systematic reviews, the York² and National Health and Medical Research Council (NHMRC) of Australia³, included areas with naturally occurring fluoride. Also, many of the health concerns raised by the public come from studies that were completed in areas with high levels of naturally occurring fluoride in their drinking water (>1.5ppm). Therefore, the HRB, in consultation with the DoH, decided to include these areas along with CWF areas.

From the initial scoping search of the literature, a number of key reports were identified. Two peer-reviewed reports using systematic searches on the topic of water fluoridation were considered to be most relevant and provided a detailed description of their methodology. The first report, *A Systematic Review of Public Water Fluoridation* was published in 2000, and is commonly referred to as the York review.² The York review team conducted their search in 25 specialist databases, including MEDLINE and Embase. They also searched the World Wide Web and the bibliographies of all included studies. Experts and authors in the field were contacted, and studies in any language were included. The full search strategy is available in the report. The York review confirmed the beneficial effect of water fluoridation on dental caries, but also suggested that this should be considered alongside the increased prevalence of dental fluorosis. Their conclusions in relation to the health effects of fluoridation will be discussed later in the review.

In 2007, an additional systematic review was published by the National Health and Medical Research Council (NHMRC) of Australia.³ The methods used in the 2007 Australian review are broadly consistent with those used in the York review and this later report builds on the evidence provided in the York review. A search of the literature was undertaken in the MEDLINE and Embase databases from January 2006 to mid-2014. In addition, the Cochrane Systematic Review and clinical trial databases were searched to help identify additional systematic reviews and original studies from 1996 onwards. All of the searches were limited to English-language publications. The authors intended that the Australian review would update the York systematic review. The Australian review supported the conclusion of the York review in terms of CWF and dental caries, and meta-analysis of additional original studies provided results consistent with those seen in the previous systematic review. Their conclusions in relation to health effects will also be considered when describing the findings of the current review.

These reviews were selected as the index reports for the current review, as they are highly respected and widely cited; are systematic reviews (with the Australian one following on from the York review), and both reviews provide detailed accounts of their methodology. While other important reviews have also been published in the area of CWF, the detailed search methods were generally not described in the reports. The selected index

reports cover the evidence on the health-related aspects of artificial water fluoridation up to and including 2006. Given the time limitation for the current review it was, therefore, decided to assess the literature on this topic from 2006 onwards, with the aim of updating the evidence of the York and Australian reviews. A large proportion of the literature on CWF deals with dental topics, and both index reviews examined the effects of CWF on the incidence of caries and of dental fluorosis, as well as the effects on systemic health. Nevertheless, the purpose of the current review as defined by the DoH relates to the health impacts of CWF; issues related to the dental benefits arising from the use of CWF or other dental matters are not included in the remit of this current report and will be covered in a future report. Therefore, the methods and search terms used in the Australian review were adapted to exclude evidence in the area of oral health, unless there was also information on general health-related topics. In line with the search methods employed in the 2007 Australian review, a search for reviews and primary research studies published from 2006 onwards in the English language was conducted, in order to answer the questions posed in this review.

Using MEDLINE, Embase and the Cochrane Systematic Review databases, the search methods and search terms employed by the NHMRC resulted in 13,246 records. The Australian researchers screened for specific study types after their initial search and excluded any that did not fit their study-type criteria. However, given the time limitations it was decided to refine the HRB search to include study-type restrictions as part of the search terms. Therefore, the databases were searched for primary research studies that have a comparator (case-control study, cohort analysis, clinical trial, evaluation study, comparative study, controlled clinical trial, observational study, cross-sectional study or randomised controlled trial). MEDLINE was also searched for reviews and systematic reviews. For details of the search strategy, search terms and exclusion criteria see Appendix 2. Although the question relating to health effects was intended to focus on the health of populations consuming fluoridated water in Europe, or in other countries with temperate climates and similar demographic as well as socioeconomic conditions, it was clear that if these limitations were applied to the search articles retrieved, much relevant information would be excluded. Therefore, these demographic and socioeconomic criteria were not applied during the screening process because much of the research on the health effects of fluoride in drinking water was carried out in countries with naturally occurring fluoride in the drinking water, whose climates are not temperate and whose socioeconomic demographics are not similar to Ireland.

The search performed by the authors of the York review was extensive, with searches of 25 databases for published and unpublished articles in all languages. Therefore, there is unlikely to be bias in the literature found for inclusion in their report. The search in the Australian review was less extensive as they searched MEDLINE, Embase and Cochrane Systematic Review databases for articles published in the English language. The HRB authors used similar methods to the Australian NHMRC search strategy. In order to ascertain the completeness of the Australian NHMRC and HRB searches, the HRB authors additionally searched CINAHL, the TRIP database and PsycINFO for relevant articles published in the English language from 1999 onwards. This search did not uncover any additional relevant material.

A subject-specific journal can be a useful tool for identifying articles relating to a particular subject. On the topic of fluoride, the journal *Fluoride* is published by the International Society for Fluoride Research. In the original search of health databases MEDLINE and Embase, the Embase search returned 19 results from *Fluoride*. MEDLINE does not index this journal. In December 2013, the Associate Director, Library Operations, for the National Library of Medicine advised that the journal *Fluoride* did not score high enough to be recommended for inclusion in MEDLINE.⁴ The score of 1.5 out of 5 was below the 3.75 or greater required for selection for MEDLINE indexing. The founders and editors of *Fluoride* are outspoken in their opposition to CWF, but state that they welcome articles and editorials on fluoridation written from either a pro- or anti-CWF perspective. Therefore, in the interest of completeness, the cumulative subject index of the journal *Fluoride* was explored, but no additional relevant papers were identified.

Search results

The combined searches yielded 3,679 papers. Having removed the duplicates (142), 3,537 articles for title and abstract screening remained. From these, 108 articles were retrieved for full text screening. Some papers were also obtained using reference harvesting from the retrieved articles; this yielded an additional 32 articles, giving a total of 140 papers. Following full text screening of these sources, a total of 48 articles were included to answer the particular question posed by the DoH. Additional articles from the search were used to inform the background and context of the review, and these are included in the bibliography. After completing the draft report, another original study (Peckham *et al.*, 2015) linking water fluoridation and hypothyroidism was published. Because of the possible clinical importance of this paper, the HRB authors decided to include it, even though it was outside the timeline of the search. As there are many non-peer reviewed reports on fluoridation, a Google search for grey literature was performed for publications, medical guidelines or position statements from nationally or internationally recognised expert bodies. Publications from these bodies relating to the subject of fluoridation are included in order to refute/validate the points from the York and Australian reviews and the evidence produced in the current report.

EndNote and EPPI-Reviewer software packages were used for reference and data management. EPPI-Reviewer was also used for preliminary screening and coding of texts. Both the screening of title and abstract and the full text screening were performed independently by two of the authors (MS and LF) and any disagreements were resolved by discussion. A data extraction tool was developed and formatted in the EPPI-Reviewer package. This tool was based on the template provided by the Australian authors and included questions relating to both individual study and study outcomes, and also relating to the quality of the study. The level of evidence was assessed by two of the authors (MS and JL) and in the event of a disagreement, a consensus was reached by discussion. A copy of the data extraction form and the criteria used to assess the quality of the studies is presented in Appendices 3 and 4.

Having extracted the information from the included articles, it emerged that many of the topics and health issues that are raised repeatedly by people who are concerned about CWF did not feature in the articles retrieved from the formal search; in addition, where articles were found, they were deemed to be unsuitable for the review by virtue of being an incorrect study type or otherwise outside the remit of the overall review. Therefore, in an attempt to address these issues, and in an effort to examine why people are concerned about these topics, it was decided to conduct further iterative searches on a range of issues. These searches were iterative rather than systematic. The MEDLINE and EBSCO databases and the Google search engine were used to undertake these more specific iterative searches using refined search terms related to each of the individual topics. These were community water fluoridation and: tea, infant formula, depression, Alzheimer's disease, neurodevelopmental issues, arthritis, and endocrine disorders. Further refining of the searching and data collection process involved reference chasing, particularly following up papers/studies that are widely cited by people who are concerned about CWF. Following content analysis, the topics that arose most frequently in the Irish context were identified, and essays on these topics are included in an addendum to the main report.

Critical appraisal and data extraction

Critical appraisal is a systematic process used to identify the strengths and weaknesses of a research article in order to assess the usefulness and validity of research findings.⁵ Katrak *et al.*,⁶ in their review of critical appraisal tools, conclude that 'there is no gold standard critical appraisal tool for any study design, nor is there any widely accepted generic tool that can be applied equally well across study types in order to assess the level and validity of evidence presented'.

There is no perfect study – the study design to use depends on the research question. In epidemiology the most rigorous design to link an exposure (such as fluoride) to an outcome (such as a health problem) would be a prospective cohort study.⁷ Prospective cohort studies are observational studies that classify the participants into an exposed group and a non-exposed group, and follow them up to determine the incidence of the outcome of interest (disease) in the exposed and, separately, in the non-exposed group. Prospective cohort studies are not used to answer the same type of research question as randomised controlled studies (RCTs). Cohort studies can be considered to be very robust for answering questions about natural history and the time-ordering of exposure and outcome. Such cohort study design is important for research on the aetiology of diseases, but it is susceptible to bias (most commonly non-participation and loss to follow-up) and confounding (other exposures that could have caused the outcome) as an alternative explanation. Retrospective cohort studies also classify the participants based on the presence or absence of an exposure, and examine their history to determine if the participant developed or did not develop the outcome. However, retrospective studies are more susceptible to bias (recall, selection, non-participation and misclassification) than prospective studies, as the outcome will have occurred before the study got underway. In addition, confounding remains an issue. The case-control study is another comparative study design, but this study design selects the participants based on their disease status (diseased and non-diseased) and examines their history to determine if they were exposed or not to the factor of interest (the odds of exposure). Once again, this study design is subject to bias (selection, non-participation, recall) and confounding. Matching is sometimes used to address some of the known confounders. Cohort studies and case-control studies can test a theory and infer causality.

Two study types commonly used in the articles included in this review are the ecological study and the cross-sectional survey. These study types are used to develop hypotheses rather than test them, and cannot infer causality. The ecological (correlational) study is a population-based study that examines the association between an exposure and an outcome in order to develop a theory; however, the people with the exposure may not be the people with the outcome (disease). In addition, it is not possible to deal with changing exposure levels over time within this study design. The cross-sectional (prevalence) survey collects the participants' status with respect to exposure (risk factors) and outcomes (diseases) at the same time, and is useful for health planning, prevalence estimation and creating theories. However, one cannot prove that the exposure occurred prior to the onset of the outcome (disease), or was the cause of the outcome. In addition, this study design is subject to interviewer and recall bias as well as confounding. Researchers can attempt to control for confounding factors with a well-designed questionnaire containing adequate questions about all known exposures and the use of multivariate modelling.

In addition to the type of study design, researchers attempt to control for chance, bias and confounding by their use of high-quality design inputs (such as adequate sample size, matching, assessing details of other exposures that could cause the outcome, interviewers blinded to the theory under examination), appropriate analytical tools (such as stratification, logistic regression) and accurate interpretation of appropriate analysis.

Quality assessment of the articles used to answer the questions in this report was performed after the screening process and was not part of the inclusion/exclusion criteria. The purpose of performing a critical analysis after deciding on the articles for inclusion is to assess each study's usefulness and validity, and determine the weight that should be given to each paper's findings in the analysis.

With regard to CWF, the fluoride intervention is at the population level; thus, the highest level of evidence would be a systematic review of prospective cohort studies. A systematic review attempts to identify, appraise and synthesise all the empirical evidence that meets pre-specified eligibility criteria to answer a given research question. Researchers conducting systematic reviews use explicit methods aimed at minimising bias, in order to

produce valid findings that can be used to inform decision-making.⁸ However, safety/harms associated with an intervention may also be assessed using other observational study types, such as a retrospective cohort or case-control study. The case-control study design is particularly suitable for rare diseases, while the retrospective cohort is particularly suitable for outcomes or diseases that develop long after the initial exposure or after prolonged exposure (e.g., cancer, osteoporosis and cardiovascular disease).³ In the fluoride literature, the cross-sectional or ecological study design is used extensively. However, these study designs cannot infer causality for the reasons already mentioned. The hierarchy of evidence used in this review is consistent with that of the Australian review³ (Table 1).

Table 1: Hierarchy of evidence for aetiology/harms

I ^a	A systematic review* of level II studies	^a A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence
II	A prospective cohort study	
III-1	All or none ^b	
III-2	A retrospective cohort study	^b All or none of the people with the risk factor(s) experience the outcome. For example, no smallpox develops in the absence of a specific virus; and clear proof of the causal link has come from the disappearance of smallpox after large-scale vaccination
III-3	A case-control study	
IV	A cross-sectional study	

Source: National Health and Medical Research Council (2007). *A systematic review of the efficacy and safety of fluoridation*. Canberra: NHMRC; Australian Government. http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/eh41_1.pdf

The quality assessment tool for the systematic reviews was adapted from the 'Health Evidence Quality Appraisal Tool' (QAT) developed by McMaster University.⁹ The tool was selected because it covers a range of appropriate assessment criteria, has been used to assess reviews, and is accompanied by detailed guidance that helps to standardise its use by different team members. The tool assessed internal validity, which is measuring the extent to which the findings answered the research question (see Appendix 4). No gold standard was available to assess the quality of primary epidemiological studies, so the HRB authors selected five criteria that measure the internal validity of primary epidemiological research. The five criteria were: research question, description of study population, sampling strategy and sample size calculation, strategies to minimise bias and methods to identify and control for confounding. For the quality measure of the included studies the review team used three broad categories: high, moderate and low. These quality levels were assigned to studies by assessing the methodological rigour employed by the study researchers when conducting the review or study.

Review limitations

The primary limitation of the review is the quality of the research included. Overall, our search indicated that there is a dearth of good quality primary research studies that examine the potential association of human health-related problems with water fluoridation. Many of the studies employed a study design that was unsuitable for inferring causality and many did not employ methods to minimise bias or control for confounders.

Due to the time restrictions, we relied on two index report to cover research in the area up to 2006, and thereafter we searched for articles that were published in the English language. Given the comprehensive nature of the searches, the review team feel it is unlikely that a key study of sufficient size and quality to change any of the findings was overlooked.

As with any search, no matter how comprehensive, the articles retrieved may be a biased collection of studies, since studies showing a statistically significant result are more likely to be published, and this may result in a positive or negative overestimate of the result.

Background

Fluoride

Fluorine is a chemical element (symbol F) and atomic number 9. Fluoride is the negative ion of the element fluorine. Any compound, whether it is organic or inorganic, that contains the fluoride ion is also known as a fluoride. Examples include CaF_2 (calcium fluoride) and NaF (sodium fluoride). Ions containing the fluoride ion are similarly called fluorides (e.g., bifluoride, HF_2^-). Water fluoridation is usually accomplished by adding sodium fluoride (NaF), fluorosilicic acid (H_2SiF_6), or sodium fluorosilicate (Na_2SiF_6) to drinking water.

There is considerable variation in the level of naturally occurring fluoride in drinking water around the world, and this variation is largely dependent on geological factors. These areas with naturally occurring fluoride in the water can be split into two groups, 1) where the naturally occurring fluoride is >1.5 ppm known as endemic areas and 2) where the naturally occurring fluoride is ≤ 1.5 ppm which is in line with the WHO permissible limit. High levels of naturally occurring fluoride in water occur in approximately 25 countries worldwide. In Asia, countries with the highest levels are India and China. In Latin America, Mexico and Argentina have the highest levels. Parts of east and north Africa also have high endemic levels of fluoride (Figure 1).

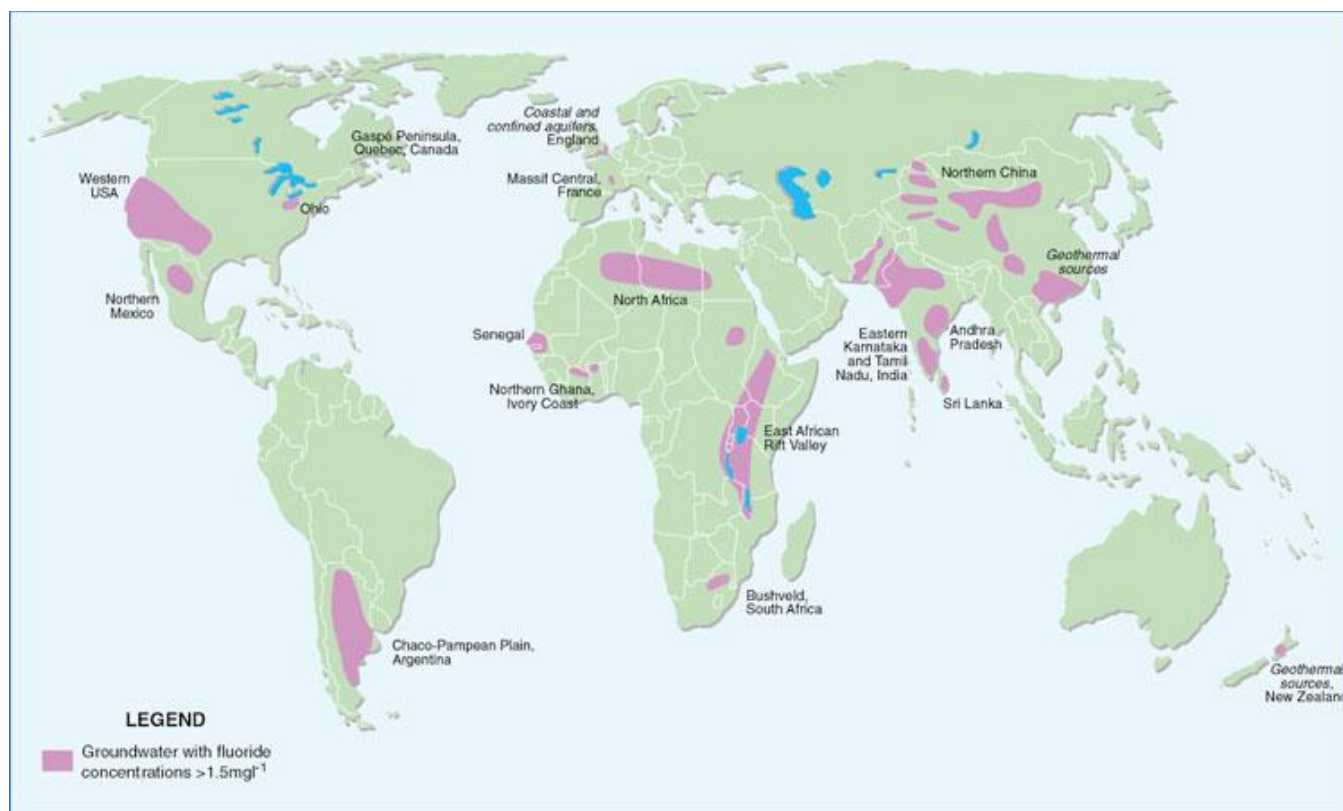


Figure 1: Map of documented occurrences of high or endemic fluoride in groundwater (>1.5 mg/L).

Source: <http://www.bgs.ac.uk/research/groundwater/health/fluoride.html>

In 1945, Grand Rapids in Michigan became the first city in the world to artificially fluoridate its drinking water, following results of epidemiological studies showing a link between raised levels of fluoride in drinking water and reduced prevalence and severity of tooth decay in local populations. During the 15-year project, researchers monitored the rate of tooth decay among Grand Rapids' almost 30,000 schoolchildren. After just 11 years, it was found that the caries rate dropped more than 60 per cent among those children who were born after fluoride

was added to the water supply. CWF entails an upward adjustment of the fluoride concentration in fluoride-poor water sources to a level that is considered optimal for dental health.

Community water fluoridation (CWF)

Community water fluoridation (CWF) was introduced in Ireland in 1964 on foot of the Health (Fluoridation of Water Supplies) Act 1960; fluoride was added at a level of 1 ppm. In 2000, water fluoridation policy in Ireland was the subject of a major review by the Forum on Fluoridation, established by the then Minister for Health and Children. In light of both international and Irish research which shows that there is an increasing occurrence of dental fluorosis, the Forum recommended the lowering of the fluoride level in drinking water from 1 ppm to a range of 0.6 to 0.8 ppm, with a target of 0.7 ppm.¹⁰ This policy was implemented in 2007.¹¹

The Fluoridation of Water Supplies in Ireland Regulations¹¹ stipulate that fluoride may be added to public water supplies either in the form of hydrofluorosilicic acid, or in such other form as may be approved by the Minister. It is further stipulated that the fluoride content of public water supplies, to which fluoride has been added, shall be determined daily at the water treatment plant. The water supply in Ireland is delivered via water supply zones (WSZs). According to the 2012 report *The Provision and Quality of Drinking Water in Ireland*, there were 3,702 WSZs in Ireland, of which 1,119 WSZs were monitored for fluoride content.¹²

The estimated number of people consuming artificially fluoridated water worldwide as of November 2012 is 377,655,000 in 25 countries, including Argentina, Australia, Brazil, Brunei, Canada, Chile, Fiji, Guatemala, Guyana, Israel, Libya, Malaysia, New Zealand, Panama, Papua New Guinea, Peru, Republic of Ireland, Republic of Korea (South Korea), Serbia, Singapore, Spain, the United Kingdom, the United States and Vietnam. These countries also have an estimated 17,910,000 people drinking naturally fluoridated water at or around the optimal level (i.e., 0.4–1 ppm), bringing the total number of people consuming optimally fluoridated water in those countries to 395,565,000 people.¹ Concerns have been raised by the public about the possible health harms that could be associated with CWF, thus, this report examines the evidence base underlying these concerns.

Policy considerations

Public health policies should be based on sound scientific evidence about risks, benefits, and economic evaluation of interventions to address a specific issue in a population. Decision-makers should also be cognisant of the impact of not employing a proven intervention. Policy-makers value systematic evidence as a basis for their decision-making, yet systematic evidence is not always available with respect to all potential adverse health effects. In Ireland, water fluoridation is considered a sound public health practice. Water fluoridation creates an environment that is conducive to promoting good oral health. It is a cost-efficient intervention that can reach large populations, without necessitating the active participation of the individuals, and it can deliver oral health benefits. In addition, it can reach a broad spectrum of people, ranging from those in low socioeconomic groups to high socioeconomic groups, and it can reduce disparities in oral health^{2,3} thereby it could be considered an essential dietary nutrient. As with all dietary nutrients it is possible to have either too little or too much in the diet. WHO presents the effects of fluoride at different doses and these are outlined in the following list.

Fluoride level in water	Effects
0.0–0.39 mg/L	Increased risk of dental caries
0.8–1.2 mg/L*	Prevention of tooth decay, strengthening of skeleton
Above 1.5 mg/L	Fluorosis: pitting of tooth enamel and deposits in bones
Above about 10 mg/L	Crippling skeletal fluorosis

Source: Water Sanitation and Health

http://www.who.int/water_sanitation_health/naturalhazards/en/index2.html

* Ireland recommends an optimal fluoride concentration of 0.7 ppm or 0.7 mg/L as this provides the best balance of protection from dental caries while limiting the risk of dental fluorosis. The calculation of this fluoride concentration in Ireland takes account of a number of considerations including fluoride from other sources. When other sources of fluoride are taken into account, Ireland is placed in the 0.8–1.2 mg/L category.

There is opposition, both in Ireland and worldwide, to the practice of artificially fluoridating water supplies. This opposition results from concerns about possible side effects that drinking fluoridated water may cause. This is a very difficult area, as it is impossible to prove beyond doubt – as with any other intervention – that absolutely no negative effects result from its use, and no risk is associated with fluoridation intervention. The scientific evidence can indicate that negative health effects are improbable, but cannot rule them out completely. Many of the concerns about adverse health effects of fluoride result from findings in endemic regions with very high levels (1.5 ppm–10 ppm) of naturally fluoridated water, two to twelve times higher than the levels of fluoride in the water in Ireland (0.6 ppm–0.8 ppm). Therefore, results from these studies cannot be equated with the situation in Ireland. In fact, studies in endemic regions compare the health of people with very high levels of fluoride in their drinking water (>1.5 ppm) to a comparison group living in a nearby area with naturally occurring fluoride within WHO permissible limits (≤ 1.5 ppm). The comparison group's exposure to fluoride in naturally fluoridated areas, although sometimes higher than levels in CWF, is classified by researchers as normal exposure or as low-risk for fluoride-related health effects.

In May 2015, the U.S. Public Health Service (PHS) updated and replaced its 1962 Drinking Water Standards related to community water fluoridation. PHS now recommends an optimal fluoride concentration of 0.7 milligrams litre (mg/L) or 0.7 ppm as this provides the best balance of protection from dental caries while limiting the risk of dental fluorosis.¹³ The calculation of this fluoride concentration takes account of a number of considerations including fluoride from other sources.

Findings

A number of potential adverse health effects have been suggested as having links with artificially fluoridated water; the latter is also commonly known as CWF. Many adverse health effects associated with fluoride in water have only been reported in areas where the levels of naturally occurring fluoride in the water are at levels higher than those occurring in CWF. The health effects identified in the peer-reviewed literature found during the HRB review team search process and on which evidence could be retrieved, were: musculoskeletal effects; intelligence quotient (IQ); cancer; cardiovascular disease (CVD), and other potential health effects comprising kidney disorders; endocrine disorders; the immune system; birth defects; and all-cause mortality.

Musculoskeletal effects

Concerns about fluoride's effects on the musculoskeletal system focus on bone mass density, skeletal fluorosis and bone fracture. Fluoride is readily incorporated into the crystalline structure of bone, and accumulates over time. Fluoride increases bone density and appears to exacerbate the growth of osteophytes present in the bone and joints, resulting in joint stiffness and pain. In severe cases, it progresses, causing skeletal fluorosis, which is a bone and joint condition associated with prolonged exposure to high concentrations of fluoride. Skeletal fluorosis is typically seen in countries with high levels of natural fluoride in groundwater (known as endemic fluoride regions). These countries experience fluoride levels which can be up to 12 times higher than the level in CWF schemes. The Scientific Committee on Health and Environmental Risks (SCHER), an official EU body, published a report on water fluoridation in 2011.¹⁴ On the subject of skeletal fluorosis it stated the following: *'Skeletal fluorosis is a pathological condition resulting from long-term exposure to high-levels of fluoride. Skeletal fluorosis, in some cases with severe crippling, has been reported in individuals residing in India, China and Africa, where the fluoride intake is exceptionally high, e.g. due to high concentration of fluoride in drinking water and indoor burning of fluoride-rich coal resulting in a high indoor fluoride air concentration. In Europe, skeletal fluorosis has only been reported in workers in the aluminium industry, fluorospar processing and superphosphate manufacturing.'*

Musculoskeletal effects and water fluoridation, up to 2006

The following section describes the findings on the effects of water fluoridation in relation to musculoskeletal effects. The two index reviews report the findings of the scientific literature up to 2006 (Table 2). The index reviews evaluated papers on bone fracture and bone development effects. Neither paper reported on skeletal fluorosis specifically.

The authors of the **York review**² conclude that water fluoridation at levels aimed at preventing dental caries has little effect on fracture risk – either protective or deleterious. A total of 18 studies investigated the association of hip fracture with water fluoride level, making 30 analyses (e.g., men only, women only, both sexes combined). Fourteen analyses found the direction of the association between water fluoridation and hip fracture to be positive (lower incidence of hip fracture with increased water fluoride level). Of these 14 analyses, five were statistically significant associations. Thirteen analyses found the direction of association to be negative (higher incidence of hip fracture), but only four of these found a statistically significant effect. Three additional analyses did not find any association. Three of the 18 studies found the direction of association positive in women, but negative in men, and one study found a negative effect in women and a positive effect in men. A total of 12 studies examined fractures at other sites. There were no definite patterns of association for any of the fractures.²

Three studies were included in the **York review**,² which examined the effects of water fluoridation on outcomes related to bone development. Two studies of otosclerosis (excessive growth of bone in middle ear, causing deafness) reported a beneficial effect of fluoridation, although no statistical analysis was presented. The study of slipped epiphyses (fractures which result in the femoral head "slipping" off the femoral neck) found the

direction of association to be positive (a protective effect) in girls, and negative (increased risk) in boys, but neither of these was statistically significant at the 5 per cent level.

The **Australian NHMRC review**³ supports the conclusion of the York review on bone fractures, although the additional primary studies included by them suggest that optimal fluoridation levels of 1 ppm may indeed result in a lower risk of fracture when compared to excessively high levels (well beyond those experienced by CWF).³ One of their additional studies also indicated that optimal fluoridation levels may also lower overall fracture risk when compared to no fluoridation (the latter was not the case when hip fractures were considered in isolation).

The authors of the two systematic review index reports concur that water fluoridation at the levels used in CWF to prevent dental caries has little effect on fracture risk – either protective or deleterious.

Table 2: Musculoskeletal effects of water fluoridation up to 2006: results of two index reports

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Index authors' conclusion
McDonagh et al.² (York review) (2000) Level of evidence: II/III/IV HRB authors' opinion of this index review: high quality	27 studies on fracture (4 prospective cohort, 6 retrospective cohort, 15 ecological, 1 case-control, 1 with both case-control and ecological study design). 2 other studies were excluded from analyses. Three additional studies on bone development	Water fluoridation (or level nearest to 1 ppm)	No water fluoridation (or lowest water fluoride level)	Fracture Bone development (two otosclerosis, one slipped epiphysis)	The original authors report that there is no consistent indication of either a harmful or protective effect of water fluoridation on fracture risk. The original authors report that there was no statistically significant indication of either a protective or harmful effect on bone development.
NHMRC³ (2007) Level of evidence: III/IV HRB authors' opinion of this index review: high quality	Six studies (three systematic reviews, three original studies)	Artificial water fluoridation or water with naturally occurring high levels of fluoride	No water fluoridation or a lower level of water fluoridation	Fracture (also bone mass density in two systematic reviews)	The Australian NHMRC review supports the conclusion of the York review on bone fractures, although the additional primary studies suggest that optimal fluoridation levels of 1 ppm may indeed result in a lower risk of fracture when compared to excessively high levels. One of their additional studies indicated that optimal fluoridation levels may also lower overall fracture risk when compared to no fluoridation (the latter was not the case when hip fractures were considered in isolation).

Musculoskeletal effects and water fluoridation in non-endemic fluoride or CWF areas, 2006–2014

Five published studies post-2006 (Table 3) examining water fluoridation and musculoskeletal malformations were identified by the search. Two of these, **Nasman *et al.***¹⁵ and **Public Health England**,¹⁶ (PHE) studied associations between CWF and bone fracture.

Table 3: Original studies of effects of lower levels of water fluoridation (artificial and natural) on musculoskeletal effects, 2006–2014

Citation, level of evidence, study quality	Location	Study type	Number of study subjects, age range	Exposure	Comparator	Outcomes	Research study authors' conclusion
Nasman <i>et al.</i> ¹⁵ (2013) Level of evidence: III-2 HRB authors' opinion: moderate-quality study to prove a theory and the implementation of the study design is adequate.	Sweden	Retrospective cohort study	473,277	Naturally occurring fluoride in the water	Individual drinking water fluoride exposure was estimated and stratified into four categories: 1) low 0.3 to 0.69mg/L 2) medium 0.7 to 1.49mg/L; and 3) high >1.5mg/L compared to 4) controls very low < 0.3mg/L	Hip fracture	Overall, the authors found no association between chronic fluoride exposure and the occurrence of hip fracture.
Public Health England ¹⁶ (2014) Level of evidence: IV HRB authors' opinion of this index review: moderate-quality study to create a theory, but not suitable to test it	England	Ecological study	Population based	CWF fluoride in drinking water at small-area level. CWF: in parts of England, the level of fluoride in the public water supply has been adjusted to 1mg/L (1 ppm). Currently, around six million people live in areas with CWF, and they were compared to people living in areas with no CWF.	Non-fluoridated small areas	Does the incidence rate of hip fracture differ in fluoridated areas compared to non-fluoridated areas both before and after controlling for confounding factors?	There was no evidence of a difference in the rate of hip fractures between fluoridated and non-fluoridated areas when the analysis was controlled for confounders. Overall, the authors found no association between chronic fluoride exposure and the occurrence of hip fracture.

Table 3 continued

Citation, level of evidence, study quality	Location	Study type	Number of study subjects, age range	Exposure	Comparator	Outcomes	Research study authors' conclusion
<p>Chacra <i>et al.</i>¹⁷ (2010)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this study: moderate quality to describe a situation and not an adequate study design to test a theory and infer causality</p>	Canada	Cross-sectional survey	92 femoral heads from Toronto and Montreal	An examination of effect of CWF on bone outcomes such as size, overall strength, hardness and bone mineralisation	Bone samples not exposed to CWF from Montreal compared to bone samples exposed to CWF in Toronto	The fluoride content, microhardness and mean density of the cancellous cores	A weak relationship was found between fluoride exposure and accumulated fluoride ($p < 0.001$) but levels, although higher, were within normal limits; the physical characteristics of bone, including stress, resulted in $R^2 = 0.05$ and this means that less than 5 % of the relationship may be attributed to fluoride.
<p>Levy <i>et al.</i>¹⁸ (2014)</p> <p>Level of evidence: II</p> <p>HRB authors' opinion: moderate-quality study and suitable to test a theory</p>	USA	Prospective cohort study	358 (25%) of a cohort of 1,382 newborns	Fluoride intake	Associations of average daily fluoride intake from birth to age 15 years with dual-energy x-ray absorptiometry bone outcomes	Relationships between daily fluoride intake and adolescents' bone measures	The findings suggest that fluoride exposures at the typical levels for most US adolescents in fluoridated areas do not have statistically significant effects on bone mineral measures.
<p>O'Sullivan and O'Connell¹⁹ (2014)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion: moderate-quality study, but not suitable to test a theory</p>	Ireland	Ecological study	A nationally representative sample of 4,977 people aged 50 and older who participated in the Irish Longitudinal Study on Ageing (TILDA)	Fluoride intake through CWF compared to non-fluoridated community water schemes	Match data from TILDA with 2006 Census data on the type of water supply in the local area, in order to assess the relationship between water fluoridation and bone density in older adults.	The bone mass density of the respondents' non-dominant foot was measured using quantitative ultrasound. The apparatus for heel evaluation measures, both broadband ultrasound attenuation and speed of sound, are used to calculate bone stiffness index (SI) which is a reflection of bone mass density	The authors report that there was no statistically significant relationship between the proportion of households with and without a fluoridated water supply and their bone health.

Nasman¹⁵ et al. examined the association between hip fracture and long-term exposure to drinking water containing fluoride in Sweden, using a well-designed population-based retrospective cohort study. All individuals born in Sweden between 1 January 1900 and 31 December 1919, and who were alive and living in their municipality of birth at the start of follow-up, were eligible for this study. Information on the study population (n = 473,277) was linked to the Swedish National In-Patient Register, the Swedish Cause of Death Register, and the Register of Population and Population Changes. The authors found no association between chronic fluoride exposure and the occurrence of hip fracture. In the very low-exposure group (or control group) the incidence of hip fracture was 810 per 100,000 person-years; in the low-exposure group the incidence of hip fracture was 670 per 100,000 person-years; in the medium-exposure group the incidence was 600, and in the high-exposure group the incidence was 860. Using the very low fluoride group as the reference group, the hazard ratios for hip fracture were 0.97 (0.94–0.99) for the low fluoride group; 0.97 (0.94–1.00) for the medium fluoride group, and 0.98 (0.93–1.04) for the high fluoride group, indicating that the incidences of hip fracture in the medium and high groups was the same as in the reference group, because they include one in the 95 per cent confidence intervals. The presence of fluoride in water is protective in the low fluoride group, as the confidence intervals around the results are below one and do not include one. The HRB authors assign a level III, as this study is a retrospective cohort study. Due to the limitations in methodology, the HRB authors rate this as a moderate-quality study.

The Public Health England¹⁶ study monitored the health effects of water fluoridation through the use of data to compare rates of selected indicators in fluoridated versus non-fluoridated areas in England. However, while this type of study is used to create a theory that there may be an association between fluoride and health outcomes, this cannot be used to infer causality. In the Public Health England study, areas supplied by water with adjusted fluoride levels are referred to as ‘fluoridated’ and those that are not as ‘non-fluoridated’. In the same study, ‘naturally fluoridated’ areas refers to areas with water that is naturally fluoridated to a level close to that hoped to be achieved by CWF schemes. The authors examined the number of hip fracture inpatient episodes per lower super output areas (LSOAs) in England between April 2007 and March 2013 recorded in hospital episode statistics (HES) as the first or second diagnosis, and coded as S 72.0; S72.1; S72.2. Duplicates, as evaluated by the unique HES identification number, were removed. A priori confounding variables examined were age (proportion of population above 65 years old) and gender (proportion of the population – male), both obtained from 2010 Office of National Statistics (ONS) mid-year estimates at 2001 LSOA level. In addition, they controlled for deprivation (measured by Index of Multiple Deprivation (IMD) 2010) and ethnicity (proportion of the population – white) from ONS 2011 census data at the 2011 LSOA level. Recent ethnicity estimates used as statistically significant changes are likely to have occurred between the 2001 and 2011 censuses, although this approach was unable to allocate a variable status to any LSOA that underwent a boundary change between these years. (LSOAs may be changed in order to maintain their characteristics of an average of roughly 1,500 residents and 650 households per LSOA.) There are now 34,753 LSOAs in England and Wales. The unadjusted or crude rate of emergency consultant inpatient episodes with hip fracture between 2007 and 2013 was 119 per 100,000 person-years at risk (PYAR) in CWF lower super output areas (LSOAs) compared to 111 per 100,000 PYAR in non-fluoridated areas LSOAs; the crude rate of hip fracture episodes was 7.2 per cent higher (95 per cent CI 4.9 per cent to 9.6 per cent; $p < 0.001$) in fluoridated compared to non-fluoridated LSOAs. However, most of this difference was accounted for by the confounding factors of age, gender, deprivation and ethnicity. The authors found that the rate of hip fracture was not statistically significantly different in fluoridated areas compared to non-fluoridated areas following adjustment for these confounding factors (0.7 per cent higher; 95 per cent CI -1.0 per cent to 2.4 per cent; $p = 0.42$). The HRB authors assign a level IV, as this study is an ecological study. Due to the limitations in methodology, the HRB authors rate this as a moderate-quality study.

The Nasman and Public Health England studies support the findings of the York and Australian reviews that artificially fluoridated water at levels between 0.4 to 1.5 ppm do not appear to have an observable effect on fracture risk.

Three studies published between 2006 and 2014 examined the associations of CWF with bone mineral density.

Chacra *et al.*¹⁷ examined the fluoride content and structural or mechanical properties of bone, and took direct measurement of bone tissue from individuals residing in municipalities with and without fluoridated water. This is an ecological study, which is suitable for developing theories rather than testing them to infer causality. Consistent with the epidemiological data, a weak relationship was observed between fluoride exposure, accumulated fluoride, and the physical characteristics of bone. The authors suggest that the variability in heterogeneous urban populations may be too high to determine the exact effects, if any, of low-level fluoride administration on skeletal tissue. They acknowledge that their analysis needed to control for confounding factors such as age (bone quality and toughness), gender (influence of oestrogen), and bone co-morbidity, but these data were not available to them. The HRB authors assign a level IV as this study is a cross-sectional study. Due to the limitations in methodology, the HRB authors rate this as a moderate-quality study.

Levy *et al.*¹⁸ noted that many of the studies on water fluoridation and bone effects did not have individual fluoride exposure measures, and so the authors computed intakes of fluoride through detailed history taking every four to six months, depending on the subjects' age. They assessed associations of average daily fluoride intake from birth to age 15 years for the Iowa Bone Development Study cohort members with dual-energy x-ray absorptiometry (DXA) bone outcomes (whole body, lumbar spine, and hip), controlling for known determinants (including daily calcium intake, average daily time spent in moderate-to-vigorous intensity physical activity, and physical maturity). The authors found no statistically significant relationships between daily fluoride intake and adolescents' bone measures in adjusted models. This type of study design avoids ecological fallacy and reduces bias. The HRB authors assign a level II, as this study is a prospective cohort study. The HRB authors rate this as a moderate-quality study because they were unable to follow up on a large number of the original cohort.

O'Sullivan and O'Connell¹⁹ (2014) examined some of the potential benefits and risks of water fluoridation for older adults. The sample was used to estimate associations between the percentage of households in a respondent's local area with a currently fluoridated water supply and the probability of the respondent having normal bone density. Past exposure of individuals to fluoridated water was not assessed; the prevalence of fluoridated water in local supplies was obtained from the 2006 Census of Ireland. The Census data indicated that there was considerable variation in the proportion of households with fluoridated water supplies, especially in rural areas. Bone mineral density was estimated from a heel ultrasound of each respondent. A range of individual variables, such as educational attainment, housing, wealth, age and health behaviours, was controlled for in the analysis. There was no statistically significant relationship between bone health and the proportion of households with a fluoridated water supply in an area.

This ecological study¹⁹ examines a nationally representative sample of 4,977 people aged 50 and older who participated in The Irish Longitudinal Study on Ageing (TILDA) survey. There was no sample size calculation or rationale for the sample size or effect size. The exposure is based on a person's current address, and only takes account of change of address if the person grew up in a rural area or lived outside the Republic of Ireland, which may result in some misclassification bias. The TILDA researchers did, however, control for confounding factors: objectively measured body mass index; ever lived outside the Republic of Ireland; exercises at least one or two days per week; ever or currently smoking; self-report of growing up in a rural area; covered by private medical health insurance; covered by medical card; age; residing in a non-completely urbanised electoral district; value of respondents' home; highest level of education completed; self-reported poor health when aged 14; self-

reported family finances when aged 14; and local authority of residence. The HRB authors assign a level IV, as this study is a single point in time analysis of data from a cohort study. Due to the limitations in methodology, the HRB authors rate this as a moderate-quality study.

Skeletal fluorosis in areas with high levels of natural fluoridation in the water (fluoride-endemic or above a threshold of 1.5 ppm)

As skeletal fluorosis is a major topic in relation to fluoridation and frequently referred to in the debate surrounding CWF, this next section will examine scientific literature that has studied this issue. Skeletal fluorosis is a condition associated with long-term exposure to drinking water containing high levels of fluoride that would in general be much higher than levels of fluoride in CWF. Although the question posed by the DoH focuses on countries in temperate climates that have CWF, a decision was made to include the evidence on skeletal fluorosis, in order to provide a context for discussion of this issue, and also to outline the very different circumstances in countries where cases of fluorosis due to natural chemicals in the water are reported. It is important to distinguish between effects of apparent fluoride toxicity at very high intakes due to uncontrolled natural water fluoridation, and effects that may occur at much lower intakes through CWF. The 2007 Australian review included four studies which examined skeletal fluorosis in areas with natural fluoride levels in the water. These four studies were all from India, and were published since 2000. The four studies reported the prevalence of malformations such as genu valgum (knock-knee), scoliosis and kyphosis (curvatures of the spine). Chronic fluoride toxicity was prevalent in these communities with fluoride concentration in well water up to 11 ppm, and the extent of natural fluoride exposure related to the prevalence of abnormalities. These observations are further complicated by the presence of calcium and vitamin D deficiency (rickets) in some of these communities, confounders which were not controlled for in the analyses. Our search identified seven studies published since 2006 that address skeletal fluorosis.

Table 4 presents seven studies that examine the effects of natural water fluoridation on skeletal fluorosis in fluoride-endemic areas.

Table 4: Original studies of effects of natural water fluoridation on skeletal fluorosis in fluoride-endemic areas (above a threshold of 1.5 ppm), 2006–2014

Citation, level of evidence, study quality	Location	Study type	Number of study subjects, age range	Exposure	Comparator	Outcomes	Research study authors' conclusion
Fewtrell <i>et al.</i>²⁰ (2006) Level of evidence: IV HRB authors' opinion of this study: low quality to describe a situation; not adequate study design to test a theory and infer causality	Global	Ecological study	Population based	Estimated levels of fluoride in drinking water	N/A	To estimate globally the level of fluoride in drinking water; estimate the number of the population exposed to levels of fluoride >1.5mg/L by WHO region; to estimate the disease burden in disability-adjusted life years (DALYs) per 1,000 population	The global burden of diseases was estimated using models to predict fluoride groundwater levels and skeletal fluorosis. The estimates were calculated by region and were between 1 and 20 DALYs per 1,000. The highest estimate was in China and the lowest estimates were in Kyrgyzstan, Niger and Senegal. The authors reported that the estimate is unlikely to be precise due to a number of data gaps.

Table 4 continued

Citation, level of evidence, study quality	Location	Study type	Number of study subjects, age range	Exposure	Comparator	Outcomes	Research study authors' conclusion
<p>Isaac <i>et al.</i>²¹ (2009)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this study: moderate quality to describe a situation. The authors do not test any theories.</p>	India	Cross-sectional survey	416 children with severe caries and local debris on teeth excluding children with deformities of the lower limb	Exposure to natural fluoride in drinking water: levels between 1 and 2.7 mg/L	N/A	Of 416 children, 24% had dental fluorosis, 11% had genu valgum, 21% had goitre (iodine deficiency), 10% had conjunctival xerosis (vitamin A deficiency). 100 children (24%) had high urinary levels of fluoride; 90% of water consumed by the residents contained high levels of fluoride.	The authors conclude that waterborne fluorosis is endemic in Kaiwara village. Endemic prevalence of fluorosis among school children was characterised by dental mottling, genu valgum, endemic goitre, and xerosis of the conjunctiva.
<p>Pandey²² (2010)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this study: low quality to describe a situation. The author does not test any theories.</p>	Central India	Cross-sectional survey	805 participants, 362 males and 443 females, all ages	Fluoride in drinking water	N/A	Overall prevalence of fluorosis was 13.7%. Prevalence of dental fluorosis was 8.2%. Both skeletal and dental fluorosis was more common in males. Dental fluorosis was higher in the 8–45 years age group, whereas prevalence of skeletal fluorosis increased with age. Genu varum (bow legs)(38.1%) and genu valgum (6.3%) were the common skeletal deformities.	The author concludes that the prevalence of overall fluorosis in the village hamlets did not seem to be linked with the level of fluoride in the drinking water, as no linear trend or dose-response was demonstrated.

Table 4 continued

Citation, level of evidence, study quality	Location	Study type	Number of study subjects, age range	Exposure	Comparator	Outcomes	Research study authors' conclusion
<p>Shorter <i>et al.</i>²³ (2010)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this study: moderate quality to describe a situation. The authors do not test any theories.</p>	Two villages in the Hai District of northern Tanzania, in which naturally occurring fluoride in the water has been identified as a problem	Cross-sectional survey	157 children living in areas with naturally occurring fluoride in the drinking water (i.e., in Tindigani and Mtakuja)	68% of children in Tindigani accessed very high-fluoride water, and 88% of children in Mtakuja accessed high-fluoride water.	N/A	One-quarter of the children in Mtakuja and 31% of children in Tindigani had skeletal deformities. More than 90% of children in both villages had dental fluorosis.	Skeletal fluorosis and dental fluorosis are major problems in this area. Deformities relating to skeletal fluorosis are common, but the reasons for individual susceptibility remain unclear and may include a low-calcium diet, ingestion of magadi (local salt) with high fluoride. Other reasons for susceptibility may include genetic factors.
<p>Rawlani <i>et al.</i>²⁴ (2010)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this study: low quality to describe a situation; not adequate study design to test a theory and infer causality</p>	Vidharbha Region, India	Cross-sectional survey	204 subjects in 50 families	The concentrations of fluoride in two different areas of the study village were 4 and 4.5 ppm.	N/A	Fifty families were screened (number of people in families not stated). 204 subjects were found to have dental and skeletal fluorosis and were included in the study. Biochemical, haematological and radiological assessments were done.	Prevalence of fluorosis in families was estimated. Of the 204 cases, 116 (56.8%) were males and 88 (43.1 %) were females. RBC count in male cases was 5.03 ± 0.49 , while in female cases it was 4.70 ± 0.47 . The alkaline phosphate level in male cases was 289.7 ± 149.1 ; in female cases it was 276.7 ± 165 . Radiological findings show thickening of inner and outer tables of skull bone in 83.9% of cases; 7.8% of cases were suffering from barrowing of long bone.
<p>Han <i>et al.</i>²⁵ (2011)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this study: low quality to describe a situation and not an adequate study design to test a theory and infer causality</p>	Two Korean cities	Cross-sectional survey	565 had a bone density scan, 99 did a 24-hour urine test, and 70 provided toenail samples.	Siwha, with naturally occurring fluoride in its water system, and Ansan, with fluoridated water system Levels not reported	To compare BMD of adults exposed to fluoride from various sources in the environment by individual level	BMD with fluoride level in serum, 24-hour urine, fingernails, and toenails. BMD has a tendency to decrease as the toenail fluoride level increases ($p = 0.082$).	The authors state that exposure to fluoride appears to have no impact on bone mineral density. However, the relationship of fluoride exposure with bone mineral density by individual and area level requires further investigation.

Table 4 continued

Citation, level of evidence, study quality	Location	Study type	Number of study subjects, age range	Exposure	Comparator	Outcomes	Research study authors' conclusion
Arvind <i>et al.</i> ²⁶ (2012) Level of evidence: IV HRB authors' opinion of this study: moderate quality to describe a situation. The authors do not test any theories	Karnataka , south-west India	Cross-sectional survey	1,544 school children of 1st to 7th standard in the rural field practice area of a medical college	Natural fluoride in drinking water: fluoride levels in natural drinking water in the study locations 0.5–3.8mg/L	N/A	Of the 26 water samples analysed, 69% revealed fluoride above the permissible limit (1mg/L).	Findings of the present study reveal a high prevalence of dental fluorosis and genu valgum among schoolchildren, and high fluoride level in the water. Further studies are needed in order to evaluate the other risk factors and reasons for gender differences.

Fewtrell *et al.*²⁰ (2006) estimated the global burden of disease (from dental and skeletal fluorosis) due to natural fluoride in drinking water (in fluoride-endemic areas above a threshold of 1.5 ppm) by combining exposure-response curves for dental fluorosis ($R^2 = 0.63$) and skeletal fluorosis ($R^2 = 0.85$) (values derived from published data) indicating that fluorosis was more likely where populations were exposed to natural fluoride. The regression analysis identified that the most effective predictors of natural fluoride concentration in groundwater were: value of gross national product (GNP); mean annual precipitation or rainfall and where the country is located, specifically 'Sear D (which includes India)' or 'Wpr B (which includes China)'. There are few data available in the literature to estimate population exposed to elevated levels of fluoride. Research conducted in India estimated that 6.9 per cent of the population is at risk of exposure to high levels of natural fluoride in drinking water. This has been used as the upper estimate of exposure. In this study, 1 per cent has been assumed as the lower estimate of exposure and 3 per cent as a mid-point estimate. The number of people estimated to be affected by dental and skeletal fluorosis in each region was as follows: Wpr B China (10,887,000), followed by Sear D India (7,889,000), Emr D Pakistan (517,000) and Afr E Ethiopia (184,000). The mid-point estimate of disease burden in DALYs per 1,000 population by WHO region was calculated for skeletal fluorosis; the estimates suggest that the greatest proportion of disease burden due to skeletal fluorosis is seen in Wpr B; Afr E (which includes Eritrea, Tanzania, Ethiopia, Kenya and South Africa) and Sear D. The authors reported that the estimate is unlikely to be precise due to a number of data gaps. The main point of this study appears to be that levels of dental fluorosis seems to correlate with levels of naturally occurring fluoride in drinking water and in fluoride-endemic areas where the burden of disease is very high. The study itself is poorly presented and adds little that explains the aetiology of the disease. The level of evidence is IV and the study quality is low.

Isaac *et al.*²¹ (2009) investigated the clinical manifestations of waterborne fluorosis from exposure to natural fluoride in drinking water by conducting a cross-sectional study of schoolchildren aged 6–13 years between first and seventh standard in Kaiwara village, Karnataka State, India. The study participants included 218 males and 198 females. No rationale was provided for the size of the sample chosen. Fluoride concentration in natural drinking water in the study locations was found to be between 1–2.7 mg/L or ppm. This is a descriptive study and there is no comparison group. The study was rated as moderate quality. The authors found that 24 per cent of schoolchildren had dental fluorosis, 11 per cent had genu valgum (knock-knee), 21 per cent had goitre (iodine

deficiency), and 10 per cent had xerosis of the conjunctiva (vitamin A deficiency). One hundred children (24 per cent) had high urinary levels of fluoride and 90 per cent of water consumed by village residents contained high levels of fluoride. The authors conclude that waterborne fluorosis is endemic in Kaiwara village. The level of evidence is IV.

The aim of the **Pandey *et al.***²² (2010) study was to estimate the prevalence of dental and skeletal fluorosis among the population and to assess the relationship between drinking water fluoride levels and prevalence of fluorosis. In 2009, they conducted a cross-sectional census survey in Gureda village, India. Water fluoride levels in the village ranged from 0.2–7.8 ppm. The survey included 805 individuals, 362 males and 443 females. The prevalence of dental fluorosis and skeletal fluorosis was assessed, based on clinical examinations of individuals. Drinking and eating habits of individuals were recorded using a checklist. Water fluoride levels of prime water sources were also determined by laboratory examination. The prevalence of dental fluorosis was 8.2 per cent. Both skeletal fluorosis and dental fluorosis were more common in males. Dental fluorosis was higher in the 8–45 years age group, whereas the prevalence of skeletal fluorosis increased with age. Genu varum (bow legs) (38.1 per cent) and genu valgum (6.3 per cent) were the common skeletal deformities. The authors describe the prevalence of bone disorders and levels of fluoride in the water, but do not correlate the two. In this low-quality study, the authors do not state whether the investigators were trained with respect to standardising the investigators' diagnostic skills. The level of evidence is IV.

Shorter *et al.*²³ (2010) conducted a cross-sectional study to assess the prevalence of dental fluorosis and deformities due to skeletal fluorosis in children attending school in the two villages in northern Tanzania. The study was judged to be of moderate quality. The subjects were 157 children from Tindigani (water source: well 23.5 ppm; borehole 25 ppm; pipe 0.4 ppm; surface 0.2 ppm; 68 per cent accessed very high-fluoride water (above a threshold of 1.5 ppm) and 118 children from Mtakuja (water source: well 5.4 ppm; pipe 0.4 ppm; 88 per cent accessed high-fluoride water). There was no rationale provided for the sample size calculation. The principal investigator was trained to score dental fluorosis, but the health staff had no specific training to ensure consistent categorisation of leg deformities. The area where the study was conducted had been identified in a pilot study as having high levels of naturally occurring fluoride in the drinking water (above the WHO-recommended level). The authors found that one-quarter of the children in Mtakuja and 31 per cent of children in Tindigani had skeletal deformities. More than 90 per cent of children in both villages had dental fluorosis. The authors did not examine the factors that may have led to fluorosis. There was no control for confounding factors; the authors did not report an analysis by nutrition status, age, or gender, and did not split subjects by access to high- and low-fluoride water sources. The level of evidence is IV.

Rawlani *et al.*²⁴ (2010) conducted a cross-sectional study to assess the prevalence of both skeletal fluorosis and non-skeletal fluorosis in cases with either skeletal or dental fluorosis in a fluoride-endemic village. The concentrations of fluoride in two different areas of the study village were 4 and 4.5 ppm (above a threshold of 1.5 ppm). Fifty families were screened for fluorosis. There were 204 fluorosis cases in the 50 families (number of people not stated in the 50 families). Of the fluorosis cases, 56.8 per cent (116) were male and 43.1 per cent (88) were female patients. This is a simple descriptive study that is concerned with the consequences of disease rather than the cause of the disease. The study is low quality and does not inform the objective of this paper. The level of evidence is IV.

Han *et al.*²⁵ (2011) recruited participants from two Korean cities: Siwha, with naturally occurring fluoride in its water system (level not provided), and Ansan, with community fluoridated water since 2001 (level not provided). All subjects took part in bone mineral density examination, which was measured by means of dual-energy x-ray absorptiometry at left heel of the subject. In order to ascertain a more detailed picture of individual fluoride exposure, serum (n = 565), 24-hour urine (n = 99), fingernail (n = 70), and toenail (n = 70) samples were

collected. The level of fluoride in the samples was measured using fluoride ion electrode (Orion Research EA940). Linear regression analysis was performed. The outcome variable was bone mineral density, and explanatory variables were fluoride concentration in the serum, urine, fingernail, and toenail samples. Confounders were age, gender, residential area, and monthly household income. The results showed no relationship between bone mineral density and fluoride level in serum, 24-hour urine, fingernail, and toenail samples. Bone mineral density had a (non-significant) tendency to decrease as the toenail fluoride level increased ($p = 0.082$). The authors conclude that, based on the results of the study, exposure to fluoride appears to have no impact on bone mineral density. However, they also state that the relationship between fluoride exposure and bone mineral density by individual and geographical area requires further investigation. The authors did not specify why they chose a sample size of 565 and no reference was made to the generalisability of study findings to the population. Nor were any details given about the study population, or attempts to minimise bias. There is an assumption that the participants from the two Korean cities had lived there all their lives. It is not stated whether the researchers examining the outcomes were aware of fluoridation levels in the cities. The confounders of age, gender, residential area, and monthly household income were identified, but it is not clear that they were controlled for in the analysis. The level of evidence is IV, and the study quality is low.

Arvind et al.²⁶ (2012) performed a cross-sectional study on schoolchildren of first to seventh standard (aged 6–13 years) in the rural area used for field practice by a medical college. In the study the levels of natural fluoride in the drinking water were between 0.5–3.8 mg/L. This study was judged to be of moderate quality. Children were examined for dental fluorosis and genu valgum. Drinking water samples were also tested for fluoride levels. The proportions of children with dental fluorosis and genu valgum were calculated by severity, age and sex. Of the 1,544 children examined, 42.1 per cent and 8.4 per cent had dental fluorosis and genu valgum, respectively. Prevalence of very mild dental fluorosis and moderate-grade genu valgum were high compared to other categories. Prevalence rates increased with age ($p < 0.05$) and were higher among girls (45.2 per cent) compared to boys (39.1 per cent) ($p < 0.05$). Of the 26 water samples analysed, 18 samples (69.2 per cent) revealed fluoride content above the permissible limit (>1.5 ppm). The authors conclude that the findings of their study reveal a high prevalence of dental fluorosis and genu valgum among schoolchildren in high water fluoride-level areas. The authors describe the situation rather than trying to assess risk factors, and they add that further studies are needed in order to evaluate the other risk factors and reasons for gender differences. The level of evidence is IV.

International reports and expert bodies' conclusions on musculoskeletal effects

Many overviews of fluoridation and its effects were identified in the HRB authors search and form the basis of the grey literature identified to inform this report.

A report titled *Fluoride in drinking water: A scientific review of the EPA's standards*²⁷ by the **United States National Research Council (NRC)** of the National Academy of Sciences reviewed research on various health effects from exposure to fluoride, including studies conducted in the 10 years prior to publication of this report in 2006. The NRC authors state that concerns about fluoride's effects on the musculoskeletal system continue to be focused on skeletal fluorosis and bone fracture. Since the previous 1993 NRC review of fluoride, two pharmacokinetic models were developed to predict bone concentrations from chronic exposure to fluoride. Predictions based on these models were used in the committee's assessments of the effects of fluoride. The purpose of the 2006 NRC report was to analyse the health effects of the maximum concentrations of fluoride prescribed under USA EPA standards, namely the Maximum Concentration Level Goal (MCLG) of 4 mg/L (ppm) and the Secondary MCL of 2 mg/L (ppm). These studies were carried out to re-evaluate American protection levels, not to establish a standard for the prevention of dental caries. These American maximum concentration levels are far higher than their CWF level (0.7 ppm or mg/L)¹³ or the levels of fluoride added to drinking water in

Ireland's CWF scheme (0.6–0.8 ppm). The conclusions of the NRC should therefore be interpreted with caution, as they examine levels of fluoride in water that are not comparable to levels in Ireland. It is important to make the distinction here that the USA EPA's drinking water guidelines are not making recommendations about adding fluoride to drinking water. The USA EPA's guidelines have determined the maximum allowable concentrations in drinking water intended to prevent toxic or other adverse effects that could result from exposure to fluoride.

With respect to skeletal fluorosis, the **NRC** concluded that, before any conclusions can be drawn, more research would be needed in order to clarify the relationship between fluoride ingestion, fluoride concentrations in bone, and stage of skeletal fluorosis. With respect to bone fractures, overall, there was consensus among the NRC committee that there is scientific evidence that under certain conditions fluoride can weaken bone and increase the risk of fractures. The majority of the committee concluded that lifetime exposure to fluoride at drinking water concentrations of 4 mg/L or higher is likely to increase fracture rates in the population, compared with exposure to drinking water fluoride concentrations of 1 mg/L (equivalent to USA CWF levels), particularly in some demographic subgroups who are prone to accumulate fluoride into their bones (e.g., people with renal disease). However, 3 of the 12 members judged that the evidence only supports a conclusion that the MCLG *might not be* protective against bone fracture. Those members judged that more evidence would be needed in order to conclude that bone fractures occur at an appreciable frequency in human populations exposed to fluoride at 4 mg/L and that the MCLG is *not likely* to be protective.²⁷

Another report of relevance is *Water fluoridation: an analysis of the health benefits and risks* produced by the **National Institute of Public Health in Quebec, Canada**²⁸ in 2007. This report mainly uses the NRC report to draw its conclusions in the area of musculoskeletal health, and concludes that it is highly unlikely that the concentration of 0.7 mg/L recommended in Quebec would be associated with musculoskeletal effects. Nonetheless, they point out that some poorly documented research hypotheses, such as the link between renal insufficiency and bone fluoride retention, are of interest, and they suggested these merit further investigation.

The European Food Safety Authority (EFSA) published a report in 2009²⁹ following a request from the European Commission. The report addresses the scientific substantiation of health claims about fluoride with regard to the maintenance of tooth mineralisation and maintenance of bone. The EFSA panel examined dietary intake of fluoride. The panel concluded that a cause-effect relationship has been established between the dietary intake of fluoride and maintenance of tooth mineralisation. They also concluded that a cause-effect relationship has not been established between the dietary intake of fluoride and maintenance of normal bone.

In response to the 2006 National Research Council (NRC) report, in 2010 the **U.S. EPA Office of Water** began a reassessment of the dose-response associated with the effects of ingested fluoride on severe dental fluorosis and bone structure.³⁰ The available data led the NRC to conclude that exposure to concentrations of fluoride in drinking water at 4 mg/L and above are suggestive of and appear to be positively associated with an increased relative risk of bone fractures in susceptible populations when compared with populations exposed to 1 mg/L. However, the USA EPA maintained that there was insufficient data to conclude that this increase in relative risk would also apply if comparisons were made with groups exposed to negligible fluoride concentrations, or if comparisons were made based on total fluoride intake rather than on the basis of drinking water concentrations.

The 2010 ***Guidelines for Canadian Drinking Water Quality: Guideline Technical Document***³¹ on fluoride concluded that skeletal fluorosis is the most serious adverse health effect clearly associated with prolonged exposure to high levels of fluoride in drinking water. Skeletal fluorosis can occur at very high exposure levels, and has rarely been documented in Canada. They also conclude that the weight of evidence from all currently

available studies does not support a link between exposure to fluoride in drinking water up to 1.5 mg/L and any adverse health effects.

The **Scientific Committee on Health and Environmental Risks**¹⁴ (SCHER) 2011 report concluded that the occurrence of skeletal fluorosis linked to high levels of fluoride in drinking water has not been reported in the EU. SCHER also concludes that there are insufficient data to evaluate the risk of bone fracture at the fluoride levels seen in areas with fluoridated water.

A review of the scientific evidence on fluoridation on behalf of the **Royal Society of New Zealand and the Office of the Prime Minister's Chief Science Advisor**³² published in August 2014 was identified by our information specialist. While this report was published after our search had concluded, a decision was made to include it as it is a comprehensive report that would likely contain all recent relevant literature on the topic and therefore could be used to validate our findings. The authors state that *'because fluoride accumulates in bones, the risk of bone defects or fractures has also been extensively analysed. While there are published studies suggesting that such associations exist, they are mostly of very poor design (and thus of low scientific validity) or do not pertain to CWF because the fluoride levels in question are substantially higher than would be encountered by individuals drinking intentionally fluoridated water.'* They conclude that, based on the available evidence, there is no appreciable risk of bone fractures arising from CWF.

Summary on musculoskeletal effects

Bone fracture, bone mass density and skeletal fluorosis are the musculoskeletal effects associated with fluoride covered in the literature.

Bone fracture incidence and bone mass density in non-endemic or CWF areas

The literature was reviewed to determine if artificial water fluoridation increases the risk of bone fracture. Over the years, a number of community-level studies compared rates of fracture, specific to age and gender, between fluoridated water and non-fluoridated water areas. Some of these studies indicated that exposure to fluoridated water increased the risk of fracture; some studies indicated that water fluoridation reduced the risk of fracture; and several studies found no effect. However, more than half of these studies were designed using an ecological approach, which means that data for individuals were based on community-level data and lacked actual exposure and outcome data specific to each individual. Focusing on the more recent original studies (2006 to mid-2014) identified by the HRB search, five studies covering the topic were identified. Two studies revealed that there was no evidence of a difference in the rate of hip fractures between fluoridated and non-fluoridated areas; one study theorised a weak relationship between fluoride exposure, accumulated fluoride, and the physical characteristics of bone; one American study suggests that fluoride exposure at the typical CWF levels for most US adolescents residing in fluoridated areas do not have statistically significant effects on bone mineral measures, and one Irish study concluded that there was no statistically significant relationship between bone health and the proportion of households with and without a fluoridated water supply. However, these studies were mainly ecological studies and cannot prove or disprove a causal link. A summary of the existing literature indicates that the relationship between fluoride in drinking water and bone health is inconsistent with no definitive proof of protective or harmful effects.

Skeletal fluorosis and bone mass density in fluoride-endemic areas

Studies have shown that fluoride ingestion at elevated levels (above a threshold of 1.5 ppm) appears to affect skeletal tissues (skeletal fluorosis) and these effects are more severe as exposure to naturally occurring fluoride increases. Very mild skeletal fluorosis is characterised by slight increases in bone mass density. The most severe form of this condition, 'crippling skeletal fluorosis', involves bone deformities, calcification of ligaments, pain, and immobility. In 1993, the United States National Research Council (NRC) reported that few cases of this

condition had been reported in the United States and that it was not considered a public health concern. Having examined the evidence available on skeletal fluorosis, it is clear that people consuming water containing levels of fluoride greater than the World Health Organization (WHO) permissible limits (>1.5 ppm) may be at risk of developing skeletal fluorosis. Outside of the endemic fluorosis belt, including countries with CWF, skeletal fluorosis is only seen in workers in the aluminium industry, fluorspar processing and superphosphate manufacturing. The studies published since 2006 that investigate skeletal fluorosis (4) or genu valgum (2) are mainly cross-sectional prevalence studies in fluoride-endemic areas, which do not attempt to examine risk factors or infer causality. The quality of the implementation of these studies is low to moderate, as generally they do not provide a rationale for the sample size or confidence intervals around the main outcome measure. In addition, they often contain investigator bias. A summary of the existing literature indicates that skeletal fluorosis is a health problem in areas with fluoride levels in drinking water greater than the WHO permissible limits (above a threshold of 1.5 ppm), but not in areas with CWF, such as Ireland.

Intelligence quotient (IQ) and other neurological conditions

In recent years, concern has been expressed about a possible association between fluoride in drinking water and low IQ levels. This concern has arisen largely from a group of studies conducted in China and other countries (India, Iran and Mexico) where fluoride in the water is naturally present at very high levels due to the particular type of rock formation. These areas are termed fluoride-endemic regions or countries. The aforementioned studies compare the IQ of children in areas with very high levels of naturally occurring fluoride in the drinking water to the IQ of children in areas of the same country or region with low levels of natural fluoride in the drinking water. The fluoride levels in the high fluoride group are higher than levels found in water with CWF schemes. The fluoride levels in the low-fluoride comparison group are generally drinking water with levels of fluoride similar to those found in countries with artificially fluoridated water or CWF. Therefore, studies that report findings indicating a lower IQ in children who are drinking fluoridated water are reaching this conclusion from the outcomes of analysis of children drinking water with very high levels of fluoride, compared to children who are drinking water containing fluoride at low levels similar to CWF levels. Another issue with studies from these countries is that since fluoride is naturally occurring, food may be contaminated with high levels of fluoride from the soil and from coal used to cook the food. The studies are of low quality in that they do not take full account of other factors that could also cause a lowering of IQ (also called confounders), e.g., nutritional status, socioeconomic status, iodine deficiency, other chemicals in the ground water (arsenic or lead). Apart from the levels of fluoride in the water, these countries are very different from Ireland with respect to climate, nutritional status, and socioeconomic status. Thus, their findings are not applicable to Ireland or other countries with CWF schemes.

The following section describes the findings from the index reports, followed by studies of the effects of water fluoridation in relation to IQ in non-endemic fluoride areas, followed by endemic regions, after which studies conducted on fluoride and other neurological conditions are summarised. The index reviews report the findings of the scientific literature up to 2006 (Table 5).

Intelligence quotient and water fluoridation, up to 2006

Table 5: Water fluoridation and IQ up to 2006: results of the York review

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Index authors' conclusion
McDonagh <i>et al.</i>² (York review) (2000) Level of evidence: IV Authors' opinion of this index review: high quality	Two studies (both cross-sectional)	Naturally fluoridated water	Low water fluoride level	IQ	Interpreting the results of studies of IQ is very difficult, due to the small numbers of English-language studies that met inclusion criteria, and also due to poor study quality.

The **York review** did not specifically examine the issue of fluoridated water and IQ, but this subject was included in a section on a variety of outcomes, including senile dementia, mortality, goitre, Down's syndrome and IQ. Two papers that investigated a possible association between water fluoridation and IQ were included; however, the authors did not draw any conclusions on water fluoridation and IQ. The **Australian NMHRC review** also did not

address the topic of water fluoridation and IQ specifically, but had a section in the review devoted to ‘other potential harms of water fluoridation’. This section included the results of the York review papers on IQ as part of its evaluation of the York review’s ‘other possible harm’ section, but no additional reviews or original studies on IQ were included by the Australian authors. Thus, they did not make any conclusions with regard to water fluoridation and IQ.

Intelligence quotient (IQ) and water fluoridation in non-endemic or CWF-fluoride areas, 2006–2014

Table 6: Original studies in non-endemic-fluoride areas examining effects of community water fluoridation and IQ, 2006–2014

Citation, level of evidence, study quality	Location	Type of study	Number of study subjects	Exposure	Comparator	Outcomes	Research study authors’ conclusion
Broadbent <i>et al.</i>³³ (2014) Level of evidence: II HRB authors’ opinion of this study: high quality and appropriate to test a theory	New Zealand	Prospective cohort	1,037, was the birth cohort for the period	Exposure to CWF at 0.85 ppm, and/or fluoride dentifrice and/or intake of 0.5-mg fluoride tablets assessed in early life	Non-CWF	IQ	The authors conclude that the findings do not support the assertion that fluoride in the context of CWF programmes is neurotoxic.

The search carried out for the current review identified one original paper in a non-endemic area (New Zealand) that aimed to clarify the relationship between CWF and IQ by **Broadbent *et al.*³³ (2014)**. This is a high-quality prospective cohort study of a general population sample born in Dunedin, New Zealand between 1 April 1972 and 30 March 1973 (Table 6). The study participants were followed for 38 years; their place of residence and whether it had a CWF scheme and other fluoride intake were assessed in early life (prior to age 5 years). IQ was assessed repeatedly between ages 7–13 years and at age 38 years. The authors found no statistically significant differences in IQ due to fluoride exposure. These findings held after adjusting for potential confounding variables, including sex, socioeconomic status, breastfeeding, and birth weight (as well as educational attainment for adult IQ outcomes). Their findings do not support the assertion that fluoride in the context of CWF programmes is neurotoxic. This study is one of the very few prospective cohort studies in this area, and it is important to point out that this type of design is appropriate for inferring causality.

Intelligence quotient (IQ) and water fluoridation in fluoride-endemic areas (above a threshold of 1.5 ppm), 2006–2014

Table 7: Reviews studies in fluoride-endemic areas (above a threshold of 1.5 ppm) examining effects of water fluoridation and IQ, 2006–2014

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Research study authors' conclusion
Tang <i>et al.</i>³⁴ (2008) Level of evidence: IV HRB authors' opinion of this review: low quality, as it did not apply methods correctly to complete a meta-analysis	This paper presents a systematic review of the literature concerning fluoride and IQ. Sixteen case-control studies that assessed the development of low IQ in children who had been exposed to different levels of naturally occurring fluoride earlier in their lives. The 16 studies were cross-sectional studies.	Naturally fluoridated water Exposed: medium- and high-fluorosis areas (definition not clear)	Non- or slight-fluorosis areas (definition not clear)	To investigate whether fluoride exposure increases the risk of low intelligence quotient (IQ) in China over the past 20 years	The authors completed a quantitative review of the case-control studies (actually cross-sectional studies) and found a consistent and strong association between the exposure to fluoride and low IQ. The meta-analyses estimated that the odds ratio of IQ in endemic fluoride areas compared with non- or slight-fluoride areas. The summarised weighted mean difference is -4.97 (95% confidence interval [CI] = -5.58 to -4.36; $p < 0.01$) using a fixed-effect model and -5.03 (95% CI -6.51 to -3.55; $p < 0.01$) using a random-effect model. The authors (incorrectly) conclude that children living in fluorosis areas have five times higher odds of developing low IQ than those who live in a non-fluorosis area or a slight-fluorosis area. The IQ was five points higher rather than five times higher.
Connett and Limeback³⁵ (2008) Level of evidence: IV HRB authors' opinion of this review: low quality, as it did not apply methods to complete a meta-analysis correctly	A systematic review of 20 ecological studies from endemic fluorosis areas. The 20 studies were cross-sectional studies.	Natural fluoride in water in China, Iran and Mexico in high, moderate and low areas. Exposed areas: fluoride between 0.88 ppm and 9.4 ppm	Areas with fluoride between 0.1 ppm and 2.01 ppm	To examine if fluoride exposure is associated with a decline in human intelligence quotient (IQ)	The authors conclude that while the evidence is not conclusive, they identified 20 ecological studies that suggest an association between high fluoride exposure and decreased IQ. The 20 studies were cross-sectional rather than ecological.
Bazian Ltd³⁶ (2009) Level of evidence: IV HRB authors' opinion of this review: Moderate quality, as it attempted to apply methods to complete a systematic review correctly.	A critical review of 20 studies. Study design not identified by author	Drinking water naturally high in fluoride, or high fluoride exposures from the use of high-fluoride coal for heating and drying grain	Endemic fluoride in water	Critical appraisal of selected studies reporting an association between fluoride in drinking water and IQ	The authors' appraisals found that the study design and methods used by many of the original researchers had serious limitations. The lack of a thorough consideration of confounding means that from these studies alone it is uncertain how far fluoride is responsible for any impairment in intellectual development reported.

Table 7 continued

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Research study authors' conclusion
<p>Choi <i>et al.</i>³⁷ (2012)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this review: low quality, as did not apply methods to complete a meta-analysis correctly</p>	<p>A systematic review and meta-analysis of 27 eligible epidemiological studies. (The authors do not describe the study type for each of the studies included, but they appear to be cross-sectional.)</p>	<p>Naturally fluoridated water. High fluoride exposure (19 drinking water, 1 well water, 3 coal burning, 3 fluorosis levels, 1 unspecified) compared to reference level</p>	<p>Investigate the effects of increased fluoride exposure and delayed neurobehavioral development of exposed children in high-fluoride areas, compared to children in low-fluoride areas</p>	<p>Children in low-fluoride areas</p>	<p>The authors report that the standardised weighted mean difference in IQ score between exposed and reference populations was -0.45 (95% confidence interval: -0.56, -0.35) using a random-effects model. Thus, children in high-fluoride areas had statistically significantly lower IQ scores than those who lived in low-fluoride areas. The authors conclude that the results support possible adverse effect of high fluoride on neurodevelopment.</p>
<p>Valdez-Jimenez <i>et al.</i>³⁸ (2011)</p> <p>Level of evidence: unable to assign</p> <p>HRB authors' opinion of this study: low quality as it does not describe how the literature was retrieved and synthesised</p>	<p>A review. The authors do not explicitly state the number of studies included in the review, but there are 18 references.</p>	<p>The objective of this review is to publicise information on the toxic potential of fluoride on the nervous system, with emphasis on populations exposed to the consumption of this chemical at concentrations outside the official standard.</p>	<p>Water with low fluoride</p>	<p>To investigate the health effects of prolonged ingestion of fluoride, particularly to the nervous system</p>	<p>The authors conclude that there are data showing that fluoride has toxic effects on the central nervous system, depending on the dose, age, and exposure time. The papers authors recommended that the geographical location of a given population and the quality of the water should be taken into consideration, so as to take preventive measures for its use and, in areas where the fluoride concentration exceeds 0.7 mg/L, to avoid the intake of fluoridated drinking water, fluoridated salt, and the use of fluoride toothpastes and articles containing fluoride.</p>
<p>Grandjean and Landrigan³⁹ (2014)</p> <p>Level of evidence: unable to assign</p> <p>HRB authors' opinion of this study: low quality, as it does not describe how the literature was retrieved and synthesised</p>	<p>Review update of the author's 2006 review. 115 studies in the review. A meta-analysis of 27 cross-sectional studies of children, mainly living in China, who were exposed to fluoride in drinking water. The comments with respect to fluoride are based on one 2012 study by Choi <i>et al.</i></p>	<p>None</p>	<p>None</p>	<p>The authors consider information about industrial chemicals and their potential link to developmental neurotoxicity.</p>	<p>According to the authors, the data in Choi <i>et al.</i> report suggest an average decrease in IQ of about seven points in children exposed to raised fluoride concentrations. The authors state that confounding from other substances seemed unlikely in most of these studies. Further characterisation of the dose-response association would be desirable.</p>

Table 7 presents six reviews examining the effects of water fluoridation and IQ in fluoride-endemic areas. These reviews were published between 2006 and 2014.

The review paper published by **Tang³⁴ et al.** in 2008 is a systematic review using meta-analysis: the studies included are all described by the authors of the review as case-control studies. Most of the papers were retrieved by the HRB authors; these papers were examined and found to be cross-sectional studies comparing prevalence of low IQ between what the authors title 'non-, slight- and high-fluorosis areas' in China. Tang *et al.* partly describe the primary studies, but they miscategorise the study type (as outlined above). They do not define the exposure terms using numeric cut-off points, and they do not provide the exact IQ cut-off points used to denote low, marginal and normal intelligence in each study. The term fluorosis is incorrectly used to describe water levels rather than the medical condition. Of the 18 studies, 6 reported non-significant results. The exposed group were subjects from medium- and high-fluorosis areas (definition not clear) and the comparison group were subjects from non- or slight- fluorosis areas (definition not clear). The weighted-pooled-standardised mean difference for the 18 studies was 5.03 (95 per cent CI -6.51 to -3.55) which is interpreted incorrectly as a five times higher odds of developing low IQ in the medium- and high-fluorosis areas when compared to the non-or low- fluorosis areas. The measurement in their table is IQ points and not odds ratios, as stated in the written results, discussion, conclusion and summary. The HRB authors maintain that Tang *et al.*'s conclusion should be five IQ points lower rather than five times higher odds of developing a low IQ. In addition, 11 of the studies in the Tang *et al.* review are also in the Choi *et al.*³⁷ (2012) review and, in the latter paper, the difference between IQ in the two groups is less than 0.5 of a standard deviation (approximately seven IQ points lower). Tang *et al.* attempted to control for studies with larger sample sizes.^{40, 41} One such study⁴² also had a large sample size, but was not excluded in the sensitivity analysis. There is no mention of controlling for confounding factors such as other chemicals (iodine, iron, lead and arsenic), age, sex, parental education, income, nutritional status etc. The authors' inclusion and exclusion criteria are not clear, and they do not complete a quality assessment for the primary studies, both of which are essential steps in conducting a meta-analysis. They do, however, test for publication bias, using a funnel plot, and confirm that publication bias is likely to be present. The HRB authors find this review to be of low quality for the reasons stated above and consider that it was inappropriate to complete a meta-analysis using the selected studies.

In 2008, a fluoride literature review was conducted by **Connett and Limeback³⁵**. The results of this review were presented in a poster format at the International Association for Dental Research 86th General Session and Exhibition conference in Toronto in 2008, and only an abstract of this review was published. The review examines fluoride and its effect on intelligence quotient (IQ). Elevated fluoride in drinking water was the primary variable, but studies where fluoride was elevated in the urine, as a result of pollution, were also included. The review included 20 original studies that met the author's inclusion criteria; the extent of fluoride exposure was reported in all but one of the studies, and nine studies reported urinary fluoride. The authors' findings provided inconclusive evidence. They identified 18 of the 20 studies as ecological studies. (However, the studies are cross-sectional in design). The authors acknowledged that most of the papers omitted important details (blinding, confounders, and randomisation). The inclusion and exclusion criteria were only partly described, and the primary studies were not assessed for quality. The HRB authors find this review to be of low quality for the reasons stated above, and they consider that it was inappropriate to complete a meta-analysis using the selected studies.

In 2009, **Bazian Ltd³⁶**, in a report for the Central Strategic Health Authority in England, examined primary studies from China, Mexico, Iran and India (which used cross-sectional study methods) to investigate whether high environmental exposure to fluoride or arsenic, or low exposure to iodine, were associated with lower IQ. The Bazian Ltd appraisals found *'that the study design and methods used by many of the researchers had serious limitations. The lack of a thorough consideration of confounding as a source of bias means that, from these*

studies alone, it is uncertain how far fluoride is responsible for any impairment in intellectual development seen. The amount of naturally occurring fluoride in drinking water and from other sources and the socioeconomic characteristics in the areas studied is different from the UK and so these studies do not have direct application to the local population of Southampton’. This statement also applies to Ireland; these studies cannot be applied to the situation in Ireland with regard to CWF, as the two situations are far from comparable. Bazian Ltd describes the lack of control for confounding as a major issue, and the company identifies the confounders as the following: differences in environmental arsenic and iodine in water, parental education, and socioeconomic measures between the populations. They further state that it is possible that some or all of the impairment in IQ can be explained by these or other unmeasured or unknown factors. They go on to say that the authors of one of the systematic reviews (Tang *et al.*³⁴) examined by them combined the results of these confounded cross-sectional surveys into summary measures by meta-analysis in a way that is not statistically appropriate or valid, and the authors’ interpretation of the results is incorrect. Bazian Ltd point out that the level of fluoride found in the high-fluoride areas in the research was generally higher than that intended for use in water fluoridation schemes, or was confounded by varying levels of other chemicals in drinking water that are not a problem in the UK (arsenic and lead). Bazian Ltd also highlight that other sources of fluoride exposure that do not exist in the UK exist in these settings – for example, burning high-fluoride coal and eating contaminated grain, which can substantially contribute to fluoride exposure. The HRB authors find this review to be of moderate quality.

Choi *et al.*³⁷ in a 2012 systematic review using meta-analysis included cross-sectional studies comparing prevalence of low IQ between low- and high-fluoride areas (19 drinking water, 1 well water, 3 coal burning, 3 fluorosis levels (not water levels) and 1 with inadequate details to specify source). There was some overlap between the quantities of fluoride in the areas classified as high-exposure areas and the areas classified as reference areas. They performed the Cochran test for heterogeneity between studies, Begg’s funnel plot, and the Egger test to assess publication bias; in addition, they conducted meta-regressions to explore sources of variation in mean differences among the studies. The weighted-pooled-standardised mean difference for the 27 studies was -0.45 (95 per cent CI -0.56 to -0.34), which shows a lowering of IQ in the high-fluoride group when compared to the reference (low-fluoride) group. However, there was overlap between the cut-off points for the high-fluoride (0.88–11.5 mg/L or ppm) and reference groups (0.2–2.35 mg/L or ppm). The authors attempted to control for high levels of arsenic and low levels of iodine by removing nine studies; moreover, they reported that the effect on IQ remained statistically significant, but the mean difference was somewhat less (-0.29, 95 per cent CI -0.44 to -0.14); they did not control for iron deficiency or high lead levels, or other chemicals associated with lowering IQ. In addition, the authors attempted to control for the method of IQ testing by excluding 11 studies that did not use the Combined Raven’s Test-Rural edition in China (CRT-RC) test. The authors also reported that the effect on IQ remained statistically significant but, once again, the mean difference was somewhat less (-0.36, 95 per cent CI -0.48 to -0.25). The authors comment that there was a consistent lowering of IQ in the intervention group in 22 of the 27 studies when compared to the reference group. However, there was some overlap between the areas classified as high-exposure areas and the areas classified as reference areas, thus making it difficult to define the cut-off at which fluoride may affect IQ levels. In addition, the authors reported that the studies were not adequate to complete a dose-response analysis, which is required in order to make more precise statements on safe levels. This review by Choi is viewed as a low-quality publication because it is based on cross-sectional studies and the quality of the studies was not assessed by Choi; such assessment is an essential part of completing a meta-analysis. In addition, the description of the studies is not complete. The control for confounding was not complete, due to the poor design of the original studies.

In 2011, **Valdez-Jimenez *et al.***³⁸ conducted a review, the aim of which was to publicise information on the toxic potential of fluoride and its effects on the nervous system, with special emphasis on populations exposed to the consumption of this chemical, whose concentration is outside the official standard. No search strategy, nor inclusion and exclusion criteria, are provided. No quality assessment of the studies they included is provided and

the primary studies are not described. There is no pooling of data; narrative information only is provided. This review is considered to be of low quality. The authors of this review conclude that there are data showing that fluoride has toxic effects on the central nervous system, depending on the dose, age, and exposure time; therefore, it is recommended by Valdez-Jimenez *et al* that the geographical location of a given population and the quality of the water should be taken into consideration, so as to take preventive measures for its use and, in areas where the fluoride concentration exceeds 0.7 mg/L, they suggest avoiding the intake of fluoridated drinking water, fluoridated salt, and the use of toothpastes and articles containing fluoride.

In 2014, **Grandjean and Landrigan**³⁹ conducted a review of existing studies and theorised that certain industrial chemicals could contribute to neurobehavioral defects; their review is an update of a previous review, which they carried out in 2006. The quality of the paper is considered to be low, and the study design cannot be determined. Search terms are provided, but not a search strategy. Inclusion and exclusion criteria are not described. The authors do not describe the primary studies included and do not assess the quality of the primary studies. Only one paragraph that is derived from the Choi 2012 review³⁷ discusses fluoride and IQ. Therefore, this paper cannot be used when summarising the evidence on IQ.

Table 8 presents six primary studies, published between 2006 and 2014, which examined the effects of water fluoridation and IQ in fluoride-endemic areas.

Table 8: Original studies in fluoride-endemic areas (above a threshold of 1.5 ppm), examining effects of water fluoridation and IQ, 2006–2014

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcome	Research study authors' conclusion
Rocha-Amador <i>et al.</i> ⁴³ (2007) Level of evidence: IV HRB authors' opinion of this study: high quality to describe a situation, but not the preferred study design to test a theory and infer causality	Mexico. 132 children aged 6-10 years	Cross-sectional study	Three rural communities with contrasting levels of fluoride and arsenic in drinking water; Salitral (fluoride 5.3+/-0.9 mg/L; arsenic 169+/-0.9 µg/L) and 5 de Febrero (fluoride 9.4+/-0.9 mg/L; arsenic 194+/-1.3 µg/L)	Moctezuma (fluoride 0.8+/-1.4 mg/L; arsenic 5.8+/-1.3 microg/L)	To explore the association between exposure to fluoride and arsenic in drinking water and intelligence in children	The authors conclude that the data suggest an increased risk of reduced IQ in children exposed to fluoride or arsenic.
Qin <i>et al.</i> ⁴⁴ (2008) Level of evidence: IV HRB authors' opinion of this study: low quality to describe a situation, and not an adequate study design to test a theory and infer causality	China	Cross-sectional study	Children aged 9 to 10.5 years with different levels of fluoride in the drinking water had their IQs tested. Exposed children high fluoride (2.1–4.0 ppm); and, low fluoride (0.1–0.2 ppm)	Normal fluoride (0.5 – 1.0 ppm)	Endemic neurotoxicity	The percentage of children with IQ scores in the top three (1–3) categories were 24.1 % in villages with 2.1–4.0 ppm fluoride in the drinking water; 27.2% in villages with 0.1–0.2 ppm fluoride, and 57.9% in villages with a 'normal' 0.5–1.0 ppm level of fluoride. For the lowest two IQ categories (4 and 5), the percentages were, respectively, 75.9%, 72.8%, and 42.1 %. The reasons for this non-monotonic pattern are not clear.

Table 8 continued

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcome	Research study authors' conclusion
Ding <i>et al.</i>⁴⁵ (2010) Level of evidence: IV HRB authors' opinion of this study: low quality to describe a situation and not an adequate study design to test a theory and infer causality	China	Cross-sectional study	Naturally occurring fluoride in the water. The children were ordered by their urine fluoride concentrations into 10 groups, with about 33 in each group. The mean value of urine fluoride concentration of the lowest group was 0.262mg/L and of the highest group was 2.956mg/L	The lowest urinary fluoride group was 0.262 mg/L	To examine the dose-response relationship of urine fluoride with IQ scores in children	The authors conclude that low levels of fluoride exposure in drinking water had negative effects on children's intelligence and dental health, and confirmed the dose-response relationships between urine fluoride and IQ scores as well as dental fluorosis.
Saxena <i>et al.</i>⁴⁶ (2012) Level of evidence: IV HRB authors' opinion of this study: low quality to describe a situation and not an adequate study design to test a theory/infer causality	India	Cross-sectional study	Naturally occurring fluoride in drinking water. 120 children exposed to ≥ 1.5 ppm of fluoride in drinking water.	50 children exposed to ≤ 1.5 ppm of fluoride in drinking water	Assess the relationship between exposure to different drinking water fluoride levels and children's IQ	The authors conclude that urinary fluoride level was associated with IQ level ($p 0.000$). The authors conclude that children living in areas with high levels of fluoride in drinking water are at risk of developing impaired intelligence.
Seraj <i>et al.</i>⁴⁷ (2012) Level of evidence: IV HRB authors' opinion of this study: moderate quality to describe a situation and not an adequate study design to test a theory and infer causality	Iran	Cross-sectional survey	Naturally occurring fluoride in drinking water. 293 children aged 6–11 years were selected who were exposed to normal fluoride (0.8 ± 0.3 ppm), medium fluoride (3.1 ± 0.9 ppm) and high fluoride (5.2 ± 1.1 ppm) in their water supplies	Low fluoride levels in drinking water (0.8 ± 0.3 ppm)	Investigate the effect of excessive fluoride intake on the intelligence quotient (IQ) of children	The authors conclude that children living in areas with higher than normal water fluoride levels demonstrated more impaired development of intelligence. Thus, children's intelligence may be affected by high water fluoride levels.
Zhang⁴⁸ (2012) Level of evidence: IV HRB authors' opinion of this study: low quality to describe a situation, and not an adequate study design to test a theory and infer causality	China	Cross-sectional survey	Naturally occurring fluoride in the water. Exposed 55 subjects to fluoride in drinking water., Fluoride levels in the table: 1.40 ± 1.3 ppm; range 0.1–1.7) and in the text: $1.40 + 1.3$ ppm, range 1.4–2.7.	68 subjects with fluoride in drinking water (0.63 ± 0.07 ppm, range 0.56–0.70). Ranges between comparison and control may overlap, as differ in table or text	To study the relationship between children's IQ and water fluoridation	The author concludes that the concentrations of urine fluoride and serum fluoride, TSH values and IQ scores were statistically significantly different in children in the high-fluoride group compared to those in the control group ($p < 0.05$) but not among males.

In 2007, a cross-sectional study was conducted by **Rocha-Amador *et al.***⁴³ to explore the association between exposure to fluoride and arsenic in drinking water and intelligence in children in Mexico. Three rural communities in Mexico with contrasting levels of fluoride and arsenic in drinking water were studied: Moctezuma (fluoride 0.8 ± 1.4 mg/L [highest parameter much higher than CWF in Ireland]; arsenic 5.8 ± 1.3 µg/L); Salitral (fluoride 5.3 ± 0.9 mg/L; arsenic 169 ± 0.9 µg/L) and 5 de Febrero (fluoride 9.4 ± 0.9 mg/L; arsenic 194 ± 1.3 µg/L). The final study sample comprised 132 children aged 6 to 10 years. Mean levels of fluoride in water were approximately 3.5 and 6 times higher than WHO recommended limits in Salitral and 5 de Febrero, respectively. Mean levels of arsenic in water were 17 and 19 times higher than WHO limits in Salitral and 5 de Febrero, respectively. These data suggest that children exposed to either high levels of fluoride or arsenic have increased risks of reduced IQ scores when compared to children drinking fluoridated water at 0.8 ± 1.4 mg/L and arsenic at 5.8 ± 1.3 µg/L. However, the design of this study precluded testing statistically the interaction between fluoride and arsenic. No sample size calculation was described or confidence intervals calculated, and therefore it is not clear how the study results can be generalised to a wider population. The authors did attempt to minimise bias, as all tests were administered at school by a trained neuropsychologist who was blinded to levels of fluoride or arsenic in participants' drinking water and urine. Overall, the methods used in this study follow good protocol, but the approach to sampling weakens the descriptive findings and the overall findings from this type of study design cannot infer causality or provide the possible separate contribution of each element. The HRB authors assign an evidence level IV to this study, and assess this study as high quality to describe a situation but not the preferred study design to test a theory and infer causality.

In 1990, **Qin *et al.***⁴⁴ investigated the effect of naturally occurring fluoride in drinking water on children's intelligence in one part of China, using a cross-sectional study design. They compared IQ of children in high-fluoride (2.1–4.0 ppm) exposure areas and low-fluoride (0.1–0.2 ppm) exposure areas with IQ in normal (0.5–1.0 ppm) exposure areas; the CWF level in Ireland is 0.6–0.8 ppm and within the normal group. After IQ testing was completed, the subjects were divided, based on the number of days since their birth. A percentage score was determined using the smoothed scale for percentage conversion of Raven's Standard Progressive Matrices scores, and the final intellectual ability ranking assigned by reference to the Raven intellectual ability rankings. For example, rank 1 referred to a standardised score greater than 95 per cent of children in the same-age theoretical norm group, i.e., high intelligence. Rank 2 refers to a standardised score of less than 95 per cent but greater than 75 percent, i.e., above average intelligence. Rank 3 refers to a standardised score of between 25 per cent and 75 per cent, i.e., average intelligence. Rank 4 refers to a standardised score of between 5 per cent and 25 per cent, i.e., below average intelligence. Rank 5 refers to a standardised score of less than 5 per cent, i.e., intellectually deficient. Children aged between 9 and 10.5 years from 22 villages in Jing County, Hubei Province, China, with different levels of fluoride in the drinking water, had their IQs tested by a trained interviewer (who did not know the fluoride levels in the drinking water used by the children tested); these children were tested using the revised version of Raven's Standard Progressive Matrices. Among 141 children in villages with 2.1–4.0 ppm fluoride in the drinking water (classified as high), 34 (24.1 per cent) had IQ scores in the top three categories (1-3) of intelligence; among 147 children in the villages with 0.1–0.2 ppm F (classified as low) 40 (27.2 per cent) had IQ scores in the top three categories (1-3) of intelligence, and among 159 children in villages with a 0.5–1.0 ppm F (classified as normal), 92 (57.9 per cent) had IQ scores in the top three categories (1-3) of intelligence. For the lowest two IQ categories (4 and 5), the percentages were 75.9 per cent for the high-fluoride exposure group, 72.8 per cent for the low-fluoride exposure group, and 42.1 per cent for the normal-fluoride exposure group. The reasons for this non-linear pattern are not clear. Age was the only confounder controlled for in this study. This is a low-quality study, as the authors did not control for confounders such as iron deficiency, iodine deficiency, exposure to high levels of lead or arsenic, and socioeconomic status (in particular, parental education and income). There was no justification for the sample size, and confidence intervals for each prevalence of low and high IQs were not calculated. The HRB authors assign an evidence level IV to this study.

A 2011 study from China by **Ding *et al.***⁴⁵ investigated the effects of low fluoride exposure on children's intelligence, and dental fluorosis, with the random recruitment of 331 children aged 7 to 14 years. Intelligence was assessed using the CRT-RC3. Mean value of fluoride in drinking water was 1.31 ± 1.05 mg/L (range 0.24–2.84). There was no sample size calculation or rationale given for the size of the sample. All confidence intervals around the mean difference in IQ score for each urinary fluoride concentration group in the multi-regression analysis crossed zero, indicating no difference between IQ levels in the two groups or an inadequate sample size. There was no attempt to minimise bias, and only partial control for confounding factors (age, iodine and arsenic). The HRB authors assigned an evidence level IV to this study because it is a cross-sectional study, which is not an adequate study design to infer causality. The HRB rate the study itself as low quality, because the rationale for the sample size was not provided and the control for confounding was partial (as it excluded other chemicals and important socioeconomic variables), and approaches to minimise bias were not presented.

Another cross-sectional study was performed by **Saxena *et al.***⁴⁶ in 2012 to assess the relationship between exposure to different drinking water fluoride levels and the intelligence of children living in Madhya Pradesh State, India. One hundred and seventy children were selected from low- (≤ 1.5 ppm) and high- (> 1.5 ppm) fluoride areas. The CWF level in Ireland is 0.6–0.8 ppm and within the low group. No rationale was given for the sample size and the authors did not describe any methods used to minimise bias. However, the authors controlled for some confounding factors (gender, socioeconomic status, education level, and nutritional status), but did not control for mineral deficiencies or mineral poisoning. Higher IQ levels were associated with lower levels of urine fluoride and vice versa. The urinary fluoride level was statistically significantly correlated with water fluoride levels. The authors conclude that children in endemic areas of fluorosis are at risk of impaired intelligence. The HRB authors rate the study quality level as low, as Saxena *et al.* did not calculate a sample size or provide a rationale for the sample size chosen. It is unclear if the study is generalisable to the population. The description of the categorical variable sociodemographic characteristics using mean and a range is statistically incorrect. In anthropometric measurement the interpretation of positive standard deviations as poor nutritional status is incorrect, because poor nutritional status would be assigned a negative standard deviation. In addition, the study design is not adequate to infer causality. The HRB authors assign an evidence level IV to this study.

In 2012, in a cross-sectional study, **Seraj *et al.***⁴⁷ investigated the effect of excessive fluoride intake on the intelligence quotient (IQ) of children living in five rural areas in Makoo in Iran. The researchers selected 293 children aged 6 to 11 years from five villages in Makoo with normal fluoride (0.8 ± 0.3 ppm, which is marginally higher than CWF levels in Ireland), medium fluoride (3.1 ± 0.9 ppm) and high fluoride (5.2 ± 1.1 ppm) in their water supplies. The five rural areas selected were similar in their general demographic and geographic characteristics, with the inhabitants having a comparable level of socioeconomic status and similar occupations. The exclusion criteria included a history of genetic disease, systemic disorders or brain trauma in the family. There was no sample size calculation or indication of the power of the study to detect difference. In order to minimise bias, the persons used to administer the IQ test were blinded to the children's fluoride status; in addition, examiner reliability was tested. The researchers did control for confounding by testing for an association between IQ and age, gender, child's educational level, mother's educational level, father's educational level, and none were statistically significant. Both arsenic and fluoride toxicity are increased by iodine deficiency, so the authors measured the amount of iodine in their drinking water and ensured that all households received iodine-enriched salts for cooking and eating purposes. The authors found a concentration of 0.08–0.1 mg/L for iodine and 0–0.5 mg/L for lead in the drinking water, which were in accordance with standard levels for these two elements, and this according to the researchers 'may accuse the high level of fluoride for the decreased IQ scores'. The authors do not control for iron deficiency or high levels of arsenic. The authors conclude that children residing in areas with higher (5.2 ± 1.1 ppm and 3.1 ± 0.9 ppm) than normal ($0.8 \pm$

0.3 ppm) water fluoride levels demonstrated more impaired development of intelligence, and thus children's intelligence may be affected by high water fluoride levels. The HRB authors assign a moderate-quality level to this study, as there was no rationale for sample size and no confidence intervals were given. Therefore, it is not possible to determine if these results can be applied to a wider population. The study authors themselves acknowledge that the study design is not adequate to infer causality. The HRB authors assign an evidence level IV to this study.

Zhang⁴⁸ set out to investigate the relationship among children's serum fluoride, urine fluoride, thyroid hormone levels and children's IQ in high-fluoride areas of China. Controlling for societal, economic, educational, geographical, and environmental factors, the author selected 68 grade 5 students from areas in Tianjin with drinking water fluoride concentrations lower than 1.0 mg/L as their control group. The CWF level in Ireland is 0.6–0.8 ppm and within the low group. In addition, they selected 55 grade 5 students from areas with fluoride concentrations higher than 1.0 mg/L as their high-fluoride group. The total number of subjects was 123. IQ testing of the subjects was performed, and water, blood, and urine samples were measured for fluoride. With respect to gender, the IQ scores of females in the high-fluoride group were statistically significantly lower than their gender counterparts in the control group (105.86 ± 15.3 v 119.28 ± 11.15 , $p < 0.001$), but the IQ score for males in the high-fluoride and control groups was not statistically significantly different (111.63 ± 10.55 v 115.69 ± 14.57 , $p > 0.05$). In the control group, 47.1 per cent of the children had an IQ above 120; a further 29 per cent had an IQ score in the range 110–119; and 2.9 per cent had IQs below 90. Whereas only 21.8 per cent of the high-fluoride group had an IQ above 120, a further 40 per cent were in the range 90–109 and 9.1 per cent had IQs below 90. Confounders were partly controlled for (age), but not gender, and it is clear from the data that female gender accounts for much of the difference in IQ. There was no rationale for the sample size calculation and no description of efforts to minimise bias. The HRB authors consider this study to be low quality. In addition, the study design is not adequate to infer causality. The HRB authors assign an evidence level IV to this study.

Neurological manifestations and water fluoridation in fluoride-endemic areas (above a threshold of 1.5 ppm)

Table 9 presents three primary studies examining the effects of water fluoridation and neurological manifestations in fluoride-endemic areas, which were published between 2006 and 2014.

In 2004, **Li et al.**⁴⁹ published the results of a study which explored the effects of excessive fluoride intake during pregnancy on neonatal neurobehavioral development and neurodevelopment toxicity. This was a cross-sectional study of 91 normal neonates delivered at the department of obstetrics and gynaecology in five hospitals in Zhaozhou County, Heilongjiang Province, China. The subjects were randomly selected between December 2002 and January 2003. The exposed group comprised 44 subjects whose mothers had high levels of fluoride (1.7–6.0 mg/L) in their drinking water, compared to 47 controls whose mothers' drinking water fluoride levels while pregnant were low (0.5–1 mg/L). The CWF level in Ireland is 0.6–0.8 ppm and within the low group. The rationale for the sample size chosen was not presented. There were statistically significant differences in the subjects' neonatal behavioural neurological assessment score between the high-fluoride group (36.48 ± 1.09) and the control group (38.28 ± 1.10). Agonistic muscle tension (ability of a muscle to contract) was impaired in the high-fluoride group (6.80 ± 0.70) compared to (7.40 ± 0.68) in the control group. The neonatal behavioural score was lower in the high-fluoride group (10.05 ± 0.94) compared to the control group (11.34 ± 0.56), and is explained by differences in the non-biological visual orientation reaction and the biological visual and auditory orientation reaction between the two groups. The authors conclude that fluoride is toxic to neurodevelopment and that excessive fluoride intake during pregnancy can cause adverse effects on neonatal neurobehavioral development. The study design is not ideal to test a theory and provide evidence of causality, and the control for confounding was inadequate. The authors controlled for mode of delivery, sex of the infant, gestational age of the foetus, birth weight and birth length. The authors did not control for confounders such as iron deficiency,

iodine deficiency, exposure to high levels of lead or arsenic, and socioeconomic status. The quality of this study as assessed by the HRB authors is low; this is because the authors did not calculate a sample size or provide a rationale for the sample size chosen. It is unclear if the study is generalisable to the population of neonates. The authors do not describe methods to minimise bias. It is not clear if the statistically significant results observed in the assessment scores are of clinical significance. The HRB authors assign an evidence level IV to this study.

Sharma *et al.*⁵⁰ (2009) conducted a cross-sectional survey of a human population exposed to low-, medium-, and high-fluoride concentrations in drinking water in villages in Sanganeer Tehsil, India. A total of 2,691 subjects were interviewed and were classified as being from low-fluoride villages (<1.0 ppm, which is equivalent to CWF levels in Ireland); medium-fluoride villages (1.0–1.5 ppm) and high-fluoride villages (1.5–6.4 ppm). Among the subjects were 1,145 children aged 12 to 18 years and 1,546 adults aged 18 years or over who were interviewed to establish their experience of various neurological ailments: headache; insomnia; lethargy; polyuria; polydipsia. There were no neurological manifestations in children in the low- and medium-fluoride villages; by contrast, in the high-fluoride villages, 9.5 per cent of the children had headache, 1.2 per cent had insomnia, and 3.2 per cent exhibited lethargy. The severity of the ailments increased with the increasing fluoride concentration in the drinking water. The authors claim that their results clearly indicate a role of fluoride in the neurological outcomes that were included in the study. However, no statistical tests were performed to determine if the differences were statistically significant among the villages, and there was no control for the myriad of other confounding factors that could cause these symptoms. There was no rationale provided for sample size calculation, and attempts to minimise bias were not described. The respondents were assigned their current living status, with no account taken of previous place of residence. The methods were not detailed enough to make a good judgement of the study methods, and in general the HRB authors rate this as a very low-quality study. The approach to analysis was very simplistic and the results provided cannot elucidate if there is or is not any possible connection between fluoride and the symptoms described. The HRB authors assign an evidence level IV to this study.

Choi *et al.*⁵¹ (2014) administered a number of age-appropriate independent tests that reflect different functional domains to determine their association with fluoride exposure and fluorosis. Their results suggest a deficit in working memory. Results of multiple regression models show that moderate and severe fluorosis was statistically significantly associated with lower total and backward digit span scores when compared to the reference combined categories of normal and questionable fluorosis. Other outcomes did not reveal any association with the fluoride exposure. The analysis included 43 eligible participants spread across three groups (using the Dean's fluorosis index), with 8 participants in the normal/questionable group, 9 participants in the very mild/mild group, and 26 participants in the moderate/severe group. The samples in each group are very small and there is no justification for the sample size chosen. Iodine deficiency is not mentioned as a possible explanatory factor. The authors discuss and rule out possible other chemical confounders (arsenic and lead) as an explanation, but do not control for the analysis. The study design employed suggests an association, but cannot infer causality. The HRB authors assign an evidence level IV to this study, and assess this study as low quality.

Table 9: Original studies in fluoride-endemic areas (above a threshold of 1.5 ppm) on the effects of water fluoridation and neurological manifestations, 2006–2014

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
<p>Li <i>et al.</i>⁴⁹ (2004)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this study: low quality to describe a situation and not an adequate study design to test a theory and infer causality</p>	China	Cross-sectional study	91 neonates were divided into two groups (high fluoride 44 subjects (1.7 – 6.0 mg/L) and 47 controls, based on the fluoride content in the drinking water of the mothers when pregnant.	47 controls, low fluoride (0.5–1 mg/L)	Differences in the neonatal behavioural neurological assessment score between the high-fluoride group and the control group	The authors report statistically significant differences in the neonatal behavioural neurological assessment score between the high-fluoride group (36.48 ± 1.09) and the control group (38.28 ± 1.10). The authors conclude that high fluoride levels are toxic to neurodevelopment.
<p>Sharma <i>et al.</i>⁵⁰ (2009)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this study: low quality to describe a situation and not an adequate study design to test a theory and infer causality</p>	India Subjects were 1,145 children aged 12 to 18 years and 1,546 adults.	Cross-sectional study	To investigate the effect of high fluoride in drinking water on neuro-behavioural patterns of a human population in villages in a fluoride endemic area. Naturally occurring fluoride in drinking water; high fluoride (1.5 – 6.4 ppm)	Low-fluoride group (<1.0 ppm), and medium-fluoride group (1.0–1.5 ppm)	Various neurological ailments, viz., headache, insomnia, lethargy, polyuria, and polydipsia (severe thirst)	The authors report that the data indicate that the largest number of cases with headache, followed by lethargy and insomnia, occurred in the fluoride-endemic village areas.

Table 9 continued

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
Choi <i>et al.</i>⁵¹ (2014) Level of evidence: IV HRB authors' opinion of this study: low quality to describe a situation and not an adequate study design to test a theory and infer causality	Southern Sichuan, China	Cross-sectional survey with 51 participants (43 completed Dean's index)	Fluoride concentration in morning urine after an exposure-free night; fluoride in well water source; and dental fluorosis status as indices of past fluoride exposure	N/A	The authors administered a battery of age-appropriate independent tests that reflect different functional domains: the Wide Range Assessment of Memory and Learning; Wechsler Intelligence Scale for Children-Revised digit span and block design; and finger tapping and grooved pegboard – and examined the association between these test results and fluoride exposure and fluorosis.	According to the authors, 60% of the subjects examined had moderate or severe fluorosis and these children were exposed to elevated fluoride concentrations in drinking water. Results of multiple regression models show that moderate and severe fluorosis was statistically significantly associated with lower total and backward digit span scores when compared to the reference combined categories of normal and questionable fluorosis. Other outcomes did not reveal any association with fluoride exposure. These results suggest a deficit in working memory.

Table 10 on the following page presents two reviews published between 2006 and 2014, which examined the effects of water fluoridation and neurological manifestations in fluoride-endemic areas.

In 2007, **Shcherbatykh and Carpenter⁵²** published a review which examined the role of metals in the aetiology of Alzheimer's disease. Only a small proportion of the paper was dedicated to fluoride. By counting the references in the fluoride section it seems that five articles were used by the authors to draw their conclusions on fluoride and Alzheimer's disease. No details on search methods were provided, nor was there any detail about the individual studies that were included. The two studies that they did include found protective effects of fluoride in drinking water on cognitive function or dementia. One study found that fluoride was not statistically significantly related to cognitive function and the two other studies reported no protective effect of fluoride. It is the opinion of the HRB authors that this review is of low quality and does not contribute to evidence of any association between fluoride in drinking water and Alzheimer's disease.

In 2009, **Blaylock and Strunecka⁵³** conducted a review which primarily investigated dysregulation of glutamatergic neurotransmission in the brain with enhancement of excitatory receptor function by pro-inflammatory immune cytokines as the underlying mechanism of the autistic spectrum of disorders. The researchers explored the role of aluminium and fluoride in this process. Approximately four studies are quoted in relation to fluoride. In addition, the authors point out that they reviewed studies which indicate that dietary excitotoxins, fluoride, and Al³⁺ can exacerbate pathological and clinical problems by worsening excitotoxicity, immune activation, and microglial priming. They conclude that their immune-glutamatergic hypothesis opens the door to a number of new modes of prevention and amelioration of these increasingly prevalent disorders. The review does not describe either paper selection or the methods used to describe the synthesis of results. Therefore, given this low-quality approach, it is impossible to use these papers to inform evidence.

Table 10: Review studies in fluoride-endemic-areas (above a threshold of 1.5 ppm) examining natural water fluoridation and neurological manifestations 2006–2014

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Research study authors' conclusion
Shcherbatykh and Carpenter⁵² (2007) Level of evidence: unable to assign HRB authors' opinion of this study: low quality, as it does not describe how the literature was retrieved and synthesised	139 references in the review, but the main concentration of the review is on the contribution of aluminium to Alzheimer's disease, and only a small proportion of references (five reviews) examine the role of silica and fluoride in the aetiology of this disease. No details given of the types of studies included in the five reviews.	The role of metals in the aetiology of Alzheimer's disease	N/A	To summarise studies which implicate a role for several chemicals (including fluoride) in contributing to or causing Alzheimer's disease	The authors state that, considering the evidence reviewed, fluoride does not either improve or disimprove cognitive function. Some of the studies reported slightly reduced risks of Alzheimer's disease or cognitive impairment in areas where higher silica or fluoride levels in drinking water were recorded. The evidence for a protective role of these substances is not convincing.
Blaylock and Strunecka⁵³ (2009) Level of evidence: unable to assign. HRB authors' opinion of this study: low quality as it does not describe how the literature was retrieved and synthesised	Not described	Environmental and dietary excitotoxins, mercury, fluoride, and aluminium in relation to autism spectrum disorder	N/A	To examine the environmental and dietary excitotoxins, mercury, fluoride and aluminium, and how they exacerbate the pathological and clinical problems associated with autism spectrum disorder	The authors suggest that the environmental and dietary excitotoxins, mercury, fluoride and aluminium, can exacerbate pathological and clinical problems by worsening excitotoxicity and microglial priming. In addition, each element has an effect on cell signalling, which can affect neurodevelopment and neuronal function.

International reports and expert bodies' conclusions on IQ and neurological manifestations

The reports identified in our grey literature search also examined the possible association between fluoride and reduced IQ. In the **NRC report²⁷** the only human studies available to the committee on the subject of fluoridation and IQ were from China, where fluoride is naturally occurring. In assessing the potential health effects of fluoride at 2–4 mg/L, the NRC committee found three studies of human populations exposed to those concentrations in drinking water, and these studies were useful for informing the committee's assessment of potential neurologic effects. The studies were conducted in different areas of China where fluoride concentrations ranged from 2.5 to 4 mg/L. Comparisons were made between the IQs of children drawn from populations with children in the same geographical region who were exposed to lower concentrations of fluoride ranging from 0.4 to 1 mg/L. The studies reported that while modal IQ scores were unchanged, average IQ scores were lower in the more highly exposed children; this was due to the fact that there were fewer children in the high IQ range. The NRC authors report that the significance of these Chinese studies is uncertain, and add that most of the papers were brief reports, and omitted important procedural details. The NRC authors conclude that while the studies lacked sufficient detail for the committee to fully assess their quality and their

relevance to US populations, the consistency of the collective results warrants additional research on the effects of fluoride on intelligence.

The reports from the **WHO**⁵⁴ in 2006; from the **National Institute of Public Health in Quebec, Canada**²⁸ in 2007; the **European Food Safety Authority (EFSA)**²⁹ in 2009 and the **U.S. EPA Office of Water**³⁰ in 2010 do not address the issue of fluoride in drinking water and IQ or neurological impairments.

The ***Guidelines for Canadian Drinking Water Quality: Guideline Technical Document***³¹ on fluoride concluded that the weight of evidence from all currently available studies does not support a link between exposure to fluoride in drinking water up to 1.5 mg/L and any adverse health effects, including those related to cancer, immunotoxicity, reproductive/developmental toxicity, genotoxicity and/or neurotoxicity. In addition, it does not support a link between fluoride exposure and IQ deficit, as there are significant concerns regarding the relevant studies, including quality, credibility, and methodological weaknesses.

The 2011 **SCHER**¹⁴ report stated that there are limited data on neurotoxicity of fluoride in humans. The SCHER authors report that it has been demonstrated that degenerative changes in the central nervous system, impairment of brain function, and abnormal development in children are caused by impaired thyroid function. Increases in serum thyroxine levels without statistically significant changes in T3 or thyroid-stimulating hormone (TSH) levels were observed in residents of regions in India and China that have high levels of fluoride in drinking water. However, these data are inconclusive according to the authors, due to the absence of adequate control for confounding factors. They conclude that available human studies do not clearly support the conclusion that fluoride in drinking water – at levels permitted in the EU – impairs children’s neurodevelopment. A systematic evaluation of the human studies does not suggest a potential thyroid effect at realistic exposures to fluoride. SCHER concludes that there is not enough evidence to conclude that fluoride in drinking water at levels permitted in the EU may impair the IQ of children. SCHER also concludes that a biological plausibility for the link between fluoridated water and IQ has not been established.

A very recent review of the scientific evidence on fluoridation was published by the **Royal Society of New Zealand and the Office of the Prime Minister’s Chief Science Advisor**³² in August 2014. The authors state that there have been a number of reports from China and other areas where fluoride levels in groundwater are naturally very high; these reports have claimed an association between high water fluoride levels and minimally reduced intelligence (measured as IQ) in children. In addition to the fact that the fluoride exposures in these studies were many (up to 20) times higher than any that are experienced in New Zealand or other CWF communities, according to the authors, the studies also mostly failed to consider other factors that might influence IQ, including exposures to arsenic, iodine deficiency, socioeconomic status, or the nutritional status of the children. Further, the New Zealand authors claimed that a shift of less than one IQ point suggests that this is likely to be a measurement or statistical artefact of no functional significance. A recently published study³³ in New Zealand followed a group of people born in the early 1970s, and measured childhood IQ at the ages of 7, 9, 11 and 13 years, and adult IQ at the age of 38 years. Early-life exposure to fluoride from a variety of sources was recorded, and adjustments were made for factors potentially influencing IQ. This extensive study revealed no evidence that exposure to water fluoridation in New Zealand affects neurological development or IQ. The report’s authors conclude that, based on the available evidence, there is no appreciable effect on cognition arising from CWF.

Summary on IQ and neurological manifestations

Non-endemic or CWF areas

There was only one study carried out in a non-endemic or CWF area (like Ireland) that examined fluoride and IQ. This was a prospective cohort study (whose design is appropriate to infer causality) in New Zealand. The study concluded that there was no evidence of a detrimental effect on IQ as a result of exposure to CWF.

Fluoride-endemic areas

There were six primary cross-sectional studies and six reviews published between 2006 and mid-2014 which examined the association between fluoride and neurological or IQ effects in endemic areas. In some of the review papers the lower and higher range of fluoride levels is not clearly specified and in other reviews the lower and higher range of fluoride levels overlap. In the six primary studies, where the fluoride levels were clearly specified, the normal level of fluoride was less than 1.5 ppm and the higher levels of fluoride were greater than or equal to 1.5 ppm, and could be as high as 10.3 ppm. These studies suggest, but do not prove, that children living in areas with naturally occurring high fluoride in the water (higher than the levels in CWF of 0.4–1 ppm) have a lower IQ compared with children drinking water with naturally occurring levels of fluoridation similar to CWF levels. It is important to state that the six primary studies and the six reviews published cannot prove a causal link. Apart from fluoride, there are other chemicals (arsenic or lead) and mineral deficiencies (iodine or iron) that could cause neurological or IQ effects, and these factors have not been comprehensively evaluated or controlled for using an appropriate study design, namely a prospective cohort study. The participants in these studies have very different socioeconomic profiles and nutritional status compared with children receiving CWF in developed countries; lower socioeconomic status and nutritional status may also lower IQ. Overall, the studies are of a low quality and of a design unsuited to prove or disprove theories. A summary of the existing literature indicates that lower IQ as a result of exposure to fluoride in drinking water is potentially problematic in areas with high levels of naturally occurring fluoride (above a threshold of 1.5 ppm), but such experiences were not reported in areas with CWF, such as Ireland.

Cancer

A possible link between water fluoridation and higher cancer mortality was claimed in the 1970s, and raised health concerns and heightened controversy surrounding the practice of CWF. In 1977, Yiamouyannis and Burk⁵⁵ reported that cancer mortality was higher in areas with artificially fluoridated drinking water (i.e.CWF) than in non-fluoridated areas. These findings were subsequently refuted by other investigators who identified problems with the study's research methodology.⁵⁶ However, because of the importance of this question, researchers have continued to examine the possibility of an association between artificially fluoridated water (i.e.CWF) and cancer in humans.

Bone cancer and osteosarcoma

Osteosarcoma is a rare primary bone cancer that usually affects young adults. The causes of primary bone cancer are not known, but there are several genetic conditions for which an increased risk of osteosarcoma is documented. Campaigners who are opposed to CWF argue that water fluoridation increases the risk of occurrence of osteosarcoma. Researchers suggest this could be theoretically possible, since bones readily take up much of ingested fluoride.^{18, 57, 58, 59, 60}

Table 11: Bone cancer, osteosarcoma and water fluoridation: results of two index reports

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Index authors' conclusion
McDonagh <i>et al.</i>² (2000) (York review) Level of evidence: III and IV HRB authors' opinion of this index review: high quality	11 studies: 8 ecological studies and 3 case-control studies	Area (or areas) with fluoridation of any level, i.e., natural or artificial	Non-fluoridated control area	Bone cancer and osteosarcoma	The review authors report that there was no clear association between water fluoridation and overall cancer incidence or mortality (for 'all cause' cancer, and specifically for bone cancer and osteosarcoma). The authors state that the evidence relating fluoridation to cancer incidence or mortality is mixed, with small variations on either side of the effect.
NHMRC³ (2007) Level of evidence: III HRB authors' opinion of this index review: high quality	One level III matched case-control study (Bassin <i>et al.</i> , 2006).	Histologically confirmed osteosarcoma cases	Matched controls	Osteosarcoma	The NHMRC authors agree with the interpretation of McDonagh on the papers included in the York review. However, they suggest that, based on the additional Bassin paper, there may be an increased risk of osteosarcoma among young males (but not among young females) as a result of water fluoridation.

The authors of the **York review**² examined 11 studies relating to bone cancer and osteosarcoma (Table 11). Of the studies presented in the York review, the direction of association between water fluoridation and bone cancer was found to be positive for young males only in one study; did not detect a relationship in eight studies, and for the remaining two studies, the data presented did not allow the HRB authors to categorise the direction

of the relationship. None of the studies found a statistically significant association. Seven studies of osteosarcoma, presenting 12 analyses, were included. Of these, the direction of association between water fluoridation and osteosarcoma incidence or mortality was found to be positive (fewer cancers) in seven analyses; negative (more cancers) in three analyses, and neither negative nor positive in two analyses. Of the six studies that presented confidence intervals around the mean differences, one found a statistically significant association between fluoridation and increased prevalence of osteosarcoma in males. According to the York review authors, this study also had the lowest validity score, 2.5 out of 8. One study contributed 4 of the 12 analyses, but did not provide confidence intervals around the mean differences. The authors of the York review conclude that no clear association exists between water fluoridation and overall cancer incidence or mortality (for 'all cause' cancer, and specifically for bone cancer and osteosarcoma). The authors of the York review state that the evidence relating fluoridation to cancer incidence or mortality is mixed, with small variations on either side of the effect.

The **NHMRC**³ Australian review found four studies in addition to the York review that examined the association between cancer and fluoridation (Table 11). Only one of these studies addressed bone cancer in that it compared the fluoride exposure of histologically confirmed osteosarcoma cases to matched controls. The authors of the **NHMRC** review state that after adjusting for significant differences at baseline between the cases and controls, the results of the Bassin *et al.*⁵⁷ 2006 study suggest an increased risk of osteosarcoma among young males (but not young females) who were exposed to water fluoridation. However, the NHMRC authors draw the attention of the reader to a letter⁶¹ to the editor of *Cancer, Causes, Control* by co-investigators of the Bassin study, in which the letter authors point out that they have not been able to replicate these findings in the broader Harvard study, which included prospective cases from the same 11 hospitals. Furthermore, the bone samples that were taken in the broader study corroborate a lack of association between the fluoride content in drinking water and osteosarcoma in the new cases. The final publication of the full study is not yet available, and its co-authors have cautioned readers not to over interpret the results of Bassin and colleagues in the interim.

Bone cancer and osteosarcoma in non-endemic fluoride areas

Table 12 presents the findings of eight primary studies carried out between 2006 and 2014. Seven of these studies were carried in non-endemic fluoride areas and one was carried out in a fluoride-endemic area. All of them examined bone cancer and osteosarcoma and its association with water fluoridation.

Table 12: Original studies carried out in non-endemic-and endemic-fluoride areas between 2006 and 2014, which examined bone cancer and osteosarcoma and its association with water fluoridation.

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
Non-endemic						
Bassin <i>et al.</i>⁵⁷ (2006) Level of evidence: III HRB authors' opinion of this study: Low-quality study, but suitable study design to test a theory	USA	Matched case-control study	Fluoride level in drinking water (≥ 0.7 ppm)	Low fluoride level in drinking water (< 0.3 ppm)	Incidence of osteosarcoma	The authors conclude that their exploratory analysis found an association between fluoride exposure (≥ 0.7 ppm compared to < 0.3 ppm) in drinking water at ages 6, 7 and 8 years, and the incidence of osteosarcoma among male adolescents but not among females adolescents. Further research is required to confirm or refute this observation.

Table 12 continued

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
<p>Kim <i>et al.</i>⁵⁸ (2011)</p> <p>Level of evidence: III</p> <p>HRB authors' opinion of this study: Low-quality study, but suitable study design to test a theory</p>	USA	Study started as a matched-case-control study, and the study was continued as an unmatched case-control study. 137 cases and 51 controls	N/A	Bone fluoride levels of osteosarcoma patients compared to such levels in two control groups: tumour controls and orthopaedic controls	Bone fluoride levels	The authors reported no statistically significant association between bone fluoride levels and osteosarcoma risk in their case-control study. The way the study was conducted does not fully comply with the study design.
<p>Comber <i>et al.</i>⁶² (2011)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this study: moderate quality study to create a theory, but not suitable to test it</p>	Ireland	Ecological study	Exposed at the time of osteosarcoma diagnosis to fluoride in drinking water	Not exposed at the time of diagnosis to fluoride in drinking	Incidence of osteosarcoma	The results of this study do not support the hypothesis or theory that osteosarcoma incidence on the island of Ireland may be statistically significantly related to public water fluoridation. However, this conclusion must be qualified, in view of the relative rarity of the cancer and the correspondingly wide confidence intervals of the relative risk estimates. This study design is suitable for developing a theory, as opposed to testing a theory.
<p>Levy and Leclerc⁶³ (2012)</p> <p>Level of evidence: IV</p> <p>HRB authors' opinion of this study: Low-quality study to create a theory, but not suitable to test it</p>	USA	Ecological study	CWF: States in which 85% or more of the population received fluoridated water between 1992 and 2006 (high-CWF States)	States in which 30% or fewer people in the population consistently received fluoridated water between 1992 and 2006 (low-CWF States)	The authors investigated the association between CWF and osteosarcoma in childhood and adolescence in the USA.	The authors state that the analysis suggests that water fluoridation status in the continental United States has no influence on osteosarcoma incidence rates during childhood and adolescence. This study design is developing a theory rather than testing a theory, and misclassification of exposure in both the fluoride and non-fluoride areas is likely to dilute any results.

Table 12 continued

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
NFIS⁶⁴ (2013) Level of evidence: IV HRB authors' opinion of this study: moderate-quality study to create a theory, but not suitable to test it	New Zealand	Ecological study	Incidence of osteosarcoma in CWF areas	Incidence of osteosarcoma in non-CWF areas	Aim was to link existing osteosarcoma incidence cases with census area unit of residence at the time of diagnosis, in order to determine whether the subjects were exposed to fluoride in drinking water or not.	The authors found that osteosarcoma is extremely rare in New Zealand. In their conclusion, they state that their findings suggest that the incidence rates in areas with CWF and without CWF are not different. This study design is developing a theory rather than testing a theory and inferring causality.
Blakey <i>et al.</i>⁶⁵ (2014) Level of evidence: IV HRB authors' opinion of this study: moderate-quality study to create a theory, but not suitable to test it	Great Britain	Ecological study	CWF and natural fluoride in drinking water at small-area level	Non-fluoridated small areas	The study objective was to examine whether increased risk of primary bone cancer (Ewing's sarcoma and osteosarcoma) was associated with living in areas with higher concentrations of fluoride in drinking water.	The authors conclude that the findings from their study do not suggest that higher levels of fluoride (whether natural or artificial) in drinking water in Great Britain lead to greater risk of either osteosarcoma or Ewing's sarcoma. This study design is developing a theory rather than testing a theory and inferring causality.
Public Health England¹⁶ (2014) Level of evidence: IV HRB authors' opinion of this study: moderate quality study to create a theory but not suitable to test it	England	Ecological study	CWF fluoride in drinking water at small-area level	Non-fluoridated small areas	Does the incidence of osteosarcoma differ in fluoridated areas compared to non-fluoridated areas both before and after controlling for confounding factors?	The authors conclude that there is no evidence to suggest a difference in the rate of osteosarcoma between fluoridated and non-fluoridated areas. This study design is developing a theory rather than testing a theory.

Table 12 continued

Endemic						
Kharb⁵⁹ (2012) Level of evidence: III HRB authors' opinion of this study: Low-quality study to create a theory but not suitable to test it	India	Case-control study	Naturally occurring fluoridated water: 10 cases diagnosed with osteosarcoma living in a fluoride-endemic area of India	10 healthy volunteers; no matching criteria and place of residence not stated	To analyse both the serum levels of fluoride in patients with osteosarcoma and the fluoride content of their drinking water	The authors conclude that their results suggest a link between fluoride exposure and osteosarcoma. However, there are many methodological issues, such as small sample size leading to low power, lack of control for confounding etc.

The **Bassin *et al.*⁵⁷** study was included in the HRB authors' search, as our search timeline began in January 2006. This paper was already included in the **NHMRC** Australian review; however, the HRB authors decided to examine and include it, due to the paper's importance in the fluoridation debate. The paper has generated much discussion and controversy since it was published. It presents partial findings of a 15-year study of fluoride and osteosarcoma by the Harvard School of Dental Medicine. The 15-year study started in 1992 and examines osteosarcoma cases that existed between 1989 and 1992, and new cases diagnosed between 1993 and 2000; all cases were identified from 11 hospitals throughout the United States. Bassin *et al.* examined the retrospective cases identified between 1989 and 1992 – people who were younger than 20 years old (103 cases) and compared them to 215 matched controls. All subjects were interviewed to determine their exposure to fluoride in their drinking water and fluoride from other sources (e.g., toothpaste, fluoride supplements etc.). Drinking water included the public supply, bottled water and well water. Bassin *et al.* categorised fluoride exposure into three groups (group 1 <30 per cent or <0.3 ppm of fluoride, group 2 3–99 per cent or 0.3 – 0.69 ppm, group 3 >99 per cent or ≥ 0.7 ppm); in addition, they used conditional logistic regression to estimate odds ratios within each group and between each group. The design of the study was case-control design, which is the only realistic design to explore the aetiology of rare diseases. Matching of cases and controls reduces confounding somewhat. The authors adjusted for some potential confounders (age, average area-based income, county population, prior use of well water, prior use of bottled water and any use of fluoride supplements). However, the authors do not state that they controlled for genetic predisposition, pre-existing bone defects, ionising radiation, alkylating agents used in chemotherapy, previous virus infection, antecedent trauma or radium in drinking water. There was no sample size calculation or effect size estimation – calculations that are required in order to detect a difference in exposure. Recall bias is likely to be an issue in this study, as the exposure was ascertained up to 18 years after the event. In addition, the interviewers were not blind to the status of the subjects i.e., whether the subject was a case or a control. Fluoride levels are area based rather than person based, and no bone fluoride samples were taken. Bone fluoride samples are a better marker of cumulative exposure. The conditional logistic regression using discordant pairs would likely have had very small samples to perform the analysis, in particular when they were split into their gender groups. Bassin *et al.* concluded that their exploratory analysis found an association between osteosarcoma and fluoride in drinking water in males, but not in females. They also acknowledged that there were limitations to their study design. The paper's authors concluded that further studies are required in order to confirm or refute the findings. The HRB authors assign a level III, as this study is a matched case-control study. The HRB authors rate this as a low-quality study.

In the same year (2006), a letter from Professor Douglass, Bassin's PhD supervisor, to the editor of the journal in which this study was published,⁶¹ urged caution in interpreting these results, as preliminary findings from the overall analysis of the cases did not show an association between osteosarcoma and fluoride in drinking water. Bone specimens had been provided by many of the subjects and, according to Douglass, preliminary analysis of

the bone specimens suggested that, in the complete study sample, the level of fluoride in bone is not associated with osteosarcoma. Since this letter was published, there has been no formal publication detailing the findings for the complete cohort.

In 2005, accusations of scientific fraud were brought against Douglass for intentionally obscuring Bassin's findings. This complaint was investigated by Harvard University, and Douglass was exonerated. In 2011, Kim *et al.*⁵⁸ (including Douglass as an author) conducted a follow-up case-control study to examine some of the concerns posed by the Bassin study, and the found no difference in the bone fluoride levels between cases and controls. However, this study also had design and execution flaws, as discussed below.

Kim *et al.*'s⁵⁸ 2011 study has already been mentioned above in relation to the Bassin *et al.* paper. The purpose of the study by Kim *et al.* was to determine if bone fluoride levels are higher in individuals with osteosarcoma. Incident cases of osteosarcoma (N = 137) and tumour controls (N = 51) were identified by orthopaedic physicians, and segments of tumour-adjacent bone and iliac crest bone were analysed for fluoride content. No rationale was given by the authors for sample size and no estimation of effect size was provided. Although the study protocol called for matching of cases and controls based on gender, age (± 5 yrs.), and distance from their medical centre, this approach was abandoned early in the study, since it proved to be a barrier to recruiting controls. Thus, all available tumour patients were recruited, and the statistical analysis was adjusted for age and gender. The authors did attempt to minimise bias by blinding the investigators to the case or control status of the bone specimens, and specimens were analysed twice; if measurements differed by more than 10 per cent, another specimen was analysed. Deer bone specimens with known fluoride concentrations were included in each batch of specimens for quality control, and confirmed the validity of the bone fluoride assay procedure. The authors also used unconditional logistic regression and conditional logistic regression to control for confounding variables. The authors controlled for age and gender, history of broken bones, other bone diseases, other cancer diagnoses, and history of receiving radiation prior to illness. The authors conclude that there was no statistically significant association between bone fluoride levels and osteosarcoma risk detected in this case-control study, based on controls with other tumour diagnoses. The HRB authors assign a level III, as this study is a case-control study. The HRB authors rate this as a low-quality study.

In 2011 **Comber *et al.***⁶² investigated the incidence of osteosarcoma in Northern Ireland and compared it with that of the Republic of Ireland in order to establish if differences in incidence between the two regions could be related to their different drinking water fluoridation policies. Data from the Northern Ireland Cancer Registry (NICR) and the National Cancer Registry of Ireland (NCRI) on osteosarcoma incidence in the respective populations were used to estimate the age-standardised and age-specific incidence rates in areas with and without CWF. No statistically significant differences were observed between fluoridated and non-fluoridated areas in either age-specific or age-standardised incidence rates of osteosarcoma. The results of this study do not support the hypothesis that osteosarcoma incidence on the island of Ireland is statistically significantly related to public water fluoridation. However, the authors state that this conclusion must be qualified, in view of the relative rarity of the cancer and the correspondingly wide confidence intervals of the relative risk estimates. This study type does not allow researchers to test a theory to link cause and effect (only to posit one), as the fluoride levels are population-based calculations rather than individual-based ones. In addition, there is likely to be crossover of an individual's place of residence between fluoridated and non-fluoridated areas, and it would be more appropriate to ascertain a history of lifetime resident addresses in order to accurately determine exposure over time. The authors have not examined other explanatory factors such as genetic predisposition, pre-existing bone defects, ionising radiation, alkylating agents used in chemotherapy, previous virus infection, antecedent trauma or radium in drinking water. The HRB authors assign a level IV as this is an ecological study. The HRB authors rate it as a moderate-quality study.

Levy and Leclerc⁶³ 2012 investigated the association between CWF and osteosarcoma in childhood and adolescence in the continental United States. They used the cumulative osteosarcoma incidence rate data from the Centers for Disease Control (CDC) Wonder database for 1999–2006, categorised by age group, sex and states. States were categorised as low (≤ 30 per cent) or high (≥ 85 per cent) according to the percentage of the population receiving CWF between 1992 and 2006; this selection process misclassifies exposure in both the fluoride and non-fluoride groups, which is likely to change the effect. The authors found no sex-specific statistical differences in the national incidence rates in the younger groups (5 to 9, 10 to 14 years), although males aged 15 to 19 years were at higher risk of osteosarcoma than females in the same age group ($p < 0.001$). Sex and age group-specific incidence rates were similar in CWF state category compared to non-CWF state category. The higher incidence rate among males aged 15–19 years versus females of the same age was not associated with the state fluoridation status. They also compared sex and age-specific osteosarcoma incidence rates between 1973 and 2007 from the SEER 9 Cancer Registries for single age groups from 5 to 19 years. There were no statistical differences between sexes for children aged 5–14 years, although incidence rates for single age groups (males aged 15–19 years) were statistically significantly higher than for females. The authors conclude that their analysis suggests that the water fluoridation status in the continental United States has no influence on osteosarcoma incidence rates during childhood and adolescence. The study conducted by Levy and Leclerc was ecological in design; therefore, fluoride exposure is estimated by area level and is not calculated at individual level. There is likely to be crossover of individuals' place of residence between fluoridated and non-fluoridated areas, and it would be more accurate to ascertain a history of lifetime resident addresses in order to accurately determine exposure over time. This study design does not allow researchers to test a hypothesis (only to posit one) because the fluoride levels are population based rather than individual-based calculations. The authors have not examined other possible explanatory factors such as genetic predisposition, pre-existing bone defects, ionising radiation, alkylating agents used in chemotherapy, previous virus infection, antecedent trauma or radium in drinking water. The HRB authors assign a level IV, as this is an ecological study. The HRB authors rate this as a low-quality study.

The National Fluoridation Information Service (NFIS)⁶⁴ report in 2013 set out to find if there is any evidence of an increased risk of the bone cancer osteosarcoma with CWF. This is a population-based ecological study in New Zealand, where only 127 new cases of osteosarcoma were registered between 1993 and 2008. The cases were identified from the cancer registry, which not only collects cancer data on cancer site and morphology, but also collects details of the age and sex of the case and the census area unit (CAU) where the case lived at the time of diagnosis. The CAUs were categorised into those served by CWF and those without CWF. The analysis found that, overall, osteosarcoma is extremely rare in New Zealand, with only 127 new cases registered between 1993 and 2008, and an average incidence of 14.1 cases in the total population per year. The peak age is 10–19 years for both sexes, and the incidence rates in areas with CWF and without CWF are not different. However, as this study is ecological in design, there is likely to be crossover of individuals' place of residence between fluoridated and non-fluoridated areas, and it would be more appropriate to ascertain a history of lifetime resident addresses, in order to more accurately determine exposure over time. This study type does not allow researchers to test a hypothesis (only to posit one), as the fluoride levels are population based rather than individual-based calculations. The authors have not examined other explanatory factors, such as genetic predisposition, pre-existing bone defects, ionising radiation, alkylating agents used in chemotherapy, previous virus infection, antecedent trauma or radium in drinking water. The HRB authors assign a level IV as this is an ecological study. The HRB authors rate this as a moderate-quality study.

Blakey *et al.*⁶⁵ (2014) examined case data on osteosarcoma and Ewing's sarcoma, diagnosed at ages 0–49 years in Great Britain (GB) (defined as England, Scotland and Wales) during the period 1980–2005. These data were obtained from population-based cancer registries. Data on fluoride levels in drinking water in England and Wales were accessed through regional water companies and the Drinking Water Inspectorate. Scottish Water provided

data for Scotland. The authors examined the relationship between incidence rates and level of fluoride in drinking water at small-area level. The study analysed 2,566 osteosarcoma cases and 1,650 Ewing sarcoma cases. Blakey *et al.* reported that the findings from this study do not provide evidence that higher levels of fluoride (whether natural or artificial) in drinking water in GB lead to greater risk of either osteosarcoma or Ewing's sarcoma.

In this ecological study, it is not clear whether the fluoride exposure in water was assigned for year of cancer diagnosis, or whether a cumulative exposure was assigned to each case for the period 1980 to 2005. There is likely to be crossover of individuals' place of residence between fluoridated and non-fluoridated areas, and it would be more appropriate, as with all ecological studies, to ascertain a history of lifetime resident addresses, in order to accurately determine exposure over time. The authors adjusted for gender, age group, the interaction gender* age group, the Townsend score and the interaction Townsend* female. No association was found between osteosarcoma and fluoride levels in drinking water ($p=0.987$). This study type does not allow researchers to test a hypothesis (only to posit one), as the fluoride levels are population based rather than individual calculations. The HRB authors assign a level IV, as this study is an ecological study. The HRB authors rate this as a moderate-quality study.

The 2014 **Public Health England**¹⁶ study conducted a population-based ecological study linking the number of osteosarcoma cases in England, recorded in cancer registries, in order to determine whether the cases were exposed to fluoride in drinking water or not. Cancer data were extracted from the National Cancer Registration Service. Osteosarcoma was considered as an indicator separately for those aged under 25 years, and those aged over 50 years, in order to reflect the bimodal distribution of incidence and differences in aetiology between age groups. Additionally, for those aged under 25 years, gender-specific analysis was performed having considered suggestions made by previous research. Following adjustment for age, gender deprivation and ethnicity, there was no evidence of a difference in osteosarcoma rates in those aged under 25 years in fluoridated compared to non-fluoridated areas (8.2 per cent higher; 95 per cent CI -9.3 per cent, 29 per cent; $p=0.38$). The results did not indicate any evidence of a difference in the rate of osteosarcoma among people aged 50 and over between fluoridated and non-fluoridated areas.

As this is an ecological study, exposure to water fluoridation is area based rather than individually based. There is likely to be crossover of individuals' place of residence between fluoridated and non-fluoridated areas and, again, it would be more appropriate to ascertain a history of lifetime resident addresses, in order to accurately determine exposure over time. The authors identified and controlled for the following confounding variables: age, gender, deprivation and ethnicity. However, the authors did not control for genetic predisposition, pre-existing bone defects, ionising radiation, alkylating agents used in chemotherapy, previous virus infection, antecedent trauma, or radium in drinking water. The HRB authors assign a level IV, as this is an ecological study. The HRB authors rate this as a moderate-quality study.

Bone cancer and osteosarcoma in endemic areas (above a threshold of 1.5 ppm)

The **Kharb**⁵⁹ 2012 study was planned to analyse serum levels of fluoride in patients with osteosarcoma, and the fluoride content of these patients' drinking water (Table 12). It compared 10 patients with osteosarcoma and 10 healthy volunteers (who served as controls). Serum and drinking water fluoride levels were estimated by ion selective electrode. The author found that the serum and drinking water fluoride levels were statistically significantly higher in patients with osteosarcoma compared to controls ($p > 0.05$, $p > 0.001$, respectively), and concluded that the results suggest a link between fluoride exposure and osteosarcoma. This study used a case-control design, but there are serious issues with respect to the study implementation, including the selection of the cases and controls. There were ten patients with osteosarcoma living in a fluoride-endemic area of India and 10 healthy volunteers whose place of residence was not reported. There was no matching of cases and controls,

and the place of residence of the controls is not stated. There is no rationale provided by the author for the particularly small sample size. There was no attempt to minimise bias and no control for confounding factors (age, gender, genetic predisposition, pre-existing bone defects, ionising radiation, alkylating agents used in chemotherapy, previous virus infection, antecedent trauma or radium in drinking water. The HRB authors assign a level III, as this is a case-control study. The HRB authors rate this as a low-quality study.

All-cause cancer in fluoride-endemic and non-endemic areas

In addition to bone cancer, the effect of water fluoridation on all-cause cancer incidence and mortality has been examined. The York review and the NHMRC reviews report on primary studies that examined this subject up to 2006 (Table 13).

Table 13: Other cancers and water fluoridation up to 2006: results of two index reports

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Index authors' conclusion
McDonagh <i>et al.</i>² (York review) (2000) Level of evidence: IV HRB authors' opinion of this index review: high quality	10 studies; 5 ecological and 5 before and after	Area (or areas) with fluoridation of any level, i.e., natural or artificial. The area with the water fluoride level closest to 1.0 ppm was chosen, and compared to the area with the lowest water fluoride level reported.	Non-fluoridated control areas	Studies that examined any cancers, excluding studies on bone cancer and osteosarcoma only	The authors state that the evidence relating fluoridation to cancer incidence or mortality is mixed, with small variations on either side of the effect.
NHMRC³ (2007) Level of evidence: IV HRB authors' opinion of this index review: high quality	York review plus three additional ecological studies	Area (or areas) with fluoridation of any level, i.e., natural or artificial	Non-fluoridated control areas	Crude incidence of cancer (total), site-specific incidence of cancer, and crude and specific cancer death rates	With regard to all cancers (excluding bone cancer), the NHMRC authors stated that there were mixed results, and that two of the three studies were of poor quality and one was of fair quality.

The authors of the **York review**² examined 10 studies relating to all-cause cancer.

All-cause cancer incidence and mortality was considered as an outcome in 10 studies containing 22 analyses. Of these analyses, 11 found the direction of association between water fluoridation and cancer to be positive (fewer cancers), and 9 found the direction of association to be negative (more cancers). Two studies found no association between water fluoridation and cancer. One study found a statistically significant negative effect (more cancers) in two of the eight subgroups investigated; this was not confirmed when other subgroups were considered. One study found a statistically significant positive effect (fewer cancers).

The authors of the York review state that there does not appear to be any association between their validity rating and the direction of the association of water fluoride exposure and cancer incidence. Of the two studies

with the highest validity scores (4.8 and 4.2), one found a statistically significant positive association and the other found a mixed effect; some analyses showed a statistically significant negative effect and others showed statistically non-significant associations in both directions. Overall, these studies do not appear to show any association between overall cancer incidence and water fluoride exposure.

The findings of the York review on cancer studies were mixed, with small variations on either side of no effect. Individual cancers examined were bone cancers and thyroid cancer, where, once again, no clear pattern of association was seen. Overall, from the research evidence presented, the York review concludes that no association was detected between water fluoridation and mortality from any cancer, or from bone or thyroid cancers specifically.

The **Australian NHMRC**³ literature review identified three additional studies that investigated the relationship between water fluoridation and cancer incidence or mortality. Again, the results of these studies showed a mixed pattern. In one study there was a suggested association between fluoride and higher cancer incidence in 23 of the 36 body sites investigated; there was a suggested association between fluoride and lower cancer incidence in four sites, and in the remaining nine sites there was no difference. Another study found that except for a higher rate of bladder cancer, cancer rates were generally similar in fluoridated and non-fluoridated areas. The third study found that the fluoride concentration in drinking water was inversely correlated with cancer incidence (i.e., the lower the fluoride levels, the higher the cancer incidence/mortality). The Australian authors deem two of these studies to be of poor quality and one to be of fair quality.

Only one additional study (Table 14) on other cancers and water fluoridation was found by the HRB authors, and this study examined the effects of water fluoridation on bone cancer (see above), bladder cancer and all cancers (excluding melanoma).

Table 14: Original studies in non-endemic fluoride areas examining other (bladder and all) cancers and fluoride, 2006–2014

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
Public Health England ¹⁶ (2014) Level of evidence: IV HRB authors' opinion of this study: moderate-quality study to create a theory but not suitable to test it	England	Ecological study	CWF fluoride in drinking water at small-area level	Non-fluoridated small areas	Does the incidence rate of invasive bladder cancers or all cancers differ in fluoridated areas compared to non-fluoridated areas both before and after controlling for confounding factors? All cancer incidence excluded non-melanoma skin cancer.	The authors conclude that there was evidence to suggest that the rate of bladder cancer was lower in fluoridated areas than in non-fluoridated areas. They also concluded that there was no evidence to suggest any association between fluoridation status and all cancer incidence. This study design is developing a theory rather than testing a theory.

Public Health England¹⁶ conducted an ecological analysis at LSOAs in England. The crude incidence density rates for cancers were calculated for aggregated indicators in fluoridated and non-fluoridated areas separately (Table 14). Cancer data were extracted from the National Cancer Registration Service. In all analyses performed, the primary exposure of interest was whether or not the person was resident in a fluoridated or non-fluoridated area at the time of their diagnosis. The case definition for bladder cancer was all primary invasive bladder cancers in England recorded in cancer registries, with date of diagnosis between 2000 and 2010 inclusive. The case definition for all cancer was all cases of cancer in England, excluding non-melanoma skin cancer recorded in cancer registries, with date of diagnosis between 2007 and 2010. The age-standardised rate of bladder cancer between 2000 and 2010 was 12.4 (95 per cent CI 12.2, 12.6) in fluoridated areas, compared to 13.0 (95 per cent CI 12.9, 13.1) in non-fluoridated areas. Following adjustment for age, gender, deprivation and ethnicity, there was strong evidence that the rate of bladder cancer was lower in fluoridated areas (8.0 per cent lower; 95 per cent CI -9.9 per cent, -6.0 per cent; $p < 0.001$). The age-standardised rate of all cancers was 402 (95 per cent CI 399 to 404) per 100,000 person-years at risk in fluoridated areas compared to 396 (395,397) per 100,000 person-years at risk in non-fluoridated areas. Following adjustment for age, gender, deprivation and ethnicity, there was no evidence of any association between fluoridation status and all cancer incidence (0.4 per cent lower; 95 per cent CI -1.2 per cent, 0.4 per cent; $p = 0.29$).

This is a population-based ecological study and its level of evidence is IV. The authors identified and controlled for the following confounding variables: age, gender, deprivation and ethnicity. There is likely to be an element of bias, in that there is a possibility of crossover of individuals' place of residence between fluoridated and non-fluoridated areas during their lifetime, and it would be more appropriate to ascertain a history of lifetime resident addresses in order to accurately determine fluoride exposure over time. The HRB authors consider this study to be of moderate quality, as it is likely to suffer from misclassification bias.

International reports and expert bodies' conclusions on cancer

The **reports** identified in our grey literature search also examined the possible association between fluoride and cancer. In the **NRC report**²⁷ the authors refer to the inherent difficulties for conducting epidemiological studies of the cancer potential of fluoride and drinking water. They continue by saying that 'the limitations severely affect the possibility of identifying relatively small effects on cancer incidence and, especially, cancer mortality. Chief among them are the latency of cancer diagnosis after exposure to causal factors, typically spanning more than 10 years and often reaching 30 years'. Many of the studies that are reviewed by the NRC are ecological studies, but some had individual calculations of fluoride exposure. The authors report that several studies had methodological limitations that made it difficult to draw conclusions. The NRC authors concluded from the studies examined that the combined literature described in their report does not clearly indicate that fluoride either is or is not carcinogenic in humans.

The reports from the **WHO**⁵⁴ in 2006 remark on the fact that studies of occupational exposure to fluoride have reported a higher incidence of, and mortality from, lung and bladder cancer and from cancers in other parts of the body. However, the WHO authors conclude that the data are inconsistent and, in a number of studies, the results can be more readily attributed to exposure to substances other than fluoride. The WHO reported that there have also been a statistically significant number of epidemiological studies examining the possible association between various cancers and exposure to fluoride in drinking water. However, the WHO state, in spite of the large number of studies conducted in a number of countries, there is no consistent, high-quality evidence to demonstrate any association between the consumption of controlled fluoridated drinking water and either morbidity or mortality from cancer.

The **National Institute of Public Health in Quebec, Canada**,²⁸ in its report, examines the potential link between water fluoridation and osteosarcoma, and concludes that most of the published studies up to that time (2007) on the topic of fluoridated water do not support the hypothesis of a link between fluoride exposure and an increased risk of developing osteosarcoma. However, they caution that given the methodological limitations of the studies, other well-controlled studies were required.

The **European Food Safety Authority (EFSA)**²⁹ report and the **U.S. EPA Office of Water**³⁰ report do not examine the potential effects of fluoridation and cancer.

The **Guidelines for Canadian Drinking Water Quality: Guideline Technical Document**³¹ on fluoride reiterated the findings of a previous Expert Panel meeting on fluoride (Health Canada 2008) which stated that the weight of evidence does not support a link between fluoride and cancer. The experts reported that it is important to avoid generalisation and overinterpretation of the results from the Bassin *et al.*⁵⁷ (2006) paper.

The **Scientific Committee on Health and Environmental Risks** in their 2011 report conclude that epidemiological studies do not indicate a clear link between fluoride in drinking water and osteosarcoma and cancer in general. They go on to say that there is no evidence from animal studies to support the link, and thus fluoride cannot be classified as carcinogenic.

The **Royal Society of New Zealand and the Office of the Prime Minister's Chief Science Advisor's** report³² in August 2014 reviewed the evidence on water fluoridation and cancer. The authors refer to the fact that the large majority of epidemiological studies have found no association between fluoride and cancer, even after decades of exposure in some populations. This includes populations with lifetime exposure to very high natural fluoride levels in water as well as high-level industrial exposures. The few studies that have suggested a cancer link with CWF suffer, they say, from poor methodology and/or errors in analysis. Bone cancers have received specific attention because of fluoride's deposition in bone. Although the Bassin *et al.*⁵⁷ study claimed an increased risk for osteosarcoma in young males, extensive reviews of these and other data do not find an association between exposure to fluoridated water and risk of osteosarcoma. Similarly, they add that data from the New Zealand Cancer Registry from 2000 to 2008 show no evidence of association between osteosarcoma incidence and residence in CWF areas. Finally, they conclude that on the available evidence, there is no appreciable risk of cancer arising from CWF.

Summary on cancer

Non-endemic or CWF areas

Concerns have been expressed about the possible carcinogenic effect of fluoride in drinking water, particularly in relation to osteosarcoma, a rare primary cancer of the bone. A number of studies have examined the association between water fluoridation and osteosarcoma, and there have also been studies that examined water fluoridation and general cancer incidence and mortality.

Between 2006 and mid-2014 a possible link between fluoride and osteosarcoma incidence has been investigated in five ecological studies and two case-control studies in non-endemic areas, and one case-control study in an endemic area. Most osteosarcomas occur in children and young adults. Teenagers are the most commonly affected age group, but osteosarcoma can occur at any age. Approximately 15 cases of osteosarcoma are diagnosed each year in Ireland, with incidence rates being slightly higher in males than in females. A number of authors report that there is biological plausibility for linking fluoride to osteosarcoma, as fluoride accumulates in bones and changes the properties of bone.^{18, 57, 58, 59, 60} Much of the concern about fluoride and its possible link

to osteosarcoma arises from the findings of a paper by Bassin *et al.* published in 2006, which concluded that their exploratory analysis found a statistically significant association between osteosarcoma and fluoride in drinking water in young males, but not in young females. They also acknowledged that there were limitations to their study design. While the study design employed has potential to infer causality, the conduct of the study has flaws and these have led to its findings being disputed. Added to this is the fact that Bassin's PhD supervisor, Chester W Douglass, the lead investigator on the complete study, published a statement in the same edition of the journal as the article, urging caution in interpreting the results. Douglass explained that preliminary results on the full study cohort did not support Bassin's findings. Subsequently, there was an allegation of fraud and suppression of Bassin's results made against Douglass. The allegations were investigated by Harvard University, and Douglass was cleared of any wrongdoing. Douglass is an author on a further study, by Kim *et al.*, which examined the same cohort of study subjects in relation to fluoride in drinking water and osteosarcoma. The researchers measured fluoride concentration in samples of normal bone adjacent to the person's tumour and found no difference in bone fluoride levels between people with osteosarcoma and people in the control group who had other malignant bone tumours. The study authors concluded that there was no statistically significant association between bone fluoride levels and osteosarcoma risk detected in that study's subjects; however, that study also had flaws in its implementation. There has been no formal publication detailing the findings for the complete cohort.

The existence of biological plausibility in relation to fluoride and bone cancer (mentioned earlier) renders the 2014 Levy *et al.* paper important to this discussion, although it does not examine osteosarcoma specifically. In the Levy *et al.* study, data were collected from a birth cohort in the Iowa fluoride study since the early 1990s. The researchers aimed to quantify fluoride intake from all sources and estimate the exact influence of fluoride intake on bone density and mineral content. The study subjects had complete accelerometry data (a technique used to study bone movement) and bone scans at age 15 years, the age at which many osteosarcomas are diagnosed. Data were analysed and adjusted for a large number of variables. The findings suggest that fluoride exposures at the typical levels for most US adolescents in fluoridated areas do not have statistically significant effects on bone mineral measures. The Levy *et al.* study is a prospective cohort, a design in which far fewer opportunities exist for bias compared with studies of weaker design, such as case-control studies, cross-sectional surveys or ecological studies. The study has extremely important strengths in that data were from a cohort that was followed longitudinally, and measures of fluoride intake were calculated for each year in individuals rather than relying on population data or long-term recall exposure. However, it is important to point out that a large number of the original cohort were lost to follow-up.

A summary of the existing literature indicates that the effects of fluoride in drinking water on osteosarcoma incidence are mixed and to date, no link has been proven. Therefore it is difficult to draw a definitive conclusion.

Fifteen studies were included in the York review on cancer incidence or mortality and an additional three studies were included in the Australian review. The current authors found only one more recent paper, the Public Health England study, which investigated this possible association. Both of the index reviews found mixed evidence in relation to fluoride in the drinking water and all-cancer incidence or mortality. The Public Health England study found no evidence of any association between fluoridation status and all-cancer incidence, with the exception of bladder cancer. The Public Health England study concluded that the analysis suggested the rate of bladder cancer may be lower in fluoridated areas than in non-fluoridated areas. Researchers have advanced hypotheses linking fluoride and all-cause cancer incidence or mortality, but there is a dearth of good quality longitudinal research available to affirm or rule out these suggested links.

Cardiovascular disease

Hypertension in humans (systolic BP >140 with diastolic >90mm/Hg) is one of the most common cardiovascular diseases, and it is an important identifiable and modifiable risk factor for atherosclerotic heart disease and stroke. The development of coronary heart disease is attributed to several well-established risk factors, but these risk factors only seem to explain half to three-quarters of the variation in the incidence of cases of coronary heart disease in most industrialised societies. Therefore, important risk factors have yet to be identified. There has been some evidence linking the long-term effects of chronic low-grade bacterial infections, such as dental caries or periodontitis, to atherosclerosis and its complications. The role of fluoride as a protective agent against dental caries is firmly established. The question then arises as to what influence, if any, fluoride in drinking water has on coronary heart disease. Since fluoride accumulates in calcified tissues, there is a suggestion that exposure to fluoride will affect aortic calcification. A number of studies indicate that fluoride may reduce aortic calcification in experimental animals and humans.

The York review did not include any studies that examined this possible association; the NHMRC review included only one study on this topic (Table 15).

Table 15: Cardiovascular disease and water fluoridation up to 2006: results index report

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Index authors' conclusion
NHMRC³ (2007) Level of evidence: IV HRB authors' opinion of this index review: high quality	One ecological study from Finland	Levels of fluoride in drinking water – V: 0.3 ppm IV: 0.15 ppm III: 0.10 ppm II: 0.064 ppm	I: 0.0064 ppm	Coronary heart disease mortality	Kaipio <i>et al.</i> 2004 suggest a small protective effect with respect to coronary heart disease mortality. However, this ecological study remains subject to many potential biases, and therefore the results should be interpreted with extreme caution.

The **NHMRC³** review found one study based in Finland that examined the association between cardiovascular disease and fluoridation. The data from this study suggest a small protective effect with respect to coronary heart disease mortality. However, this ecological study remains subject to many potential biases, and therefore the results should be interpreted with extreme caution. If there is an effect of fluoridation on coronary heart disease, it is possible that the mechanism is indirect, via a reduction in dental infections.

Cardiovascular disease in fluoride-endemic areas (above a threshold of 1.5 ppm)

From our search, we identified six papers that examined fluoride in drinking water and its impact on cardiovascular disease (Table 16). All of the studies were conducted in countries where fluoride occurs naturally in the water and generally at higher levels (>2 ppm) than is present in CWF schemes (0.4–1 ppm).

Table 16: Original studies in fluoride-endemic areas (above a threshold of 1.5 ppm) examining cardiovascular disease and water fluoridation, 2006–2014

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
Varol <i>et al.</i>⁶⁶ (2010) Level of evidence: III HRB authors' assessment: though suitable to test a theory, the conduct of the study was low quality	Isparta, with naturally occurring fluoride present in water at high levels (2.74 ppm \pm 0.64) in southwest Turkey	Matched case-control	63 cases of dental fluorosis	45 controls from non-endemic areas; matched for age, sex and BMI.	To assess aortic elasticity in patients with dental fluorosis	The authors conclude that the results of their study demonstrate that elastic properties of ascending aorta are impaired in patients with dental fluorosis exposed to naturally occurring fluoride in drinking water.
Varol <i>et al.</i>⁶⁷ (2010) Level of evidence: III HRB authors' assessment: though suitable to test a theory, the conduct of the study was low quality	Isparta, with natural occurring fluoride present in water at high levels (2.74 ppm \pm 0.64) in southwest Turkey	Matched case-control	63 cases of dental fluorosis	45 controls from non-endemic areas; matched for age, sex and BMI.	To assess ventricular systolic, diastolic, and global functions in patients with dental fluorosis	The authors conclude that the results show that patients with chronic fluorosis had left ventricular diastolic and global dysfunctions.
Amini <i>et al.</i>⁶⁸ (2011) Level of evidence: IV HRB authors' assessment: low quality study to develop a theory and not suitable design to infer causality	Iran	Ecological study	Naturally present fluoride in water	Level of fluoride in the groundwater	To examine the relationship between fluoride in groundwater and blood pressure among the Iranian population	The authors found an increase of hypertension prevalence and the mean systolic blood pressure with increasing levels of fluoride in the groundwater of the Iranian population. This study design is developing a theory rather than testing a theory, and so this is only a suggested relationship. The authors do not control for confounding factors.

Table 16 continued

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
<p>Sun⁶⁹ (2013)</p> <p>Level of evidence: IV</p> <p>HRB authors' assessment: moderate-quality study to describe a situation, but not appropriate design to test a theory</p>	Fluoride endemic areas in China	Cross-sectional study	A total of 487 subjects randomly recruited in China were divided into four groups by the concentrations of fluoride in their drinking water. Consumption levels of drinking water fluoride were 1.55 ± 0.22 mg/L (mild), 2.49 ± 0.30 mg/L (moderate), and 4.06 ± 1.15 mg/L (high), respectively.	Compared to 0.84 ± 0.26 mg/L (normal)	The authors investigated the relationship between mild to high water fluoride exposure and essential hypertension as well as plasma endothelin-1 levels	The authors concluded that the study not only confirmed the relationship between excess fluoride intake and essential hypertension in adults, but it also demonstrated that high levels of fluoride exposure in drinking water could increase plasma ET-1 levels in subjects living in fluoride-endemic areas. The authors controlled for age, gender, smoking, alcohol, BMI and plasma ET 1.
<p>Ostovar⁷⁰ (2013)</p> <p>Level of evidence: IV</p> <p>HRB authors' assessment: low quality study to develop a hypothesis and not appropriate design to test a theory</p>	Iran	Ecological correlation study	Endemic fluoride in drinking water	Level of endemic fluoride	The authors examined the relationship between fluoride level in drinking water and the prevalence of hypertension in people living in villages of Bushehr Province, southern Iran.	The authors found a moderate negative correlation ($r = -0.58$) between the prevalence of hypertension and water fluoride level. There was no control for confounding factors.
<p>Liu⁷¹ (2014)</p> <p>Level of evidence: IV</p> <p>HRB authors' assessment: moderate-quality study to describe a situation, but not appropriate design to test a theory</p>	China	Cross-sectional study	Level of natural fluoride in drinking water: normal concentration group <1.2 mg/L; mild-concentration group $1.21-2$ mg/L; moderate concentration group $2.01-3$ mg/L; high concentration group ≥ 3.01 mg/L	The normal (as defined by the authors) fluoride level in drinking water <2 mg/L	Assess the relationships between developing carotid artery atherosclerosis through consuming high fluoride in drinking water and its possible mechanism, using the baseline data collected from 585 study subjects	The authors state that the findings of the study revealed a statistically significant positive relationship between excess fluoride exposure from drinking water and prevalence of carotid artery atherosclerosis in adults living in fluoride-endemic areas. The possible mechanism was that excess fluoride induced the decreasing level of glutathione peroxidases, causing the systemic inflammation and endothelial activation by oxidative stress. The authors controlled for age, gender, smoking, alcohol, BMI, blood pressure, triglyceride and cholesterol.

Varol et al.⁶⁶ (2010) examined 63 patients (36 males/27 females) with endemic fluorosis and 45 (30 males/15 females) age-, sex-, and body mass index-matched healthy controls were included in this study in order to examine the relationships between naturally occurring fluoride in drinking water and developing carotid artery atherosclerosis. Aortic stiffness indices, aortic strain, aortic distensibility and aortic strain index were calculated from the aortic diameters measured by echocardiography and blood pressure obtained by sphygmomanometry. As expected, the urine fluoride levels of fluorosis patients were statistically significantly higher than control subjects. In contrast, statistically significantly higher aortic strain index measures were observed in fluorosis cases than in the controls (3.4 ± 0.6 vs. 3.0 ± 0.4 ; $p < 0.001$, respectively). According to the authors, the results demonstrate that elastic properties of ascending aorta are impaired in patients with fluorosis who live in fluoride endemic areas.

The study design was a matched (age, sex and body mass index) case-control, and was assigned an evidence level III. There was no sample size calculation described. No rationale was given for the sample size chosen, and the desired effect size was not stated. Cases were diagnosed according to the clinical diagnosis criteria, as described by Wang et al.:⁷² i) living in the endemic fluorosis region since birth; ii) having mottled tooth enamel, indicating dental fluorosis; iii) consuming water with fluoride levels above 1.2 mg/L (normal 1 mg/L); iv) a urine fluoride level greater than 1.5 mg/L (normal <1.5 mg/L). The study's exclusion criterion was the presence of any known cardiac and lung disease, hypertension, angina pectoris, atrial fibrillation or any other arrhythmias, diabetes mellitus, chronic renal and hepatic diseases, serum electrolyte imbalance. The controls were matched with the cases for age, sex and BMI, in order to reduce the level of confounding. There were no other adjustments for known confounders, which could have been accomplished using conditional regression analysis rather than multi-linear regression. The study design is a matched case-control study, but it was described and analysed by the authors as a cross-sectional study. The HRB authors assign a low quality score, as the study analysis did not follow that required by the study design, and so would question the validity of the analysis.

The same case-control study by **Varol et al.**⁶⁷ in 2010 was used to assess the impact of chronic fluorosis on left ventricular diastolic and global functions. Basic echocardiographic measurements, left ventricular diastolic parameters and left ventricular myocardial performance index (MPI) were measured. The urine fluoride levels of patients with fluorosis were statistically significantly higher than those of control subjects. Isovolumic relaxation time (IVRT) and deceleration time (DT) were statistically significantly higher in fluorosis patients than in controls (for IVRT 106.9 ± 15.6 ms vs 96.7 ± 12.2 ms; $p < 0.001$ and for DT 211.7 ± 30.7 ms vs 188.0 ± 30.0 ms; $p < 0.001$, respectively). Myocardial performance index was statistically significantly higher in fluorosis patients than in controls (0.62 ± 0.15 ms vs 0.49 ± 0.10 ms; $p < 0.001$, respectively). The authors conclude that chronic fluorosis patients were likely to develop left ventricular diastolic and global dysfunctions.

The same strengths and limitations apply to the Varol 2010 study as to the previous paper by Varol, which presented analysis from the same study, but focused on a different aspect of CVD. The HRB authors rate this study as low quality, and question the validity of the analysis.

Amini et al.⁶⁸ (2011) set out to examine the relationship between fluoride in groundwater resources (GWRs) of Iran and the blood pressure of the Iranian population. In order to do so, they employed an ecological study design. The mean fluoride data of the GWRs (as a surrogate for fluoride levels in drinking water) were derived from a previously conducted study. The hypertension prevalence and the mean of systolic and diastolic blood pressures (SBP and DBP) of the Iranian population by different provinces and genders were statistically significant. Positive correlations were found between the mean concentrations of fluoride in the GWRs and the prevalence of hypertension in males ($r = 0.48$, $p = 0.007$), females ($r = 0.36$, $p = 0.048$), and overall ($r = 0.495$, $p = 0.005$). Also, statistically significant positive correlations between the mean concentrations of fluoride in the GWRs and the mean systolic blood pressure of males ($r = 0.431$, $p = 0.018$), and a borderline correlation with

females ($r = 0.352$, $p = 0.057$) were found. Amini *et al.* conclude that the increase in the prevalence of hypertension and the mean systolic blood pressure suggested an association with the increased fluoride level in the Iranian GWRs.

This study used a population-based ecological design. Bias is likely to be an issue here, as being a population-based study, we do not know definitively if the people who drank the high-fluoride water are the people who have high blood pressure. There was some control for confounding factors, but such control was not adequate. The authors attempted to validate their findings by correlating the fluoride water concentrations with the caries free index. As expected, the r -value for six-year-old children was 61 per cent. For nine-year-old children, it was 52 per cent, indicating a moderate correlation, but not necessarily causality. The HRB authors assign a level IV, as this study is an ecological study. The HRB authors rate it as a low-quality study.

Sun *et al.*⁶⁹ (2013) investigated the relationships between high fluoride in water exposure and essential hypertension as well as plasma endothelins (ET)-1 levels in a cross-sectional study. A total of 487 residents aged 40 to 75 years were randomly recruited from eight villages in Heilongjiang Province in China. The study subjects were divided into four groups according to the concentrations of fluoride in their water. Consumption levels of drinking water fluoride for normal, mild, moderate, and high-exposure groups were 0.84 ± 0.26 mg/L; 1.55 ± 0.22 mg/L; 2.49 ± 0.30 mg/L; and 4.06 ± 1.15 mg/L, respectively. The prevalence of hypertension in each group was: 20.16 per cent, 24.54 per cent, 32.30 per cent, and 49.23 per cent, respectively. There were statistically significant differences between all the groups; namely, with the increase in water fluoride concentrations, the risk of essential hypertension increased. Statistically significant differences were observed in the plasma ET-1 levels between the different groups ($p < 0.0001$). In the multivariable logistic regression model, high water fluoride concentrations (Fluoride ≥ 3.01 mg/L, OR(4/1)=2.84), age (OR(3/1)=2.63), and BMI (OR(2/1)=2.40, OR(3/1) = 6.03) were closely associated with essential hypertension. The authors conclude that the study results confirmed the relationship between excess fluoride intake and essential hypertension in adults, but it also demonstrated that high levels of fluoride exposure in drinking water could increase plasma ET-1 levels in subjects living in fluoride-endemic areas.

This was a cross-sectional study of 487 residents. Individuals were selected for the study based on the following criteria: adults aged 40 to 75 years; residents living at the same address for at least 10 years who drank the water from tube wells or small wells for more than 10 years. Excluded were adults with other diseases such as a past medical history of diabetes mellitus, fasting blood glucose ≥ 7.0 mmol/L, coronary heart disease, stroke, carotid atherosclerosis, secondary hypertension, kidney disease, liver disease, respiratory disease, emaciation, or long-term use of drugs, as well as those who had a family history of hypertension. No rationale was given for the sample size chosen. The investigators endeavoured to minimise bias by training the research staff and medical practitioners before the field survey was conducted. The training module contents included the purpose of this study, the survey procedures, how to implement the questionnaire and the methods of measurement, etc. When subjects underwent a physical examination, they were also interviewed using a standard questionnaire; data on demographic variables (age, sex, and marital status), smoking and drinking status, sources of potable water, total duration for drinking the water and health information were obtained. The authors used a multivariable logistic regression model that assigned sex, age, water fluoride concentrations, smoking status, drinking status, body mass index, and plasma ET-1 levels as fixed factors; they assigned essential hypertension as the dependent variable, in order to explore the relationships between all the factors. The HRB authors assigned this study an evidence level IV, as it had a cross-sectional design. They noted that it was of moderate quality, as there was no sample size calculation and it was not clear if the study was generalisable. This type of study design is suitable to suggest a relationship, but is not suitable to prove a causal link.

Correspondence from **Ostovar *et al.***⁷⁰ in the *International Journal of Occupational and Environmental Medicine* in 2013 reports on an ecological correlation study which examined the relationship between fluoride level of drinking water and the prevalence of hypertension in people living in villages of Bushehr Province, southern Iran. Fluoride concentration in drinking water with an approximate range 0.2–2.2 mg/L, as well data on the prevalence of hypertension, were collected for 91 villages in Bushehr Province, southern Iran. These villages were home to 160,150 inhabitants (80,661 males and 79,489 females). The prevalence of hypertension was calculated by dividing the number of patients with hypertension by the total population in each village (all ages) whose data were extracted from the provincial health centre surveillance system. The hypertension prevalence ranged from 0.3 per cent to 30.3 per cent. Using a weighted least square linear regression analysis, the authors report a statistically significant negative correlation (-0.58) between the hypertension prevalence and water fluoride level, indicating that as the fluoride level increased, the prevalence of hypertension decreased.

This was a population-based ecological study. As such we do not know definitively if the people who drank the high-fluoride water are the people who have low blood pressure. There was no control for confounding factors and no inclusion or exclusion criteria mentioned. The HRB authors rate this as a low-quality study and reiterate that this type of study design can only suggest a relationship. The HRB authors assign it a level IV.

Liu *et al.*⁷¹ (2014) set out to assess the relationship between excess fluoride intake from drinking water and carotid atherosclerosis development in adults in fluoride-endemic areas of China. Cross-sectional analysis was conducted to assess the relationships between developing carotid artery atherosclerosis through consuming high levels of fluoride in drinking water. In this cross-sectional analysis, 585 study subjects were divided into four groups, based on the concentrations of fluoride in their drinking water. The range of fluoride concentrations was: normal-concentration group (less than 1.20 mg/L), mild-concentration group (1.21–2.00 mg/L), moderate-concentration group (2.01–3.00 mg/L), and high-concentration group (more than 3.01 mg/L). The prevalence rate of carotid artery atherosclerosis in the subjects in each group was found to be 16.13 per cent; 27.22 per cent; 27.10 per cent; and 29.69 per cent, respectively. Statistically significant difference between the prevalence of carotid artery atherosclerosis in the mild-, moderate- and high-fluoride exposure group, compared to the normal-concentration group, was observed ($p < 0.05$). In addition, it was found that elevated intercellular cell adhesion molecule and reduced glutathione peroxidases were associated with carotid artery atherosclerosis in fluoride- endemic areas. Liu *et al.* conclude that the findings of the study revealed a statistically significant positive relationship between excess fluoride exposure in drinking water and the prevalence of carotid artery atherosclerosis in adults living in fluoride-endemic areas. The possible mechanism, according to Liu *et al.*, was that excess fluoride induced the decreased level of glutathione peroxidases causing the systemic inflammation and endothelial activation by oxidative stress.

There were 585 subjects in this cross-sectional study. No rationale was given for the number of subjects chosen, and the researchers did not complete a power calculation to estimate prevalence. The inclusion/exclusion criteria were adults aged above 40 years old living at the same address for at least 10 years and consuming water from the same tube wells or shallow wells for more than 10 years. Prior to carrying out the study, the researchers attempted to minimise bias through training the survey personnel, including some medical doctors; in addition, a standard questionnaire was developed to collect the study data. Adults with certain medical conditions (diabetes mellitus, coronary heart disease, stroke, kidney disease, liver disease, respiratory disease, emaciation) were excluded from the study. Also excluded were people with a history of long-term use of drugs. According to Liu *et al.*, all selected subjects for this study had similar dietary habits and yearly incomes. The authors controlled for confounding factors, including sex, age, smoking status, alcohol consumption, blood glucose, systolic blood pressure, diastolic blood pressure, BMI, triglyceride, total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol. The authors analysed the risk factors for atherosclerosis using a logistic regression model, in order to determine the contribution of each of the risk

factors (while controlling for the other risk factors) in the development of atherosclerosis. The HRB authors assign this study an evidence level IV, based on its design. The study is deemed to be of moderate quality.

International reports and expert bodies' conclusions on cardiovascular disease

The **reports** identified in our grey literature search were examined for their conclusions on the possible association between fluoride and cardiovascular disease.

The **NRC report**²⁷ 2006, the **WHO report**⁵⁴ 2006, the **National Institute of Public Health in Quebec report**²⁸ 2007 the **European Food Safety Authority (EFSA) report**,²⁹ the **U.S. EPA Office of Water**,³⁰ the **Guidelines for Canadian Drinking Water Quality: Guideline Technical Document**³¹ and the **Scientific Committee on Health and Environmental Risks**¹⁴ do not address the issue of water fluoridation and its possible effects on cardiovascular disease.

The **Royal Society of New Zealand and the Office of the Prime Minister's Chief Science Advisor's report**³² in August 2014 reviewed only one research study on the possible link between water fluoridation and cardiovascular disease, i.e., the Liu⁷¹ study conducted in China and discussed above. The report describes the findings of the Liu study, but could not draw general conclusions based only on one study.

Summary on cardiovascular disease

Non-endemic or CWF areas

Only one of the index reports examined a study concerned with the possible effect of drinking fluoridated water on the associated risk of cardiovascular disease in non-endemic areas similar to Ireland. The ecological study included in the index report was conducted in Finland, and the results suggested a slight protective effect of fluoride with respect to coronary heart disease. The HRB authors did not find any more recent studies in CWF areas on this topic.

Fluoride-endemic areas

The six studies the HRB authors found and reviewed were all completed in areas where fluoride is naturally occurring in groundwater and generally at higher levels than those found in water with CWF (0.4–1 ppm), like Ireland. The higher levels of naturally occurring fluoride varied across the six studies, with moderate and higher levels being defined as greater than 2 ppm with no upper limit. Two case-control studies in Turkey found that high levels of natural water fluoridation decreased aortic elasticity and contributed to cardiac dysfunction; however, it is difficult to make definitive statements based on these studies, as their execution is judged to be of low quality. Three studies, two in Iran and one in China, examined water fluoridation and its link with hypertension. Two of these were ecological studies and one was a cross-sectional study; two found a higher prevalence of hypertension with increased fluoride level, and one found the opposite. However, due to the study design employed in all three studies, it can only be suggested that a relationship may exist between fluoride and blood pressure levels. The sixth study, a cross-sectional study in China, reported a positive correlation between atherosclerosis prevalence and water fluoride concentration; however, it is a suggested correlation, not a proven cause and effect. A summary of the existing literature indicates that the evidence is inconsistent and lacking in methodological rigour.

Other potential health effects

Other health concerns linked with fluoridation of drinking water have been raised, and our search uncovered a small number of studies carried out since 2006 that address some of these issues. Apart from the index reports, five studies that examine a variety of other potential health effects from water fluoridation were discovered. The topics discussed in this section are: kidney disorders (in CWF areas and areas of high naturally fluoridated water), birth defects, endocrine disorders, immune system disorders and all-cause mortality.

Kidney disorders and fluoride

Two studies examining kidney disorders in CWF areas, and one review examining the same topic in naturally fluoridated water areas, were found. The York review examined a variety of other possible negative effects, but kidney disorders were not a subject of any of their included studies.

Table 17: Other potential health effects and water fluoridation: kidney disorders up to 2006, results of the NHMRC review

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Index authors' conclusion
NHMRC³ (2007) Level of evidence: IV HRB authors' opinion of this index review: high quality	One level IV cross-sectional study. India	Naturally occurring fluoridated water at levels of 3.5–4.9 ppm	Naturally occurring fluoridated water at levels of 0.5 ppm	Kidney stone prevalence	Kidney stone prevalence: Endemic: 750/100,000 Non-endemic: 163/100,000

The **NHMRC review³** included only one study relating to kidney disorders, a cross-sectional study by Singh *et al.*⁷³ (2001). This study estimated the kidney stone prevalence among Indians living in areas where the water is naturally fluoridated at a high level (3.5–4.9 ppm) and compared the overall prevalence to areas where the natural fluoride level was 0.5 ppm (Table 17). The kidney stone prevalence was almost five times higher (OR 4.63 (2.07–7.92)) in the high-fluoride areas when compared to the low-fluoride areas. The Australian authors concluded that the study of Singh *et al.* involved fluoride concentrations which would not be observed in water in Australia, and that the study quality was poor.

Table 18: Additional review papers examining other potential health effects and water fluoridation: kidney disorders: 2006–2014

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Research study authors' conclusion
Ludlow⁷⁴ (2007) Level of evidence: IV HRB authors' opinion of this narrative review: high quality	47 references, papers from 1954 to July 2006. Most papers are from 1980s–1990s.	CWF	No CWF	Summarise recent literature relating to the health effects of fluoridation of community water supplies for people	The poor evidence quality and deficient methodological rigour of the identified studies means that no definitive conclusions regarding the association between consumption of optimally fluoridated community water and chronic kidney disease can be made

Ludlow *et al.*⁷⁴ conducted a narrative review of the literature relating to the renal health effects of CWF in 2007, with the aim of summarising the recent literature. Their search strategy focused on the OVID platform (MEDLINE, PreMEDLINE, PsycINFO), the Cochrane Library, the National Research Registers (United Kingdom and United States) and other databases of controlled clinical trials (Table 18). Databases were searched from inception until 1 July 2006. The reference list of each article retrieved by the electronic database search was hand-searched, in order to find other relevant articles. Articles not published in the English language were excluded. The Google Internet search engine and grey literature directories (such as the New York Academy of Medicine: Grey Literature Page) were also used to identify unpublished reports. The authors included only articles that provided relevant information to answer the questions. Where possible, Ludlow *et al.* made a distinction between investigations concerning the optimal concentration of fluoride in drinking water (≤ 1.5 ppm), and higher than optimal fluoride levels (> 1.5 ppm). The authors do not present their quality assessment tool, but they provide a summary of their quality assessment. According to Ludlow *et al.*,⁷⁴ the quality of the included studies was typically in the moderate to weak range, with a lack of methodological rigour and inadequate control of potential sources of bias. The review identified a distinct lack of high-level evidence in the form of randomised controlled trials or cohort studies, with the majority of studies consisting of case-series, case-reports or comparative studies utilising historical controls. Ludlow *et al.* conclude that the poor evidence quality and deficient methodological rigour of the identified studies meant that no definitive conclusions regarding the association between consumption of optimally fluoridated community water and chronic kidney disease could be made.

Table 19 presents two primary studies on other potential health effects, kidney disorders and water fluoridation, carried out between 2006 and 2014.

The Public Health England¹⁶ study aimed to link the number of patients admitted to hospital with kidney stones with area of residence at the time of their diagnosis, in order to determine whether they were exposed to fluoride in drinking water or not. The indicator studied was the number of first and second diagnosis of kidney stones among emergency inpatient consultant episodes per LSOA in England, recorded in hospital episode statistics between April 2007 and March 2013. A priori confounding variables examined were age, gender, deprivation, and ethnicity. The rate of kidney stones was almost 8 per cent (7.9 per cent lower; 95 per cent CI - 9.6 per cent to -6.2 per cent; $p < 0.001$) lower in fluoridated areas compared to non-fluoridated areas, following adjustment for age, gender and deprivation and ethnicity. Public Health England concludes that there was evidence that the rate of kidney stones was lower in fluoridated areas than in non-fluoridated areas both before and after controlling for confounding factors.

This ecological study was designed to monitor the health effects of water fluoridation through the use of data to compare rates of selected indicators in fluoridated versus non-fluoridated areas in England. However, this type of study design is used to create a theory of association between fluoride and health outcomes, but cannot be used to infer causality. There may be bias in this study, as there is likely to be crossover of individuals' place of residence between fluoridated and non-fluoridated areas, and it would be more accurate to ascertain a history of lifetime resident addresses in order to accurately determine fluoride exposure over time. Public Health England identified and controlled for the following confounding variables: age; gender; deprivation; ethnicity. The HRB authors assign an evidence level IV to this study, based on its design, and they classify the study quality as moderate.

Table 19: Additional original studies examining other potential health effects: kidney disorders and water fluoridation, 2006–2014

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
Public Health England¹⁶ (2014) Level of evidence: IV HRB authors' opinion of this study: moderate quality study to create a theory, but not suitable to test it	England	Ecological study	CWF fluoride in drinking water at small-area level	Non-fluoridated small areas	To link the number of patients admitted to hospital with kidney stones with area of residence at the time of their diagnosis, in order to determine their exposure to fluoride in drinking water	There was evidence that the rate of kidney stones was lower in fluoridated areas than in non-fluoridated areas both before and after controlling for confounding factors.
Chandrajith⁷⁵ (2011) Level of evidence unable to classify, as design is unclear. HRB authors' opinion of this study: low-quality	Sri Lanka	A mix of case series and cross-sectional survey	Fluoride content in water and its link with chronic kidney disease of unknown aetiology. Drinking water from high-prevalence endemic regions was analysed for trace and ultratrace element contents. (means for 5 areas ranged between 0.50-1.41 mg/L and maximums for 5 areas ranged between 1.18-5.30 mg/L)	Drinking water from non-endemic regions was analysed for their trace and ultratrace element contents	To identify possible aetiologies and risk factors for chronic kidney disease (CKD) patients from the north central region of Sri Lanka, with a particular focus on geo-environmental factors	The authors conclude that no single geochemical parameter in the drinking water could be clearly and directly related to the CKD aetiology on the basis of the elements examined in this study.

Chandrajith *et al.*⁷⁵ conducted a study in Sri Lanka in 2011. Population screening had been carried out using a multi-stage sampling technique which indicated the point prevalence of chronic kidney disease with uncertain aetiology to be 2 per cent and 3 percent among those over 18 years of age. Drinking water collected from high-prevalence (means for 5 areas ranged between 0.50-1.41 mg/L and maximums for 5 areas ranged between 1.18-5.30 mg/L) and control region (mean 1.03 mg/L and maximum 1.68 mg/L) was analysed for its trace and ultratrace element contents, including the nephrotoxic heavy metals cadmium and uranium. The authors state that results indicate that the affected regions contain moderate to high levels of fluoride (means for 5 areas ranged between 0.50-1.41 mg/L and maximums for 5 areas ranged between 1.18-5.30 mg/L). The cadmium contents in drinking water, rice from affected regions and urine from symptomatic and non-symptomatic patients were much lower, indicating that cadmium is not a contributing factor for chronic kidney disease with uncertain aetiology in Sri Lanka. One hundred and eight water samples were tested in regions where chronic kidney disease of uncertain aetiology is endemic; 14 water samples from non-endemic regions were tested. Also tested were 135 water samples from wells used by patients with chronic kidney disease of uncertain aetiology who were attending renal clinics.

The study design seems to be a mixture of case series and cross-sectional. The study population is described, but is very confusing, and does not correlate with the results presented. There was no clear description of the methods used. No rationale was given for the sample size chosen, and no attempts to minimise bias were described. There was some attempt to control for other chemicals, as confounders, that may have caused chronic kidney disease. The HRB authors are unable to assign this low-quality study an evidence level, as the study design is unclear.

Endocrine disorders and fluoride

Endocrine disorders can refer to many different health problems, including thyroid disease and diabetes. Concerns have been raised in relation to possible endocrine gland dysfunction and fluoridated water. The glands that have been mentioned in relation to this are the pancreas (diabetes), the thyroid (goitre, hypothyroidism and hyperthyroidism), the pituitary gland (hyperpituitarism and hypopituitarism) and the pineal gland (a number of disorders). The York review² examined three studies which investigated water fluoridation in relation to effects on goitre (Table 20). The NHMRC report³ did not uncover any further studies that examined water fluoridation and endocrine disorders.

Table 20: Other potential health effects and water fluoridation: endocrine disorders up to 2006, results of index report

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Index authors' conclusion
McDonagh <i>et al.</i>² (York review) (2000) Level of evidence: IV HRB authors' opinion of this index review: high	Three cross-sectional, studies	Water fluoridation level	Non-fluoridated control area	Goitre prevalence	One study found a statistically negative association of combined low iodine/high fluoride with goitre. The other two studies did not detect a statistical association.

Goitre or enlarged thyroid was considered by the **York²** team, but they only found three studies relating to this disorder. Of these, one found a statistically significant association with water fluoride level and the other two did not find this association. Therefore, findings on goitre were mixed and, once again, no clear pattern of association was seen. The authors of the York review conclude that, overall, there was no association detected between water fluoridation and the prevalence of goitre.

One paper concerning the effects of water fluoridation and endocrine disorders was identified from our search. However, after completion of the draft report, another original study⁷⁶ linking water fluoridation and hypothyroidism was published. Because of the possible clinical importance of this paper, the HRB authors decided to include it, even though it was outside the timeline of the search (Table 21).

Table 21: Additional original studies examining other potential health effects and water fluoridation: endocrine disorders, 2006–2014

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
Peckham⁷⁶ (2015) Level of evidence: IV HRB authors' opinion of this study: low quality	England	Ecological	People living in areas with fluoride levels in drinking water at a target concentration of 1 ppm	People living in non-fluoridated areas < 0.3 ppm	To study the effects of fluoride in the water on the prevalence of hypothyroidism in GP practices	The authors concluded that higher levels (0.7 – 1 ppm) of fluoride in drinking water provide a useful contribution for predicting prevalence of hypothyroidism in GP practices in England.

Peckham and colleagues⁷⁶ examined the association between levels of fluoride in water supplies for GP practice locations and prevalence of hypothyroidism at the same practice location, in order to determine if there was an association between fluoride in water and the prevalence of hypothyroidism (Table 21). They included 7,935 general practices for the national study. The authors state that 10 per cent of the people in England live in areas where drinking water contains natural fluoride, or where artificial fluoride has been added at a target concentration of 1 ppm (1 mg/L). For the national study (first model) the authors do not describe how many GP practices were situated in the fluoridated areas or the non-fluoridated areas. The second model restricted data to the West Midlands (fluoridated) and Greater Manchester (non-fluoridated) areas. Of note it would appear a bigger proportion of the Greater West Midland area is situated in the UK goitre belt compared to the Greater Manchester area, which would result in a higher prevalence of hypothyroidism regardless of the water fluoridation status. The number of GP practices included in this model is not stated for either of these areas; it appears that there may have been 946 GP practices in total in the sub-analysis. They controlled for three possible confounding factors: age over 40 years, female gender and level of deprivation.

Women over the age of 40 years are the most likely population subgroup to develop hypothyroidism. Studies from developed countries on the potential association of socioeconomic status with iodine supply and the risk for thyroid disorders are sparse. Other factors that influence the development of hypothyroidism are: iodine intake, treatment for hyperthyroidism, radiation therapy, thyroid surgery and certain medications. Therefore, all these factors – and not just age and gender – need to be controlled for when examining an association. In addition, Peckham et al. do not provide any insight on the time course of hypothyroidism which is unlikely to develop instantaneously.

At a national level, after adjusting for the confounders, age, gender and deprivation index, Peckam *et al.* found that higher levels of fluoride in drinking water at GP practice level predicted a higher prevalence of hypothyroidism. The odds of a GP practice recording high levels of hypothyroidism (in the upper tertile) were 1.37 (95 per cent 1.12–1.67) times higher in areas with fluoride levels between >0.3 and ≤0.7 mg/L and 1.62 times (95 per cent CI 1.38–1.90) higher in areas with fluoride in greater than 0.7 mg/L, than for GP practices in areas with fluoride ≤0.3 mg/L. The authors also found that GP practices located in the West Midlands (a wholly fluoridated area) were nearly twice (1.94, 95 per cent CI 1.39–2.70) as likely to report high hypothyroidism prevalence than those in Greater Manchester (non-fluoridated area). The HRB authors consider that this study demonstrates an association between fluoride in water at GP practice locations and the prevalence of hypothyroidism in the same practices. The HRB authors acknowledge that this study suggests that fluoride in water may be linked to the development of hypothyroidism, but observational epidemiological studies (such as cohort and case-control study designs) are required in order to prove causality. When that has been done, the observed association must be judged against established causation criteria in order to determine whether it is

scientifically valid to draw a causal inference. Therefore, an observed statistical association between a risk factor and a disease does not necessarily lead us to infer a causal relationship. A judgement about whether an observed statistical association represents a cause-effect relationship between exposure and disease requires inferences beyond the data from a single study, such as the Bradford-Hill criteria⁷⁷ listed below.

The Bradford-Hill viewpoints⁷⁷ are widely used in epidemiology as a framework with which to assess whether an observed association is likely to be causal. These viewpoints include: strength of the association, replication and consistency of the association, specificity of the association (one-to-one relationship between cause and outcome), temporality (exposure must have occurred before disease symptoms appeared), dose-response relationship, biological plausibility (study results are consistent with existing knowledge) and consideration of alternative explanations.

Peckham and colleagues describes their study as a cross-sectional study design, but the HRB authors consider the design of this study, based on the methods described, to be ecological. A cross-sectional study is based on individuals, and the data on exposure and outcome are collected at the same point in time from each individual. The approach to data collection in Peckham's study is population based rather than individual based, and the data come from two separate sources that cannot link the hypothyroidism status of the individual attending the GP practice to that individual's personal exposure to fluoride in drinking water. In addition, the exposure to fluoride is based on the location of the principal general practice rather the person's place of residence, and the data come from two different time periods. Ecological studies can create a hypothesis or theory rather than infer causality.

Peckham *et al.* acknowledge that 'ecological bias exists in the study in that it assumes that aggregated statistics, that is, GP practice hypothyroidism registers, are representative of the individuals living in the area. CodePoint location coordinates may not give a precise location of practices, as they are created by taking an average of the coordinates of all the individual addresses in the postcode, then snapping to the nearest of those addresses. The coordinates of that address are taken as representative of the whole postcode. Also, patients registered with a practice may also be distributed over a wide area, covering a number of WSZs and, therefore, the fluoride level for the practice postcode may not be accurate for practice patients. In addition, many GP practices have branch surgeries in different geographical locations but the data sets do not distinguish between branch and main practices so all data are attributed to the main practice and thus the WSZ of the main practice'. Thus, there is a considerable risk of misclassification of exposure in this study.

In the interpretation section of the discussion, the authors comment that 'this study only included data on diagnosed hypothyroidism, and it is possible that in fluoridated areas there would be a proportion of the population who will suffer from subclinical hypothyroidism'. However, although not stated in their paper, this would also be true for non-fluoridated areas.

The HRB assign this study an evidence level IV, based on the design. The quality level is low. There are three reasons for assigning a low-quality rating. First, the study design assigned was incorrect. Second, the control for confounding was incomplete. Third, the authors infer a causal relationship rather than a theoretical relationship.

Immune system disorders and fluoride (above a threshold of 1.5 ppm)

Neither of the chosen index reports uncovered any studies on the possible association between fluoride in the drinking water and immune system disorders. The search performed for the current review identified one study dealing with this subject.

Table 22: Additional original studies examining other potential health effects and water fluoridation: immune system disorders (above a threshold of 1.5 ppm), 2006–2014

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
Hernandez-Castro⁷⁸ (2011) Level of evidence: IV HRB authors' opinion of this study: low quality	Mexico	Cross-sectional	61 people (39 males and 22 females) with no symptoms of an immune disease exposed to over 2 ppm fluoride in their drinking water	None	Fluoride mainly through drinking water, and its effect on different immune parameters, mainly T regulatory cells	The authors conclude that their data suggest that F exposure exerts a complex and relevant effect on T regulatory cells in humans.

Hernandez-Castro *et al.*⁷⁸ conducted this study in 2011 to examine the effects of fluoride on different immune parameters, mainly T regulatory cells in people whose exposure to fluoride is mainly from drinking water (Table 22). T regulatory cells are a component of the immune system that suppresses immune responses of other cells. This is an important 'self-check' built into the immune system to prevent excessive reactions. Regulatory T cells come in many forms, with the most well understood being those that express cluster differentiation (CD): CD4, CD25, and foxp3 (named CD4+CD25+ regulatory T cells). The T helper cells (Th cells) are a type of T cell that play an important role in the immune system, particularly in the adaptive immune system. Regulatory T cells are involved in shutting down immune responses after they have successfully eliminated invading organisms; they are also involved in preventing autoimmunity cells. Mature Th cells express the surface protein CD4 and are referred to as CD4+ T cells. CD4+ T cells are commonly divided into regulatory T (Treg) cells and conventional T helper (Th) cells. Th cells control adaptive immunity against pathogens and cancer by activating other effector immune cells. Treg cells are defined as CD4+ T cells in charge of suppressing potentially deleterious activities of Th cells. The authors found a negative correlation between urinary fluoride and percentage of CD4(+)CD25(+) Treg cells ($r=-0.55$, $P<0.001$). This means that a defective function of these cells was detected in 30 per cent of individuals exposed to fluoride. In contrast, a positive association between levels of CD4(+)TGF-beta(+) or CD4(+)IL-10(+) Treg lymphocytes (interleukin10 or IL-10 is an important suppressive cytokine, produced by a large number of immune cells) and fluoride urine concentration was detected. In addition, a negative correlation was detected between the urinary levels and the proportion of apoptotic cells in peripheral blood mononucleated cell or T cells or monocytes ($P<0.05$ in all cases). Finally, no apparent association between F exposure and toll-like receptor 4/CD14 expression or the synthesis of tumour necrosis factor-alpha was detected. The study authors conclude that their data suggest that F exposure exerts a complex and relevant effect on Treg cells in humans.

This cross-sectional study of 61 subjects from a community in the state of Durango, Mexico, where the population is exposed to fluoride levels of over 2.0 ppm in drinking water had no comparison group and no indication as to the reason the particular sample size was chosen and no justification for the sample size. Attempts to eliminate bias were not mentioned, and some possible confounding factors were controlled for in the analysis (age and gender and time of residence), but there was no control for other factors that could modify Treg cells. The HRB authors assign an evidence level IV to this study, based on the design. They classify it as low quality.

Birth defects and fluoride

Birth defects can include many different congenital anomalies. In relation to water fluoridation, the most concern has been expressed about the possible association of fluoridation and the occurrence of Down syndrome in babies born to women exposed to fluoride. The York review examined six studies which explored this association. The NHMRC reported on one further review of this topic and one additional primary study.

The **York team**² reviewed six studies which examined the association between Down syndrome and water fluoride level (Table 23). Three studies found a negative direction of association, one found a positive direction of association, one found no association, and the sixth found a positive direction of association for one set of data and a negative direction of association for the other. None of the three studies that found a negative direction of association presented any measure of statistical significance. The one study that found a positive direction of association did present variance data and failed to find a statistically significant association. The study that found a positive direction of association in one set of data and a negative direction of association in the other did not find a statistically significant association in either direction.

The **NHMRC review**³ examined two systematic reviews and one additional primary research study (Table 23). The results of the additional primary study supported the findings of the two systematic reviews, indicating no difference in stillbirths and congenital abnormalities in fluoridated and non-fluoridated areas (with the exception of clefts, which were statistically significantly lower in the fluoridated areas).

Table 23: Other potential health effects and water fluoridation, birth defects up to 2006: results of index reports

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Index authors' conclusion
McDonagh <i>et al.</i> ² (York review) (2000) Level of evidence: IV HRB authors' opinion of this index review: good	Six primary studies. All ecological study designs	Water fluoridation level	Non-fluoridated control area	Prevalence of Down syndrome/ congenital anomalies	The authors conclude that there was insufficient evidence to reach conclusions.
NHMRC ³ (2007) Level of evidence: IV HRB authors' opinion of this index review: high quality	Two reviews (York review and Whiting <i>et al.</i>) in which both reviewed the same six primary studies One additional primary study (Lowry <i>et al.</i> 2003)	Artificial water fluoridation or water with naturally occurring high levels of fluoride	No water fluoridation or a lower level of water fluoridation	Down syndrome/ congenital anomalies	The NHMRC review findings indicated no difference in congenital abnormalities in fluoridated and non-fluoridated areas (with the exception of clefts, which were statistically significantly lower in the fluoridated areas).

The **Public Health England**¹⁶ study was ecological in design. Cases of Down syndrome, by lower tier local authority, were obtained from the National Down Syndrome Cytogenetic Register. The case definition included all cases of Down syndrome in England, including: live births; stillbirths (24+ weeks' gestation); late miscarriages (20-23 weeks' gestation) and terminations of pregnancy with foetal anomaly which were recorded between 2009 and 2012 (Table 24). Almost every baby with clinical features suggesting Down syndrome, as well as any antenatal diagnostic sample from a pregnant woman suspected of carrying a Down syndrome baby, received a

cytogenetic examination, since the definitive test for the syndrome is detection of an extra chromosome 21 (trisomy 21). All clinical cytogenetic laboratories in England and Wales submit a completed form for each such diagnosis and its variants to the National Down Syndrome Cytogenetic Register. Cases of Down syndrome were categorised according to year of outcome for live births, stillbirths (24+ weeks' gestation) and late miscarriages (20–23 weeks' gestation) and by expected year of outcome for terminations of pregnancy with foetal anomaly. Between 2009 and 2012, there were 6,619 cases of Down syndrome out of 2,727,300 live births in England; a prevalence of 24.3 per 10,000 live births (95 per cent CI: 23.7 to 24.9). The prevalence was 21.7 (95 per cent CI: 20.0 to 23.4) per 10,000 live births in fluoridated local authorities (658/303,818) compared with 24.6 (95 per cent CI: 24.0 to 25.2) per 10,000 live births in non-fluoridated local authorities (5,961/2,423,482). The average maternal age was higher in the non-fluoridated local authorities (29.3 years; 95 per cent CI: 29.30 to 29.31) compared with the fluoridated local authorities (28.4 years; 95 per cent CI: 28.37 to 28.41). In the Poisson regression model, adjusting for the total number of births but not including any adjustment for maternal age, the incidence rate in fluoridated local authorities compared to non-fluoridated was 12 per cent lower (95 per cent CI -19 per cent to -4 per cent; $p < 0.01$); whereas in the model fitted with expected births as a measure of the exposure, i.e., adjusting for maternal age, there was no evidence of an association between fluoridation and Down syndrome (2 per cent higher; 95 per cent CI -6 per cent to 10 per cent; $p = 0.68$). There was no evidence of a difference in the rate of Down syndrome in fluoridated and non-fluoridated areas.

Table 24: Additional original studies examining other potential health effects and water fluoridation: birth defects, 2006–2014

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
Public Health England¹⁶ (2014) Level of evidence: IV HRB authors' opinion of this study: moderate-quality study to create a theory but not suitable to test it	England	Ecological	CWF	Non-CWF areas	Is there a difference between the rates of Down syndrome births in fluoridated and non-fluoridated areas?	The authors concluded that there was no evidence of a difference in the rate of Down syndrome in fluoridated and non-fluoridated areas.

This study conducted by Public Health England¹⁶ was a population-based ecological study. The authors provided a comprehensive description of the study population. There is a potential for bias, as there is likely to be crossover of individuals' place of residence between fluoridated and non-fluoridated areas, and it would have been more appropriate to ascertain a history of lifetime resident addresses in order to accurately determine exposure over time. The authors did control for confounding factors to the best extent possible. The risk of a Down syndrome birth is highly associated with maternal age. Therefore, this variable was considered as an a priori confounder. The HRB authors assign a level IV to this study based on design, and they consider it to be of moderate quality.

All-cause mortality and fluoride

All-cause mortality refers to deaths from any cause, and some studies investigated if there was a higher death rate from all causes in areas with water fluoridation, compared to areas without fluoridation. The York review examined five such studies (Table 25). The Australian NHMRC review found no additional studies to the York review examining all-cause mortality and fluoride.

Table 25: Other potential health effects and water fluoridation: all-cause mortality up to 2006, results of index reports

Citation, level of evidence, study quality	Number and type of studies included	Exposure	Comparator	Outcomes	Index authors' conclusion
McDonagh <i>et al.</i>² (York review) (2000) Level of evidence: IV HRB authors' opinion of this index review: good	Five studies; two before and after studies and three of unknown design	Water fluoridation naturally or artificially at or near 1 ppm	Non-fluoridated control area	Difference in all-cause mortality rates	The authors conclude that there was no association between water fluoridation and all-cause mortality rates.

In the **York review**,² five studies examined the association between all-cause mortality and water fluoride exposure. Three studies found the direction of association of water fluoridation and mortality to be negative (more deaths); one found the direction of association to be positive (fewer deaths); and one found no association. Once again, no measures of the statistical significance of these associations were provided. However, for two of the studies that found a negative direction of association, the point estimate was 1.01, which is unlikely to have reflected a statistically significant effect.

Table 26: Additional original studies examining other potential health effects and water fluoridation: all-cause mortality, 2006–2014

Citation, level of evidence, study quality	Location	Type of study	Exposure	Comparator	Outcomes	Research study authors' conclusion
Public Health England¹⁶ (2014) Level of evidence: IV HRB authors' opinion of this study: moderate-quality study to create a theory, but not suitable to test it	England	Ecological	Artificial water fluoridation	No fluoridation	All-cause mortality	The authors conclude that the study showed some evidence of lower all-cause mortality in fluoridated versus non-fluoridated areas. The overall effect size was very small, and this is likely to have occurred as a result of chance, or possibly confounding.

The **Public Health England¹⁶** study conducted an ecological study comparing all-cause mortality rates in fluoridated areas to non-fluoridated areas in England (Table 26). The total all-cause mortality, recorded as the

count of deaths, was obtained at LSOA level from the Office of National Statistics (ONS) data for the period January 2009 to January 2012. These three years were used, as mortality was relatively stable during this period following reductions over preceding years. A priori confounding variables examined were: age, gender, deprivation and ethnicity. Following initial univariate analysis, multivariable models were constructed to test the association between fluoridation status and the all-cause mortality, adjusted for a priori confounding variables. There was some evidence that all-cause mortality was lower in fluoridated LSOAs compared to non-fluoridated LSOAs (1.4 per cent lower; 95 per cent CI -2.6 per cent, -0.3 per cent; $p=0.02$) following adjustment for age, gender and deprivation; and also some evidence that it was lower following additional adjustment for ethnicity (1.3 per cent lower; 95 per cent CI -2.5 per cent, -0.1 per cent; $p=0.04$). PHE conclude that the study showed some evidence of lower all-cause mortality in fluoridated versus non-fluoridated areas. The overall effect size was very small, and this is likely to have occurred as a result of chance, or possibly due to other unidentified confounding factors.

This is a population-based ecological study. The authors tried to control for confounding factors to the best extent possible. However, there is likely to be bias present because of crossover of individuals' place of residence between fluoridated and non-fluoridated areas, and it would be more appropriate to ascertain a history of lifetime resident addresses in order to accurately determine fluoride exposure over time. The HRB authors assign a level IV to this study based on design, and consider it to be of moderate quality.

International reports and expert bodies' conclusions on other potential health effects

The **reports** identified in our grey literature search were examined for their conclusions on the possible association between fluoride and other harms.

The **NRC report**²⁷ (2006) considered other possible health effects, including effects on the gastrointestinal system, kidneys, liver, and the immune system. According to the authors, there were no human studies on drinking water containing fluoride at 4 mg/L (the maximum contaminant level goal set by the US EPA) in which gastrointestinal, renal, hepatic, or immune effects were carefully documented. Case reports and in vitro and animal studies indicated that exposure to fluoride at concentrations greater than 4 mg/L can be irritating to the gastrointestinal system, affect renal tissues and function, and alter hepatic and immunologic parameters. Such effects are unlikely to be a risk for the average individual exposed to fluoride at 4 mg/L in drinking water.

The **WHO report**⁵⁷ (2006), on other possible health effects stated that a number of epidemiological studies have been carried out to examine other possible adverse outcomes as a consequence of exposure to fluoride, either from drinking water or as a result of an individual's occupation. Studies on the association between exposure of mothers to fluoride in drinking water and adverse pregnancy outcomes have shown no increased risk of either spontaneous abortion or congenital malformations. No reasonable evidence of effects on the respiratory, haematopoietic, hepatic or renal systems that could be attributed specifically to fluoride exposure has emerged from studies of occupationally exposed populations. In addition, such studies have failed to produce convincing evidence of genotoxic effects. The WHO pointed out that the majority of fluoride is excreted via the kidneys, and therefore it is reasonable to assume that those with impaired renal function might be at greater risk of fluoride toxicity than those who do not have impaired renal function.

The **National Institute of Public Health in Quebec report, 2007**,²⁸ the **European Food Safety Authority**²⁹ (EFSA) report and the **U.S. EPA Office of Water**³⁰ do not address the issue of water fluoridation and its possible effects on 'other' potential health harms outside of those harms mentioned in the previous sections.

The **Guidelines for Canadian Drinking Water Quality: Guideline Technical Document**³¹ examined possible links between water fluoridation and otosclerosis, urolithiasis (kidney stones), and parathyroid hormone levels. Overall, the results show that adverse health effects are usually associated with high levels of fluoride in drinking water. The **NRC Expert Committee on Fluoride in Drinking Water**²⁷ (as quoted by the Canadian Guidelines document) did not find any human studies on drinking water containing fluoride at 4 mg/L where gastrointestinal, hepatic, or immune effects were carefully documented. Based on Health Canada's review of available science, as supported by the Expert Panel Meeting on fluoride, the weight of evidence does not support a link between exposure to fluoride in drinking water up to 1.5 mg/L and any adverse health effects, including immunotoxicity, reproductive and/or developmental toxicity, genotoxicity, and/or neurotoxicity.

The **Scientific Committee on Health and Environmental Risks**¹⁴ examined the possible link between water fluoridation and genotoxicity and reproductive and developmental defects (in addition to skeletal fluorosis, carcinogenicity and neurotoxicity). Regarding genotoxicity, they wrote that there are conflicting reports on genotoxic effects in humans. An increase in sister chromatid exchanges and micronuclei has been reported in peripheral lymphocytes from patients with skeletal fluorosis or residents in fluorosis-endemic areas in China and India, whereas no increased frequency of chromosomal aberrations or micronuclei were observed in osteoporosis patients receiving sodium fluoride treatment. The quality of the former studies is questionable. On reproductive and developmental defects they report that few human studies have suggested that fluoride might be associated with alterations in reproductive hormones and fertility, but the experimental animal studies used were of limited quality and no reproductive toxicity was observed in a multi-generation study. SCHER concludes that fluoride at concentrations in drinking water permitted in the EU does not influence the reproductive capacity.

The **Royal Society of New Zealand and the Office of the Prime Minister's Chief Science Advisor's**³² report stated that a number of other alleged effects of CWF on health outcomes have been reviewed, including effects on reproduction, endocrine function, cardiovascular and renal effects, and effects on the immune system. The New Zealand Chief Science Advisor's concluded that the most reliable and valid evidence to date for all of these effects indicates that fluoride in levels used for CWF does not pose appreciable risks of harm to human health.

Summary on other potential health effects

In relation to a possible link between exposure to water fluoridation and a number of other health effects, the literature search did not provide enough evidence on any particular outcome to make an evidence-based statement. These health effects relate to kidney disorders (two primary studies); hypothyroidism (one primary studies); immune system disorders (one primary study); birth defects (one primary study); and all-cause mortality (one primary study).

On the topic of hypothyroidism there was one primary study. Peckham *et al.*, in an ecological study, found a statistically significant association between water fluoride levels of greater than 0.3 ppm and the prevalence of hypothyroidism in GP practices.

In summary the findings of the ecological study-by-Peckham et al suggest that fluoride in water may be linked to the development of hypothyroidism. The published studies examining other possible negative health effects (renal stones, Downs syndrome and all-cause mortality) provide no evidence of harmful outcomes in CWF areas.

Overall conclusion

Non-endemic or CWF areas

In summary the literature found no strong evidence that CWF is definitively associated with negative health effects. However, the evidence base examining the association between health effects and community water fluoridation is scarce. It is mainly based on ecological studies and a small number of prospective cohort studies. Ecological studies are not adequate to infer causality.

Having examined the evidence, and given the paucity of studies of appropriate design, further research would be required in order to provide definitive proof, especially in relation to bone health (osteosarcoma and bone density) and thyroid disease (hypothyroidism).

Fluoride-endemic areas

In geographical areas where there is a naturally occurring high level of fluoride in drinking water (> 1.5 ppm), the health concerns have a somewhat different emphasis; these areas do not include CWF areas like Ireland. There are strong suggestions that high levels of naturally occurring fluoride in water may be associated with negative health effects, in particular, skeletal fluorosis and lowering of IQ. In addition, there are some indications that high levels of naturally occurring fluoride in water may also be associated with cardiovascular disease. However, the evidence base examining the association between health effects and high fluoride exposure emanates from low quality studies of inappropriate study design.

Addendum: Essays on other fluoride-related topics

Introduction and method for essays

Rationale

People concerned about CWF have presented an array of additional topics/health effects that they believe to be associated with adverse health effects pertaining to the consumption of, and exposure to, fluoridated water. These concerns are often only tenuously linked to effects from fluoridated water and have been neither proven nor disproven by scientific evidence. Due to the lack of scientific evidence on these topics, they were not addressed by the articles retrieved from the formal search for this review.

Topic selection

In order to address the fears about CWF, particularly in Ireland, discussion on the subject was aggregated and the most frequent topics identified. General discussion in relation to issues concerning CWF was collected from a number of sources: newspaper and magazine articles; Dáil debates; social media accounts of anti-fluoridation activists; websites of activists; and self-published reports by activists. When this information was collated and aggregated, a content analysis was undertaken in order to identify the most prevalent topics, particularly in relation to the debate in Ireland. As such, a number of prominent topics were identified for further investigation, with the purpose of examining the texts in order to understand if there is any evidence of a link between these issues and CWF. The findings are presented in a series of essays in the addendum on the topics of CWF and the following: tea, infant formula, depression, Alzheimer's disease, neurodevelopmental issues, arthritis, and endocrine disorders.

Search strategies

The search strategy employed for gathering the information necessary to write the essays was somewhat different to the formal search strategy employed for the overall review. Evidence on the topics for consideration in these essays either did not feature in the results of the formal search, were outside the time limits of the search, or the articles found in the search were deemed to be unsuitable for the review by virtue of being an inappropriate study type (e.g., single case series) or otherwise outside the remit of the overall review. Nevertheless, formal databases, MEDLINE and EBSCO, were used to undertake more specific iterative searches, using refined search terms related to each of the individual topics: community water fluoridation and tea, infant formula, depression, Alzheimer's disease, neurodevelopmental issues, arthritis, and endocrine disorders. Further refining of the searching and data collection process involved reference chasing, particularly following up papers/studies that were widely cited by anti-fluoridation activists in their debate. These papers were analysed in relation to their contribution to the fluoridation debate, and any statements relating to health effects were examined, and conclusions and/or comments on their efficacy drawn accordingly.

Fluoride and tea

The tea plant, *camellia sinensis*, absorbs and accumulates fluoride through the soil into the leaves of the tree over time.⁷⁹ The levels of fluoride in tea leaves and stems increases with age: therefore, the older the plant, the higher the levels of fluoride present.⁷⁹ Fluoride is easily released through the infusion process of brewing tea, which has led to tea being considered a major source of fluoride. The level of fluoride consumed by tea drinkers increases further when the tea is made with fluoridated water.

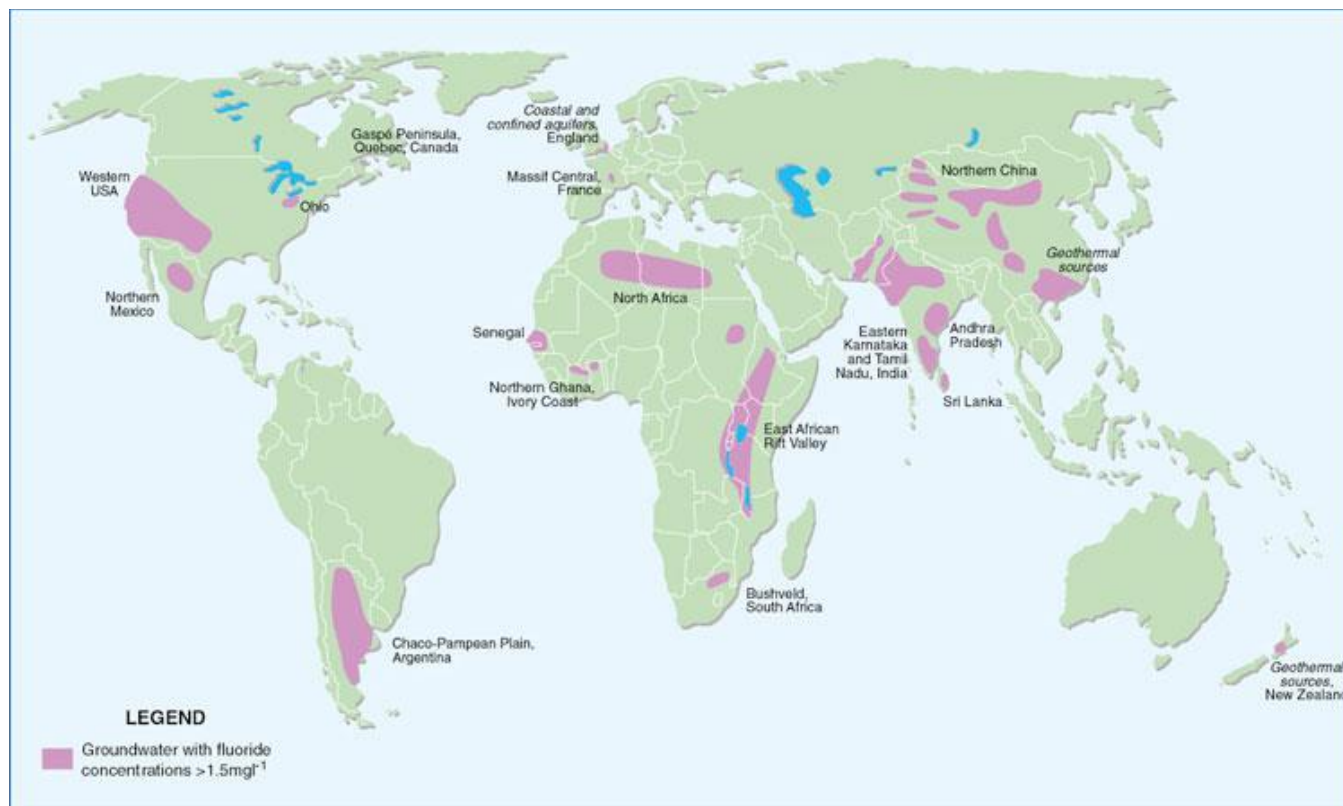


Figure 1: Map of documented occurrences of high-fluoride groundwater (>1.5 mg/L).

Source: <http://www.bgs.ac.uk/research/groundwater/health/fluoride.html>

Waters with high fluoride content are found mostly in calcium-deficient groundwater in particular rock types, such as granite and gneiss. Groundwater with high fluoride concentrations occurs naturally in many areas of the world, including large areas of Africa, China, the Middle East and southern Asia (India, Sri Lanka). One of the best known high-fluoride belts on land extends along the East African Rift from Eritrea to Malawi. There is another belt from Turkey through Iraq, Iran, Afghanistan, India, northern Thailand and China. The Americas and Japan have similar belts. Countries of the world with documented occurrences of high-fluoride groundwater (>1.5 mg/L) are displayed in Figure 1.

The main tea growing countries in Asia are China, India and Sri Lanka and, to a lesser extent, Iran and Turkey. In Africa, the major tea-growing countries are Kenya, Malawi, Rwanda, Tanzania, and Uganda. In South America, tea is grown in Argentina and Brazil, while in Europe it is grown in Russia and Georgia.⁸⁰ There is considerable crossover between the tea-producing countries and countries with occurrences of high-fluoride groundwater, particularly in China, India and the Rift valley area in Africa (see Figure 2). The majority of tea exported around

the world, is grown in these fluoride-endemic areas, and therefore will have accumulated fluoride that is naturally present in tea.

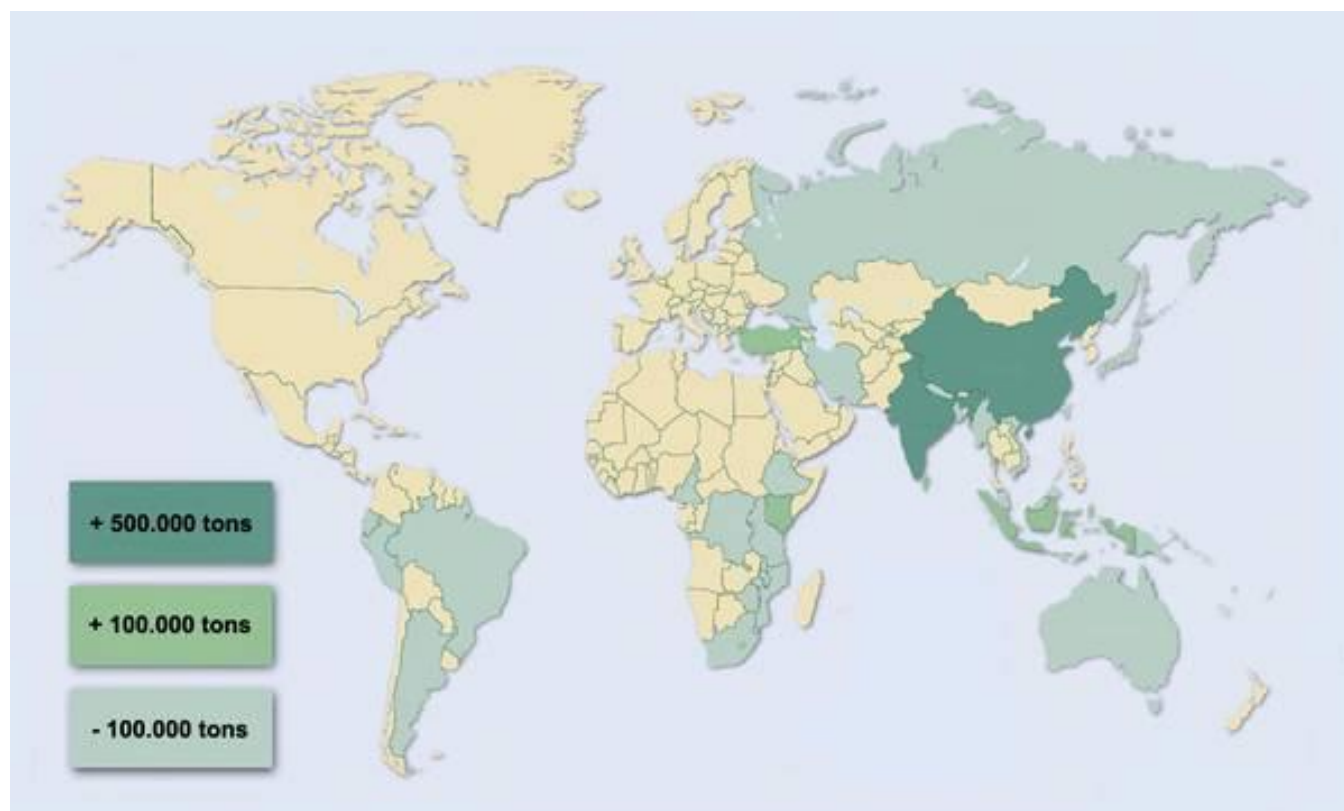


Figure 2: World map of tea growing areas. Source: <http://www.jfstea.com/en/products.html>

As discussed above, fluoride can be present in water as a naturally occurring phenomenon. However, in order to prevent dental caries, in areas with low fluoride levels in water it has also been added to water supplies via CWF schemes up to a level of 1.5 mg/L. In Ireland, the level of fluoride added to water via CWF is between 0.6–0.8 mg/L. The subject of tea and its potential to contribute to excessive levels of fluoride is often raised when discussing issues surrounding CWF. Many opponents of CWF cite tea drinking as leading to a chronic overexposure to fluoride, and they link high levels of tea consumption with a risk of fluoride toxicity.⁸¹ People concerned about the effects of CWF fear that high consumption of tea, particularly with fluoridated water, can cause skeletal fluorosis and other bone-related health effects.

Tea is the most consumed beverage in the world after water.⁸² As Ireland is known for having one of the highest per capita tea consumption rates in the world, those who oppose CWF are particularly concerned about the perceived negative effects of fluoride levels present in tea made with fluoridated water. However, there is a dearth of scientific evidence in this area. The formal systematic search of the literature, described elsewhere in this report, did not uncover evidence to link tea consumption with negative health effects of CWF. In relation to any harmful health effects of fluoride in tea, the systematic search resulted in one article, on brick tea consumption in Mongolia.⁸³ Nonetheless, other material relating to this subject relating specifically to tea and fluoride was found by performing iterative searches of the literature in an effort to uncover any information concerning health-related links between tea and fluoride. These are discussed in more detail here.

Fluoride levels in tea

The tea plant naturally accumulates fluoride from the soil and can contain '196ug (micro grams) per 2g dry tea (around one teabag) ... although the fluoride can exceed this if fluoridated water is used during brewing'.⁸²

Fluoride can be present in water as a result of a naturally occurring phenomenon, or it can be added artificially, through CWF schemes, up to a level of 1.5 mg/L. In Ireland, hydrofluorosilicic acid (HFSA) is added to the public water supply, so that after the addition of HFSA, the water must contain no more than 0.8 mg/L, and no less than 0.6 mg/L of fluoride.¹¹

In a 2007 review of black tea relating to seven key areas of health including dental health and bone health, the reviewers state that the number of studies on bone health and dental caries was small and indicated a positive effect of tea, less convincingly in the case of dental health due to lack of large human studies. For bone health, this positive effect was hypothesised to be due to several likely mechanisms. These included: the contribution of tea to dietary fluoride which could alleviate osteoporotic progression; the impact of flavonoids on bone mineral content; inhibition of bone resorption by tea extracts; involvement in bone mineral metabolism. However, the authors conclude that for bone and dental health there was insufficient evidence to make any recommendations about intake.⁸²

A study by Chan *et al.* assessed the exposure to fluoride from the consumption of tea by analysing the fluoride concentrations in a range of tea products.⁸⁴ Each tea sample was prepared in an Erlenmeyer flask and involved brewing 2g of dried tea in 100ml of boiling deionised water (water with all ions removed, including fluoride) which was subsequently incubated between 85 and 90°C in a water bath.⁸⁴ Individual infusions were timed at 2, 10 and 30 minutes. The authors reported that fluoride levels in all tea infusions ranged from 0.43 to 8.85 mg/L, with an average of 3.8 mg/L in a tea infusion brewed for two minutes.⁸⁴ Oolong/Pu'er and pure blends of tea had the lowest fluoride content, while black and green tea blends had the highest fluoride content, particularly economy brands of tea, such as supermarket own-brand types.⁸⁴

The Chan study is regularly cited by those concerned about CWF and tea, as it points to the potentially high levels of fluoride in tea and emphasises that the older the tea leaves the higher the levels of fluoride present. The media response to the Chan study gave rise to headlines such as 'Cheap tea raises risk of bone and teeth problems' in *The Telegraph* on 24 July 2013, and 'Could cheap tea bags make you ill? Study reveals they contain high fluoride levels that could damage teeth, bones and muscles' from *The Daily Mail* on 29 July 2013. Criticising the media response as relatively alarmist, Ruxton, in an article in 2014, points out issues surrounding the accuracy of the Chan study in terms of the levels of fluoride measured and the methods by which the measurements were carried out.⁸⁵ Ruxton explains that comparison of figures from Chan's study with other sources serves to emphasise the variation across different teas; she also cites a previous study which has highlighted the lack of a standardised method to determine fluoride levels.⁸⁶ Commentary on the Chan study by McArthur also noted that the analytical method selected for the study, and the small amount of water used to make up the tea infusions, served to overestimate the fluoride content.⁸⁷

Tea and fluoride toxicity

Prolonged large-scale consumption/ingestion of fluoride, as with most chemicals (even those that the body requires for nutrition), may cause toxicity. High consumption of fluoride in countries that have high levels of naturally fluoridated water, greater than 1.5 mg/L, has been associated with skeletal fluorosis. Studies have noted that skeletal fluorosis occurs when an individual has consumed above 10mg of fluoride almost every day over a period of one or two decades.⁸⁸⁻⁹⁰ Other reports have suggested that, 'in temperate climates, no cases of clinical skeletal fluorosis have been associated with fluoride levels up to 4 mg/L in drinking water'.⁸⁵ Nevertheless, "processed tea" is seen as another potential source of high levels of fluoride.^{91, 92} Studies have sought to link fluoride toxicity and skeletal fluorosis with heavy consumption of tea.^{88, 91, 93-96} In Ireland, these papers have

been summarised and the main points reiterated by Waugh.⁹⁷ In the paper by Yi and Cao⁹³ they summarise six separate single cases of fluorosis, each of which relate to high consumption of tea. The case report by Izuora *et al.*⁹⁴ refers to a woman who presented with skeletal fluorosis from consuming extraordinarily high volumes (1-2 gallons daily for more than three decades, equivalent to 3.7–7.5L per day) of economy brand brewed tea. This gave her a daily fluoride intake of 14.6–29.3 mg. The 2005 paper by Whyte *et al.*⁹⁵ discusses the case of a 52-year-old woman who presented with skeletal fluorosis and disclosed drinking 1-2 gallons of double-strength instant tea daily throughout her whole adult life. They calculated that her total fluoride exposure was 37–74mg per day. The 2008 case report by Whyte *et al.*⁹¹ refers to the case of a 49-year-old woman who presented with skeletal fluorosis from consuming, since the age of 12, extraordinarily high volumes of instant tea (2-3 gallons daily) that was made with community fluoridated water. The authors of the 2008 study conclude that while an extra-strength mix of instant tea using fluoridated water was also contributory, *'the instant tea powder was clearly the principal source'*⁹¹ of their patients' high fluoride consumption. Another single case report with similarly unique factors is the Joshi *et al.*⁹⁶ paper which refers to a woman with excessive toothpaste consumption (brushing her teeth up to 10 times per day) who also consumed large quantities of tea daily over 5–10 years; this involved consuming six 240ml cups of standard breakfast tea daily, which would have provided 10.9mg of fluoride per day. Johnson *et al.*⁸⁸ reported on four case studies, each of which involved excessively high consumption of tea, some within relatively extraordinary scenarios: *'Predisposing clinical features appear to include renal insufficiency with reduced fluoride excretion and a tendency toward obsessive compulsive drinking behaviors, such as those associated with anorexia nervosa and other psychiatric disorders.'*⁸⁸

Single case studies,^{88, 91, 93-96} as referred to in this essay, do not provide evidence of fluoride toxicity from average tea consumption. In general, the studies did not describe average tea consumption. Furthermore, conclusions of cause and effect cannot be drawn from any study without a comparison group. In addition, many confounding factors may be present which are not, and could not be, controlled for without a comparison group and an appropriate set of statistical approaches.

Conclusion

The studies quoted in this essay do not provide sufficient information to demonstrate a link between skeletal fluorosis or any type of fluoride toxicity and the consumption of usual levels of tea; this includes the consumption of tea that has been made with community fluoridated water. Tea leaves contain proportionally higher levels of fluoride than CWF water that is used to make it.

Fluoride and infant formula

Opponents of CWF cite infant exposure to fluoride, through the consumption of bottle-fed infant formula, as a worrying health issue. A number of medical conditions have been postulated to be linked with the possibly high level of fluoride intake in bottle-fed babies; such conditions include gastrointestinal disorders, respiratory illnesses, cancers, advanced sexual maturity (related to fluoride impacting negatively on thyroid function), autism and other neurological/neuropsychiatric impairments, diabetes, obesity, organ failure and sudden infant death syndrome (SIDS).⁹⁷⁻⁹⁹ Low rates of breastfeeding in Ireland have increased concerns about any health risks associated with the fluoride intake of bottle-fed babies, but there is a dearth of conclusive evidence to support these claims. However, fluoride is not listed as an ingredient on the main brands of infant formula (SMA, HIPP, Cow and Gate, Aptimil) available in Ireland.

Breastfeeding is widely recognised and recommended, nationally and internationally, as the best feeding practice for infants, particularly in the first six months of their lives. The promotion of breastfeeding is a part of health policy in Ireland. In 2012, 46.6 per cent of mothers were recorded as exclusively breastfeeding their infants on discharge from hospital.¹⁰⁰ However, 44.7 per cent of very young babies were recorded as being bottle fed with infant formula.¹⁰⁰ As the rates of bottle feeding are relatively high, the Food Safety Authority of Ireland (FSAI) has issued guidelines on safe formula feeding, recommending the preparation of infant formula powder reconstituted with boiled and cooled tap water.¹⁰¹ The Centers for Disease Control and Prevention (CDC) in the USA also advise reconstituting infant formula with community fluoridated water. However, they warn that the exclusive consumption of infant formula reconstituted with fluoridated water may increase chances for dental fluorosis. For this reason, they recommend that parents who are concerned about fluorosis can use low-fluoride bottled water – which is labelled as deionised, purified, demineralised, or distilled – to reconstitute infant formula for some of the feeds.¹⁰² Despite relatively high levels of bottle feeding with infant formula, breastfeeding remains recommended as the most appropriate mode of infant feeding for those concerned about the fluoride intake of their infants; ‘even at very high fluoride intakes by mothers, breast milk still contains very low concentrations of fluoride compared to other dietary fluoride sources’.²⁷

The majority of research undertaken on fluoride and infant formula focuses on the prevalence of dental fluorosis as a result of high consumption of fluoride (in water). The body of studies on fluoride and infant formula tend to cover two major areas. The first area examining the fluoride content of infant formula (made with both non-fluoridated and community fluoridated water),^{103, 104} and the second area examining whether the fluoride content of infant formula increases the risk of and severity of dental fluorosis.¹⁰⁵ Recommendations from these studies are inconsistent, with some advocating continued use of fluoridated water to reconstitute infant formula and others recommending the use of non-fluoridated water, or monitoring the amount of fluoridated water used. Iterative searches of the literature, which were focused on looking for information on adverse health effects of infant formula that was reconstituted with fluoridated water, found some articles that examine levels of fluoride intake in bottle-fed babies. However these studies do not relate fluoride intake to health issues. Other material found included articles which examined bottle-fed infants consuming formula reconstituted with CWF water and some health effects (for example, studies related to SIDS that were outside the formal search time period). However, the majority of information retrieved concerned bottle-fed infants and the development of dental fluorosis; that is outside the remit of this report, as the question posed only relates to non-dental health effects. There is a dearth of conclusive evidence-based studies demonstrating harmful, non-dental health effects from consumption of infant formula reconstituted with community fluoridated tap water.

Studies on infant formula and fluoride

One study aimed to evaluate associations between infant formula feeding and dental caries or dental fluorosis in a sample of Australian children.¹⁰⁵ The study examined the experience of seven groups of children: three groups

of children in a non-fluoridated area (exclusive breastfeeder, user of formula for less than six months, and user of formula for longer than six months or more), and on four groups in a CWF area (exclusive breastfeeder, user of formula with non-fluoridated water, user of formula with fluoridated water for less than six months and user of formula for longer than six months). The authors found that there was a correlation between the prevalence of dental fluorosis (mostly mild fluorosis) and infant formula feeding.¹⁰⁵ What is interesting in the context of bottle feeding is that the authors report that the positive correlation was 'statistically significant only in **non-fluoridated** areas, between infant formula [users for 6+ months] and prevalence of mostly very mild or mild fluorosis';¹⁰⁵ this is possibly due to the fluoride content in the formula itself. In the overall conclusion, the authors state that 'Infant formula use was associated with higher prevalence of fluorosis in **non-fluoridated** areas but not in fluoridated areas. Type of water used for reconstituting infant formula in **fluoridated areas** was associated with caries experience.' Children in **fluoridated areas** who were fed infant formula with **non-tap** water had the highest level of primary caries experience, although the sample size was small (n = 16). The other three groups in **fluoridated areas** had relatively similar and lower mean caries experience. Of interest is the suggestion that the infant formula itself, rather than the fluoride content of the water, was the highest risk factor for fluorosis. The authors recommended further research in order to better understand the impact of early childhood fluoride consumption/exposure and the risks and benefits of fluoride use.¹⁰⁵

A 2014 paper by Zohoori *et al.*¹⁰⁶ presents a summary of a study which completed a detailed assessment of the dietary fluoride intake of infants living in community fluoridated and non-fluoridated areas in the UK, in order to determine if the total daily fluoride intake (TDFI) exceeded the upper limit of 0.1mg/kg body weight per day, the level above which the risk of dental fluorosis increases. This was undertaken using full-day food diaries on three consecutive days, and followed up using parent questionnaires and interviews on the fourth day. Analysis of the fluoride content of both ready-to-feed and homemade food/drink items actually consumed by infants was also undertaken.¹⁰⁶ The mean dietary intake of fluoride found in the study for infants living in fluoridated areas was 0.103mg/kg body weight per day and in non-fluoridated areas was 0.021mg/kg body weight per day.¹⁰⁶ The main conclusion of the study states that infants living in fluoridated areas, in general, may receive a fluoride intake, from diet only, of more than the suggested optimal range for total daily fluoride intake. However, it is important to note that 'almost all infants living in non-fluoridated area received less than optimal fluoride exposure to help prevent dental caries'.¹⁰⁶ Zohoori *et al.*¹⁰⁶ confirmed that it is imperative to undertake an estimation of total daily fluoride intake at both individual and community levels when recommendations for the use of fluoride, to maximise reduction in dental caries while minimising risks of dental fluorosis, are being considered.

Arguments of anti-fluoridation campaigners

The negative health effect most frequently found in studies examining the fluoride content of formula-fed infants relates to the increased risk for dental fluorosis. Yet, people opposed to CWF have speculated that infant formula consumption, especially when reconstituted with community fluoridated water, creates a heightened risk of fluoride toxicity, which may lead to the development of a range of negative health effects. The conditions emphasised in this regard include gastrointestinal disorders, respiratory illnesses, cancers, advanced sexual maturity (related to fluoride impacting negatively on thyroid function), autism and other neurological/neuropsychiatric impairments, diabetes, obesity, organ failure and SIDS.^{97-99, 107} Nevertheless, these theoretical associations between the consumption of infant formula reconstituted with fluoridated water and adverse health conditions, other than dental fluorosis, have not been documented in any scientific study found in our search, have not been investigated, and are therefore neither proven nor unproven. There is no scientific evidence that we could find to support these claims.

In considering a link between fluoride consumption and SIDS, a 1999 study from New Zealand by Dick *et al.*¹⁰⁸ utilised 'a nationwide case-control database of sudden infant death syndrome' to evaluate fluoride exposure status. In the study, they controlled for the method of infant feeding (breast or reconstituted formula) and

concluded that 'exposure to fluoridated water prenatally or postnatally at the time of death did not affect the relative risk of sudden infant death syndrome'.¹⁰⁸ This study was summarised in the NRC report²⁷ and also referenced by the 2014 health effects of water fluoridation report from New Zealand³² to highlight a lack of any evidence inferring causality between infant formula reconstituted with fluoridated water and SIDS. The speculation about a connection between SIDS and the fluoridated water consumption of bottle-fed babies^{97, 98} remains theoretical, as there is no generally accepted scientific knowledge to demonstrate any association.

Conclusion

Fluoride is not listed as an ingredient on the main brands of infant formula (SMA, HIPP, Cow and Gate, Aptamil) available in Ireland. Studies which demonstrate a correlation between infant formula consumption and the prevalence of dental fluorosis are presented in this essay. One study's findings indicate that the fluoride content in the infant formula rather than in community fluoridated water of the study country may lead to the development of mild dental fluorosis. None of the remaining studies considered could provide sufficient information to infer causality of any other adverse health conditions through the consumption of infant formula reconstituted with community fluoridated water.

Fluoride and depression

In recent years, people concerned about CWF in Ireland have sought to link depression with the consumption of community fluoridated water. The links they describe consist of disjointed use of statistics on the prevalence of depression in Ireland, the numbers of antidepressant prescriptions, and the hypothesised link between hypothyroidism and fluoride, which leads them to conclude that fluoride causes depression.⁹⁷ The drive to link fluoride with depression is intensified by a prominent campaigner in Ireland^{109, 110} who claims to have been cured of a lengthy period of depression following the removal of community fluoridated water from her diet.

For people concerned about CWF, the debate in relation to fluoride and depression centres on the effects of fluoride on the thyroid gland.¹¹¹ Discussions on causal associations between depression and fluoride are chiefly reproduced and perpetuated through the social media accounts of campaigners^{112, 113} also through blogs by like-minded individuals/organisations¹¹⁴ as well as some newspaper¹¹⁵ and magazine articles.¹⁰⁹

The Fluoride Action Network (FAN) website references the USA report of the National Research Council (NRC), Committee on Fluoride in Drinking Water from 2006²⁷ as demonstrating substantial evidence that fluoride exposure can impact on thyroid function in some individuals. The NRC report was published to examine the health effects of fluoride in areas of the USA where there are high levels of naturally occurring fluoride in water;²⁷ it was not concerned with the much lower levels of fluoride found in community fluoridated water. The NRC report stated that fluoride acts as an endocrine disruptor insofar as it alters 'normal endocrine function or response'.²⁷ The NRC found that the most significant 'endocrine effects of fluoride exposures in experimental animals and in humans include decreased thyroid function, increased calcitonin activity, increased parathyroid hormone activity, secondary hyperparathyroidism, impaired glucose tolerance, and possible effects on timing of sexual maturity'.²⁷ Thus, FAN uses these potential adverse health effects on the thyroid gland that may result from high levels of fluoride (4 mg/L of naturally occurring fluoride in water), as described in the NRC report, to make a case for fluoride causing depression in areas where the community drinking water is fluoridated, even though the levels are not comparable. In Ireland, the fluoride level added to water is between 0.6 and 0.8 mg/L.

All iterative searches for evidence of a link between fluoride and depression returned no relevant results. One animal study was retrieved, and is discussed briefly in this essay. But, to put this animal study in context, some background information on the whole issue of iodine deficiency, hypothyroidism and fluoride and how this is related to depression is discussed first.

Iodine deficiency, hypothyroidism, fluoride and depression

Hypothyroidism, which is more commonly known as an underactive thyroid, is a common endocrine disorder in which the thyroid gland does not produce enough thyroid hormone.¹¹⁶ It is known that hypothyroidism can produce signs and symptoms of depression.¹¹⁷

Iodine deficiency is recognised as the most common cause of primary hypothyroidism, particularly in developing countries.¹¹⁶ Those leading the campaign to cease CWF in Ireland state that, at certain fluoride levels, fluoride may replace iodine, particularly where there is already an iodine deficiency.⁹⁷ They believe that because iodine can be replaced with fluoride, this can cause hypothyroidism. Due to the stated symptoms of hypothyroidism, which can include depression, people then extrapolate this information to say that fluoride consumption is a cause of depression.

Fluoride and depression study

As previously highlighted, the only study retrieved that purports to directly link fluoride intake with depression was one recent animal study. The study aimed to examine if the exposure of immature mice to fluoride would affect their emotional behaviour and cognition.¹¹⁸ The study exposed the mice, from a young age, to sodium fluoride (NaF) in water; '48 mice were randomly divided into control group (distilled water), Group L (low fluoride, 2 mg/L NaF), Group M (mid-fluoride, 5 mg/L NaF) and Group H (high fluoride, 10 mg/L NaF)'.¹¹⁸ After four weeks the researchers used a number of physical-based cognition test methods on the mice to examine the effects of the fluoride intake. From the results of these tests, the authors concluded that developmental fluoride exposure is likely to induce anxiety and depression-like behaviours in adult mice.¹¹⁸ Nevertheless, they were extremely unclear about how these results translate to human subjects, but still suggested that it proved neurotoxic risks for humans. They also recommended that a large-scale epidemiological study be undertaken with humans to establish if such effects would be problematic.¹¹⁸

Conclusion

It is obvious that there are people who are concerned about the possibility that drinking community fluoridated water is linked to/causes depression. Given that CWF has been in operation for many decades in some countries, one would have expected that any link between fluoride and depression would have been detected after this length of time, and that there would be scientific studies and resulting evidence of any link between drinking community fluoridated water and depression in humans. However, this is not the case, and we are left with speculations and, unfortunately, no proof to support claims of a causal link between fluoride intake and depression, or no evidence to disprove this theory.

Fluoride and Alzheimer's disease

Age and family history have been identified by scientists as the major risk factors for Alzheimer's Disease.¹¹⁹ Scientists believe that genetics and lifestyle factors, such as diet/nutrition, alcohol consumption, lack of physical activity and smoking, may also have a part to play.¹²⁰ A 1998 study by Varner *et al.* was the catalyst for concerns being raised about a potential causal link between fluoride and Alzheimer's disease.¹²¹ However, it has been highlighted that there were several flaws in the experimental design of the Varner study which prevented any definitive conclusions being drawn.¹¹⁹

Research on aluminium and fluoride in incidences of Alzheimer's disease

It has been suggested that metals, particularly aluminium, are involved in the aetiology of Alzheimer's disease, but the extent of their effect has not been measured.⁵² Accordingly, there is a hypothesis that other substances, such as fluoride, can alleviate¹²²⁻¹²⁴ and/or exacerbate¹²¹ the perceived negative effect of aluminium on Alzheimer's disease. Studies undertaken by Forbes *et al.*, which looked at any possible linkages between fluoride and aluminium in relation to Alzheimer's disease as part of the Ontario Longitudinal Study of Aging, generated a number of papers, one of which (1991) is referenced here.¹²⁴ In a letter to the Editor of *The Lancet*, the authors referred to preliminary results from the Ontario Longitudinal Study of Aging. One of the key findings was that men living in areas 'where drinking water aluminium concentrations are high and fluoride concentrations are low are about three times more likely to have some form of mental impairment, compared with those living in areas where aluminium concentrations are relatively low and fluoride concentrations high.'¹²⁴ The levels of fluoride concentrations are not stated in this letter.

A 2001 review by Flaten¹²⁵ discusses eight studies by Forbes (including the one discussed above), and a number of other studies, which contend that their findings support the assumption that higher fluoride levels (4.2 ppm) in drinking water in conjunction with lower aluminium levels can lower incidences of Alzheimer's disease. However, Flaten¹²⁵ describes the studies, including those by Forbes, as having too many methodological flaws, including inconsistent methods and data, and thus are not strong enough to be considered conclusive evidence.

Arguments of anti-fluoridation campaigners

Anti-fluoridation campaigners opposed to CWF in Ireland have concerns that fluoride may cause Alzheimer's disease. The most prominent discussion of these alleged associations are presented in a 2012 report by Waugh,⁹⁸ which incorrectly connects separate sentences/sections from the NRC report;²⁷ resulting in a picture being portrayed of a more direct link between fluoride and Alzheimer's disease. The report omits to mention that the NRC report examined health effects in areas of the USA with high levels of naturally occurring fluoride.²⁷ The NRC report did not examine the health effects of lower levels of fluoride similar to levels in community fluoridated water, and their results therefore are not comparable to CWF.

An example of how ideas can be open to misinterpretation is when a sentence is taken out of context and joined to another sentence (equally out of context) to portray a stated association between fluoride and Alzheimer's disease. This is exemplified in Waugh's report⁹⁸ where excerpts from the NRC report²⁷ are conflated to convey a different message than that intended in the original (NRC) report: e.g., the following direct quote from page 111 of Waugh's 2012 report:⁹⁸ 'it is apparent that fluorides have the ability to interfere with the functions of the brain....fluorides also increase the production of free radicals in the brain through several different biological pathways. These changes have a bearing on the possibility that fluorides act to increase the risk of developing Alzheimer's disease'.⁹⁸

The first sentence as presented in Waugh's report, (before the four dots), is at the beginning of the Recommendations section of the Neurotoxicity and Neurobehavioral Effects chapter in the NRC report,²⁷ at the end of page 222. When in context, it reads: 'On the basis of information largely derived from histological, chemical, and molecular studies, it is apparent that fluorides have the ability to interfere with the functions of the brain and the body by direct and indirect means. To determine the possible adverse effects of fluoride, additional data from both the experimental and the clinical sciences are needed.'²⁷

The second part of the excerpt from Waugh's report, after the four dots, appears earlier on page 222 of the NRC report²⁷ in a section on Neurochemical and Biochemical Changes, which is part of the Neurotoxicity and Neurobehavioral Effects chapter. When in context, it reads: 'Fluorides also increase the production of free radicals in the brain through several different biological pathways. These changes have a bearing on the possibility that fluorides act to increase the risk of developing Alzheimer's disease. Today, the disruption of aerobic metabolism in the brain, a reduction of effectiveness of acetylcholine as a transmitter, and an increase in free radicals are thought to be causative factors for this disease. More research is needed to clarify fluoride's biochemical effects on the brain.'²⁷

When read in context from the NRC report²⁷ the two sections illustrate a possible relationship between fluoride consumption and risk of Alzheimer's disease, while firmly pointing out the need to undertake more specific research in this area. It is important to remember also that this possible relationship is in the context of higher levels of naturally occurring fluoride in water (>1.5 ppm) which the NRC were investigating and not the lower levels, ≤1.5 ppm, which exist in community fluoridated water.

Conclusions

Potential risks of Alzheimer's disease from fluoride intake are not considered to be high, within the broader spectrum of research on causes of Alzheimer's disease, as there is little research undertaken in the area of fluoride and this condition. The York review in 2000 examined additional studies which looked at other possible negative effects of fluoride on health.² Among these, they considered studies undertaken on Alzheimer's disease, but stated that the quality of the studies was very low (evidence level C: lowest quality of evidence, high risk of bias).² Accordingly, there is no generally accepted scientific knowledge that identifies the consumption of fluoridated water as a risk factor for Alzheimer's disease.¹¹⁹

Fluoride and neurodevelopmental disorders

A possible association between fluoride and neurodevelopmental disorders, such as autism and attention deficit hyperactivity disorder (ADHD), has been a concern voiced by anti-fluoridation campaigners. The main body of this HRB report contains a section on the evidence relating to IQ and neurological manifestations. However, the search produced no scientific papers that dealt specifically with the possible connection between fluoride and the development of autism and ADHD. There is a dearth of published scientific studies in this regard. Yet, this association between fluoride and neurodevelopmental disorders (even though there is no scientific evidence to prove it) is continually perpetuated predominantly via campaigners' social media accounts,¹²⁶ magazine articles¹²⁷ and other web-based discourse, as well as orally through public campaigning. The paper widely cited to authenticate the possible link between fluoride and autism or ADHD is the 2014 paper by Grandjean and Landrigan, titled 'Neurobehavioural effects of developmental toxicity'.³⁹ This article contains no evidence of any direct causal association between fluoride and these neurodevelopmental disorders.

The subject of the paper by Grandjean and Landrigan³⁹ is the identified increase in industrial chemicals being classed as neurotoxins. In theory, neurotoxins are potentially capable of causing developmental disorders, including intellectual and learning disabilities such as autism and ADHD. The only specific mention of fluoride in the Grandjean and Landrigan paper refers to the 2012 paper by Choi *et al.*³⁷ The Choi *et al.*³⁷ paper was a meta-analysis of 27 cross-sectional surveys published in Chinese journals on the neurotoxic effects on children's IQ in areas of high levels of endemic fluoride in the groundwater (in China, Mongolia and Iran). High levels of endemic fluoride refer to levels of fluoride greater than 1.5 mg/L as compared to optimally community fluoridated water which, in the case of Ireland, is between 0.6 and 0.8 mg/L. The 2012 meta-analysis by Choi *et al.*³⁷ provided results which they (Choi *et al.*) believe to 'support the possibility of adverse effects of fluoride exposures on children's neurodevelopment'³⁷ predominantly related to IQ levels. The Choi *et al.* review has already been discussed in the main body of the report in relation to IQ. Many of the studies in the Choi *et al.* review did not control for the presence of other chemicals in the water that could lower IQ; nor did they control for other causes of low IQ, such as iron or iodine deficiency. After publication of the Choi *et al.* paper, and a plethora of refutations, Choi and Grandjean¹²⁸ subsequently conceded that using results from cross-sectional surveys in endemic areas (in China, Mongolia and Iran) could not allow them 'to make any judgement regarding possible levels of risk at levels of exposure typical for [community] water fluoridation in the USA'.¹²⁸ This also means that their results were not relevant to Ireland, since the levels of fluoride added to water in Irish CWF remain lower than those of the USA.

Fluoride and autism

Another concern for people opposed to CWF is the development of autism. There is very little evidence for this theory on the subject of fluoride and autism, yet the theory continues to flourish. Speculative associations in the Irish context by Waugh⁹⁷ (2013) centre on a belief that 'the highest incidences of autism are also to be found in countries where the population are exposed to artificially fluoridated drinking water'.⁹⁷ Waugh⁹⁸ (2012) also shares the belief of some people opposed to CWF who claim that 'environmental toxins may be partly responsible for the increase in autism', hence their related belief that 'it is not inconceivable therefore, in the absence of proper scientific assessment, to consider that fluoride may be a contributory factor'.⁹⁸

On this topic, Blaylock and Strunecka⁵³ suggest that fluoride, and aluminium, have a part to play in contributing to autism or autistic spectrum disorders (ASD). In a 2009 paper they assert that 'some symptoms of ASD such as the sleep problems and the early onset of puberty suggest abnormalities in melatonin physiology and dysfunctions of the pineal gland'⁵³ and they postulate that this is the mechanism by which fluoride is linked to autism. However, this is a theoretical link and the authors provide no evidence to support their theory.

Fluoride and ADHD

As with autism, there is no evidence which provides a direct causal association between fluoride and attention deficit hyperactivity disorder (ADHD). Nonetheless, Masters¹²⁹ in an article published in 2012 has attempted to highlight an association between fluoride and ADHD. Masters states that 'while reliable epidemiological data on ADHD or ADD are not available, geographical data are consistent with the hypothesis that behavioural dysfunctions related to lead, manganese, and other toxic chemicals are significantly higher in communities using silicofluorides in water treatment than in those not using these chemicals',¹²⁹ but provides no proof of causality.

A study on rats by Mullenix *et al.*¹³⁰ (1995) is regularly cited as proof of a causal association between fluoride and neurodevelopmental disorders such as ADHD. The study's objective was to evaluate the neurotoxic potential of sodium fluoride in an animal model, and the authors suggest that 'a generic behavioral pattern disruption as found in this rat study can be indicative of a potential for motor dysfunction, IQ deficits and/or learning disabilities in humans'.¹³⁰ The study was highlighted by an editorial in the journal *Fluoride*, which interpreted its findings as giving further weight to studies that list fluoride as a neurotoxin.¹³¹ However, the study by Mullenix was refuted as ambiguous by Ross and Daston (1995); this was due to the lack of appropriate control groups and a lack of control for confounding factors, as there are many other factors that may explain the observation. These authors suggest that it is disingenuous to claim neurotoxicity of fluoride in this regard as 'both positive and negative control materials should be evaluated, and the results linked with well-characterised functional and morphological indices of neurotoxicity'.¹³²

Conclusion

There are indications that high levels of naturally occurring chemicals (fluoride >1.5 ppm, lead and arsenic) in groundwater may be linked to lower IQ in endemic countries. However, in studies that have been conducted in a scientific and thorough manner in a CWF country, there is no evidence of such effects. For example, in a prospective cohort study conducted in 2012 (Broadbent *et al.*³³) in New Zealand, where there is a national programme of CWF, no link was found between fluoride in the water and IQ (see page 39 of the main body of the report). Any existing published studies which claim to show a causal association between fluoride and neurodevelopmental disorders such as autism or ADHD are ambiguous, and are not of a design that is adequate to prove or disprove these associations. However, in fluoride-endemic countries (where levels are greater than 1.5 ppm) there are studies which suggest that fluoride may have adverse effects on the development of neurodevelopmental disorders, but the study designs used do not allow the authors to provide definitive proof; it should be noted that the levels of fluoride exposure in drinking water in endemic countries are higher than those in CWF (0.6 ppm–0.8 ppm) in Ireland.

Fluoride and arthritis

Arthritis describes more than 100 diseases and conditions that affect the body's joints and surrounding tissue. Arthritis is a condition characterised by inflammation of the joints, causing pain and immobility, and can range in severity from mild to acute.¹³³ According to Arthritis Ireland, approximately 915,000 people, including 1,100 children, are currently living with arthritis in Ireland.¹³³ The two most common types of arthritis are osteoarthritis and rheumatoid arthritis.

Types of arthritis

Osteoarthritis is the most common arthritic condition, and affects the hips, knees, hands and feet, but can affect other joints.¹³⁴ It is associated with ongoing wear and tear of joints and, typically, affects people over the age of 45; nevertheless, younger people can suffer from osteoarthritis too. The symptoms can vary in severity from person to person or between affected joints.¹³⁴

Rheumatoid arthritis is an autoimmune disease, and occurs because the body's immune system, which normally fights infection, starts to attack healthy joints'.¹³⁵ It is a long-term condition and the symptoms include inflammation of the joints, which causes pain, swelling and stiffness.¹³⁵ The joints can become damaged by the intensity of the inflammation over time, as can the cartilage and nearby bone.¹³⁶ Rheumatoid arthritis is known to be three times more common in women than in men. The exact mechanism for the initiation of rheumatoid arthritis is unknown. However, it is postulated that there could be a genetic predisposition to the condition in some patients with the disease.¹³⁶

Perceived associations between arthritis and fluoride

People concerned about CWF believe that the consumption of fluoridated water is associated with the development of arthritis. One purported theory centres on a belief that there is a higher incidence of arthritis in countries with CWF. For example, in a magazine article (Hot Press), Declan Waugh stated 'rheumatoid arthritis, which is an inflammatory response of the immune system, is 60% higher in southern Ireland [than in Northern Ireland]. The highest incidences of rheumatoid arthritis are correlated very clearly with fluoridated countries'.¹³⁷ People who are not in favour of CWF, claim that those suffering from arthritis, or arthritic symptoms, are 'in fact suffering from low-grade fluoride poisoning',¹³⁸ which causes skeletal fluorosis.

Skeletal fluorosis

Skeletal fluorosis is a condition that can emerge from chronic high-level exposure to fluoride; it is typically found in fluoride-endemic countries. Skeletal fluorosis is reviewed in more detail in the main body of this HRB report (from page 34-39). While most studies identified an association between skeletal fluorosis and exposure to high levels of naturally fluoridated water, multiple other sources of fluoride could contribute to the development of skeletal fluorosis; such sources include food, water, air (due to pollution). The World Health Organization has explained the aetiology and symptoms of skeletal fluorosis in simple terms: 'In skeletal fluorosis, fluoride accumulates in the bone progressively over many years. The early symptoms of skeletal fluorosis include stiffness and pain in the joints. In severe cases, the bone structure may change and ligaments may calcify, with resulting impairment of muscles and pain'.¹³⁹ Therefore, many of the symptoms of the earlier stages of skeletal fluorosis can be confused with arthritis.

A report in the USA by the NRC reviewed studies which explored any possible relationship between fluoride and arthritis. One study found that fluoride exacerbated the symptoms of rheumatoid arthritis, and another found that fluoride was 'well tolerated', with no evidence of any worsening of the arthritis.²⁷ While acknowledging that

fluoride could exacerbate existing rheumatoid arthritis symptoms, the studies examined found 'no indications that fluoride had a causal relationship with rheumatoid arthritis'.²⁷ They also highlight a study by Savas *et al.*¹⁴⁰ which linked fluoride exposure with osteoarthritic symptoms. The study focused on a fluoride-endemic area in Turkey where they investigated osteoarthritis in the knees of patients with skeletal fluorosis, and compared them to patients who had osteoarthritis of the knee but did not have skeletal fluorosis. The NRC reported Savas *et al.* as concluding that 'Turkish patients with demonstrated endemic fluorosis had a greater severity of osteoarthritic symptoms and osteophyte formation than age- and sex-matched controls'.²⁷

Are there any reported associations between arthritis and fluoride?

People concerned about CWF reference a number of other papers as proving an association between fluoride and arthritis. For example, a 1980 paper by Alhava *et al.*¹⁴¹ has been cited in this regard. The Alhava paper, while not specifically about arthritis, discusses the impact of fluoride on cancellous bone strength and bone density. The main finding of the study was that 'bone mineral density and cancellous bone strength, did not show any statistically significantly beneficial effects of fluoridation, apart from some evidence that fluoridation may preserve mineral density and bone strength better in women with chronic immobilizing diseases'¹⁴¹ from the community water fluoridated area compared to the non-fluoridated control area. This suggests a beneficial effect for fluoridation, as the authors themselves state that there is a loss of bone mineral in chronic diseases, which results in physical inactivity.

A paper by Cook,¹⁴² also cited by those concerned about CWF, focuses on the association between consumption of tea in a fluoridated area and the prevalence of arthritic symptoms. The Cook paper¹⁴² centres on a single case report with no comparator; no conclusion can be drawn from examining one case in isolation. Therefore, this paper does not suffice as adequate evidence or proof. Furthermore, many confounding factors may be present which are not, and could not be, controlled for without a comparison group and an appropriate set of statistical measures.

Another paper, by Gupta *et al.*¹⁴³ draws attention to the fact that the early stage of skeletal (or bone) fluorosis is not clinically obvious. Often, the patient may initially complain about 'vague pains in the small joints of the hands, feet, and lower back'¹⁴³ and that such cases may be 'misdiagnosed as rheumatoid arthritis or ankylosing spondylitis'.¹⁴³ Teotia *et al.*'s paper states that 'the underlying metabolic abnormality in skeletal fluorosis is excess of fluoride and calcium in the bones which is due to an excess fluoride intake'.¹⁴⁴ They highlight how many patients in endemic fluoride areas are misdiagnosed with arthritis, and often treated for rheumatoid arthritis due to the early fluorosis stage symptoms of 'stiffness, backache, and joint pains'¹⁴⁴ before receiving a correct diagnosis of skeletal fluorosis.

None of the papers discussed here prove a causal association between fluoride and arthritis; even in endemic areas where arthritic symptoms tend to be symptoms of skeletal fluorosis rather than arthritis itself, and certainly none demonstrate an association between community fluoridated water and the development of arthritis.

Conclusion

This essay set out to explore why there is a concern about CWF causing or exacerbating arthritis. The issues around the topic have been discussed, and an attempt made to demonstrate how either correctly or incorrectly conclusions have been drawn. It is easy to understand why there is confusion between arthritis and skeletal fluorosis given that some of the symptoms overlap. There is no definitive research evidence to support claims of a causal link between fluoride intake and arthritis, but it is known that long-term exposure to high levels of fluoride may cause skeletal fluorosis.

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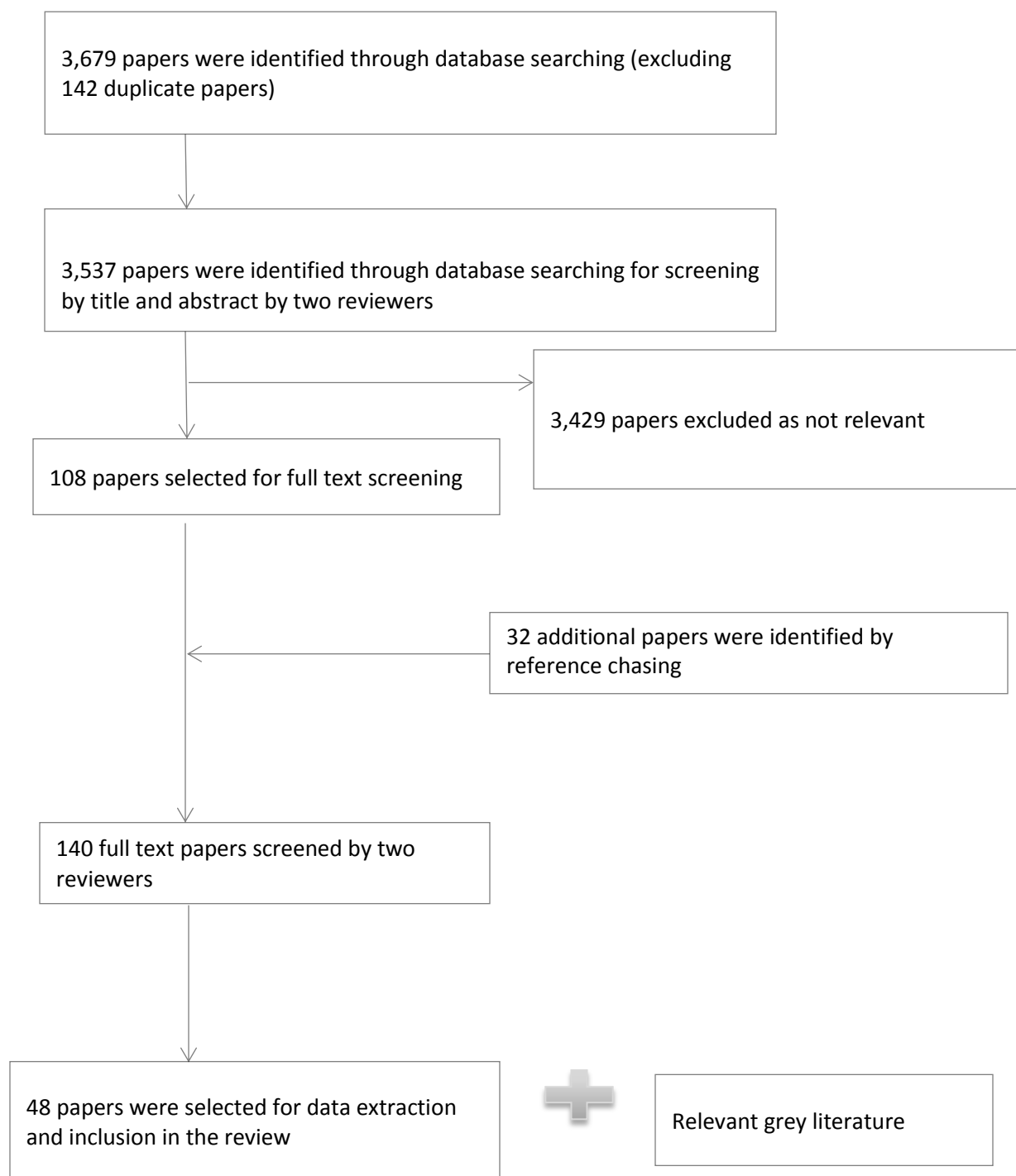
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Appendix 1 Flow chart of searches and screening process



Appendix 2 Search strategy used to find articles

The search strategies are presented in the table below. To maximise the retrieval rate, the search strategies combined Medical Subject headings (MeSH terms) or other controlled vocabulary terms with text words. The aim of the search strategy was high precision and recall. The following electronic databases were searched:

- Embase (through the OVID platform)
- MEDLINE (through the OVID platform)

All abstracts were screened by two reviewers. In the case of disagreements or where it was unclear from the abstract whether it should be included, the full paper was retrieved for a more detailed evaluation by both reviewers.

Database	Search terms	Number of papers or reports retrieved for screening
MEDLINE In-Process & Other Non-Indexed Citations and MEDLINE 1946 to July 2014 and Embase 1974 to July 2014	1. (fluorid* or fluorin* or flurid* or florin*).ti. or (fluorid* or fluorin* or flurid* or florin*).ab. or (fluorid* or fluorin* or flurid* or florin*).sh. 2. limit 1 to (english language and humans and yr="2006 - Current") 3. case control study/ 4. cohort analysis/ 5. clinical trial/ 6. "systematic review"/ 7. evaluation study/ 8. comparative study/ 9. controlled clinical trial/ 10. observational study/ 11. Case-Control Studies/ 12. Cohort Studies/ 13. Randomized Controlled Trial/ 14. Evaluation Studies/ 15. cross sectional study/ 16. 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 17. 2 and 16 18. remove duplicates from 17	2,887

Database	Search terms	Number of papers or reports retrieved for screening
MEDLINE	1. (fluorid* or fluorin* or flurid* or florin*).ti. or (fluorid* or fluorin* or flurid* or florin*).ab. or (fluorid* or fluorin* or flurid* or florin*).sh. 2. limit 1 to (english language and humans and yr="2006 - Current") 3. limit 2 to "review articles"	792
Additional searches 1999 - current [mid-June] <ul style="list-style-type: none"> • CINAHL and PsycINFO databases • TRIP database • <i>Fluoride</i> the topic-specific journal was searched – low-quality journal not indexed in MEDLINE 	Adjusted search string	No additional articles were obtained
Total		3,679
Total articles screened following the removal of duplicates		3,537

Exclusion criteria.

Not a clinical study
 Wrong intervention
 Wrong outcomes
 Dental health only
 Diagnostic tests/imaging studies
 Fluoride as a therapeutic agent
 Studies focused solely on organic pollutants
 Comparisons of water fluoridation levels with no health outcomes
 Surveys of opinions on fluoride and water fluoridation
 Measurements of urinary excretion levels
 Not in English

3520 studies were excluded based on the criteria above

Appendix 3 Extraction form

Main criteria	Sub-criteria
Study title and year	
Number and type of studies included in review	
Study type	
Sample size	
Study population	Exposed or cases Comparison group or controls (reference group)
Intervention/aetiology	
Study outcomes	
All studies quality criteria	Research Question
Systematic reviews/ Meta-analysis quality criteria	Search strategy Inclusion and exclusion criteria Quality assessment Primary studies described Method of pooling Summary result Heterogeneity Publication bias
Primary studies quality criteria	Description study population Sample size calculation/rationale/CI Minimise bias Control for confounding
Level of evidence	
Comment	

Appendix 4 Quality assessment tool for reviews

Health Evidence™
Helping public health use best evidence in practice

Quality Assessment Tool – Review Articles

Instructions for completion:

Please refer to the attached dictionary for definition of terms and instruction for completing each section. For each criteria, score by placing a check mark in the appropriate box

First Author: _____

Year: _____

Journal: _____

Reviewer: _____

CRITERION	YES	NO
Q1 Did the authors have a clearly focused question [population, intervention (strategy, and outcomes(s))]?		
Q2 Were the appropriated inclusion criteria used to select primary studies?		
Q3 Did the authors describe a search strategy that was comprehensive?		
<p><i>Circle all strategies used:</i></p> <ul style="list-style-type: none"> health databases psychological databases social science databases educational databases other hand searching key informants references lists unpublished 		
Q4 Did search strategy cover an adequate number of years?		
For question 5, 6, and 8, please choose the column relating to the appropriate methodology. Strike a line through the column that does not apply.		
<p>Q5. Quantitative reviews:</p> <p>Did the authors describe the level of evidence in the primary studies included in the review?</p> <p>Level I RCTS only</p> <p>Level II on-randomised, cohort, case-control</p> <p>Level III uncontrolled studies</p>	<p>Q5. Quantitative reviews:</p> <p>Do the authors provide a clear description of the range of methods in each of the primary studies included in the review?</p>	
<p>Q6 Quantitative reviews:</p> <p>Did the review assess the methodological quality of the primary studies, including:</p> <p>(Minimum requirement: 4/7 of the following)</p> <ul style="list-style-type: none"> Research design Study sample Participation rates Sources of bias (confounders, respondent bias) Data collection (measures of independent/dependent variables) Follow-up/attrition rates Data analysis 	<p>Q6 Quantitative reviews:</p> <p>Did the review assess the methodological quality of the primary studies, including:</p> <p>(Minimum requirement: 4/7 of the following)</p> <ul style="list-style-type: none"> Suitability of methodology/paradigm to the research question Sampling (selection of participants/settings/documentation) Clear description of context, data collection and data analysis Rigor: <ul style="list-style-type: none"> Audit trail Some coding by 2 or more coders, if appropriate Deviant case analysis *negative cases) Respondent validation (member checking) Triangulation Reflexivity (research and research process) Relevance (credibility, consistency, applicability, transferability) 	
Q7 Are the results of the review transparent?		
<p>Q8 Quantitative reviews:</p> <p>Was it appropriate to combine the finding of results across studies?</p>	<p>Q8 Quantitative reviews:</p> <p>Is there a description of how reviewers determined results were similar enough cross studies to compare or combine them?</p>	
Q9 Were appropriate methods used for combining or comparing results across studies?		
Q10. Do the data support the author's interpretation?		
TOTAL SCORE:		

Quality Assessment Rating:
(Circle one)

Strong (high)
(total score 8 – 10)

Moderate
(total score 5 – 7)

Weak (Low)
(total score 4 or less)

