COMMISSION DE LA SANTÉ ET DES SERVICES SOCIAUX
Déposé le 2013-04-23
No. : CSSS – 023
Secrétaire Cédric Drouin

Harvard Fluoridation study- published in Environmental Perspectives

** excerpt below taken from a listserve**
Based on Pew's analysis, the article in Environmental Health Perspectives (EHP) does not raise valid concerns about water fluoridation, as practiced in the U.S. The article reviewed studies of IQ scores for children living in areas of China , Mongolia and Iran where the water supplies have unusually high, natural fluoride levels. In many cases, the high-fluoride areas were significantly higher than the levels used to fluoridate public water systems in the U.S. In fact, the high-fluoride areas in these foreign countries reached levels as high as 11.5 mg/L, which is more than 10 times higher than the optimal level used in the U.S.

The EHP offers a meta-analysis, and its credibility hinges on whether good-quality studies are reviewed. Yet the article's co-authors admit that "each of the [studies] reviewed had deficiencies, in some cases rather serious, which limit the conclusions that can be drawn." Although the studies compared high-fluoride with low-fluoride areas, the authors acknowledge that "the actual exposures of the individual children are not known."

It's worth noting that a 2009 animal study found that even at fluoride levels that were up to 230 times higher than the typical human consumes, there was "no evidence of learning deficits in any of the fluoride-exposed groups."

In addition, the authors acknowledge that the average standardized mean difference (0.45) in IQ scores between the high- and low-fluoride groups "may be within the measurement error of IQ testing."

Finally, the co-authors recognized that these foreign studies did not rule out a variety of confounding factors that could have influenced these IQ scores. Given the small difference in IQ scores, it's possible that arsenic levels, school quality, nutrition, parents' educational levels and other factors could have shaped the results. The authors also added that "reports of lead concentrations in the study villages in China were not available"— another factor that could not be discounted. A British research team reviewed similar Chinese data and reported that "water supplies may be contaminated with other chemicals such as arsenic, which may affect IQ."
http://www.fluoridesandhealth.ie/documents/

Documents from Ireland
Irish Expert Body on Fluorides and Health
http://www.fluoridesandhealth.ie/documents/Appraisal_of_Waugh_report_May_2012.pdf
http://www.fluoridesandhealth.ie/documents/

An example of a Health Region's Fluoridation Report The Regional Municipality of Halton's Review of Fluoridation
http://sirepub.halton.ca/cache/2/ohpgkarfptwcev55f5zw3s55/145392
10172012041108649.PDF

ENVIRONMENTAL HEALTH PERSPECTIVES

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Developmental Fluoride Neurotoxicity: A Systematic Review and Meta-Analysis

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http://dx.doi.org/10.1289/ehp.1104912

Online 20 July 2012

NIEHS
National Institute of Environmental Health Sciences

National Institutes of Health
U.S. Department of Health and Human Services

Developmental Fluoride Neurotoxicity: A Systematic Review and Meta-Analysis

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Running Title: Fluoride neurotoxicity

Key Words: Fluoride, Intelligence, Neurotoxicity

Acknowledgments:

We thank Dr. Vasanti Malik, Harvard School of Public Health, for the helpful advice on the meta-analysis methods.

This study was supported by internal institutional funds.

The authors declare that they have no competing financial interest.

List of Abbreviations:

CI, confidence interval

CNKI, China National Knowledge Infrastructure

SE, standard error

SMD, Standardized mean difference

TOXNET, Toxicology Data Network

Abstract

Background: Although fluoride may cause neurotoxicity in animal models and acute fluoride poisoning causes neurotoxicity in adults, very little is known of its effects on children's neurodevelopment.

Objective: We performed a systematic review and meta-analysis of published studies to investigate the effects of increased fluoride exposure and delayed neurobehavioral development.

Methods: We searched the MEDLINE, EMBASE, Water Resources Abstracts, and TOXNET databases through 2011 for eligible studies. We also searched the China National Knowledge Infrastructure (CNKI) database, as many studies on fluoride neurotoxicity have been published in Chinese journals only. In total, we identified 27 eligible epidemiological studies with high and reference exposures, endpoints of IQ scores or related cognitive function measures with means and variances for the two exposure groups. We estimated the standardized mean difference (SMD) between exposed and reference groups across all studies using random effects models. We conducted sensitivity analyses restricted to studies using the same outcome assessment and having drinking water fluoride as the only exposure. Cochran test for heterogeneity between studies, Begg's funnel plot and Egger test to assess publication bias were performed. Meta-regressions to explore sources of variation in mean differences among the studies were conducted.

Results: The standardized weighted mean difference in IQ score between exposed and reference populations was -0.45 (95% CI -0.56 to -0.35) using a random-effects model. Thus, children in high fluoride areas had significantly lower IQ scores than those who lived in low fluoride areas. Subgroup and sensitivity analyses also indicated inverse associations, although the substantial heterogeneity did not appear to decrease.

Conclusions: The results support the possibility of an adverse effect of high fluoride exposure on children's neurodevelopment. Future research should include detailed individual-level information on prenatal exposure, neurobehavioral performance, and covariates for adjustment.

Introduction

A recent report from the US National Research Council (NRC 2006) concluded that adverse effects of high fluoride concentrations in drinking-water may be of concern and that additional research is warranted. Fluoride may cause neurotoxicity in laboratory animals, including effects on learning and memory (Chioca et al. 2008; Mullenix et al. 1995). A recent experimental study where the rat hippocampal neurons were incubated with various concentrations (20 mg/L, 40 mg/L, and 80 mg/L) of sodium fluoride *in vitro* showed that fluoride neurotoxicity may target hippocampal neurons (Zhang et al. 2008). Although acute fluoride poisoning may be neurotoxic to adults, most of the epidemiological information available on associations with children's neurodevelopment is from China, where fluoride generally occurs in drinking water as a natural contaminant, and the concentration depends on local geological conditions. In many rural communities in China, populations with high exposure to fluoride in local drinking water sources may reside in close proximity to populations without high exposure (NRC 2006).

Opportunities for epidemiological studies depend on the existence of comparable population groups exposed to different levels of fluoride from drinking water. Such circumstances are difficult to find in many industrialized countries, as fluoride concentrations in community water are usually no higher than 1 mg/L, even when fluoride is added to water supplies as a public health measure to reduce tooth decay. Multiple epidemiological studies of developmental fluoride neurotoxicity were conducted in China due to the high fluoride concentrations that are substantially above 1 mg/L in well-water in many rural communities, although microbiologically safe water has been accessible to many rural households as a result of the recent five-year plan (2001-2005) by the Chinese government. It is projected that all rural residents will have access to safe public drinking water by 2020 (World Bank 2006). However, results of the published studies have not been widely disseminated. Four studies published in English (Li et al. 1995; Lu et al. 2000; Xiang et al. 2003; Zhao et al. 1996) were cited in a recent report from the National Research Council (NRC 2006), while the World Health Organization has considered only two (Li et al. 1995; Zhao et al. 1996) in its most recent monograph on fluoride (WHO 2002).

Fluoride readily crosses the placenta (ATSDR 2003). Fluoride exposure to the developing brain, which is much more susceptible to injury caused by toxicants than is the mature brain, may possibly lead to damage of a permanent nature (US EPA 2011). Based on the considerations of health risks, and in response to the recommendation of the National Research Council (NRC 2006), the U.S. Department of Health and Human Services (HHS) and the U.S. Environmental Protection Agency (EPA) recently announced that HHS is proposing to change the recommended level of fluoride in drinking water to 0.7 mg/L from the currently recommended range of 0.7 to 1.2 mg/L, and EPA is reviewing the maximum amount of fluoride allowed in drinking water, which currently is set at 4.0 mg/L (US EPA 2011).

To summarize the available literature, we performed a systematic review and meta-analysis of published studies on increased fluoride exposure in drinking water and neurodevelopmental delays. We specifically targeted studies carried out in rural China that have not been widely disseminated, thus complementing the studies that have been included in previous reviews and risk assessment reports.

Methods

Search Strategy

We searched MEDLINE (National Library of Medicine, Bethesda, MD; http://www.ncbi.nlm.nih.gov/pubmed), EMBASE (Elsevier B.V., Amsterdam, the Netherlands; http://www.embase.com), Water Resources Abstracts (Proquest, Ann Arbor, MI; http://www.csa.com/factsheets/water-resources-set-c.php), and TOXNET (National Library of Medicine, Bethesda, MD; http://toxnet.nlm.nih.gov) databases to identify studies of drinking water fluoride and neurodevelopmental outcomes in children. In addition, we searched the China National Knowledge Infrastructure (CNKI; http://www.cnki.net) database to identify studies published in Chinese journals only. Keywords included combinations of "fluoride" or "drinking water fluoride", "children", "neurodevelopment" or "neurologic" or "intelligence" or "IQ". We also used references cited in articles identified. Records were searched from 1980 to 2011. Our literature search identified 39 studies, among which 36 (95%) were studies with high and reference exposure groups, and 3 (7.7%) studies were based on individual-level measure of exposures. The latter showed dose-related deficits were found but were excluded because our meta-analysis focused on studies with the high and low exposure groups only. In addition, 2 studies were published twice, and the duplicates were excluded.

Inclusion criteria and Data Extraction

The criteria for inclusion of studies included studies with high and reference fluoride exposures, endpoints of IQ scores or other related cognitive function measures, presentation of a mean outcome measure and associated measure of variance [95% confidence intervals (CI) or standard errors (SEs) and numbers of participants]. Interpretations of statistical significance are based on an alpha level of 0.05. Information included for each study also included the first author, location of the study, year of publication, and numbers of participants in high-fluoride and low-fluoride areas. We noted and recorded the information on age and gender of children, and parental education and income if available.

Statistical Analysis

STATA (version 11.0; StataCorp, College Station, TX) and available commands (Stern 2009) were used for the meta-analyses. A standardized weighted mean difference (SMD) was computed using both fixed-effects and random-effects models. The fixed-effects model uses the Mantel-Haenszel method assuming homogeneity among the studies, while the random-effects model uses the DerSimonian and Laird method, incorporating both a within-study and an additive between-studies component of variance when there is between-study heterogeneity (Egger et al. 2001). The estimate of the between-study variation is incorporated into both the standard error of the estimate of the common effect and the weight of individual studies, which was calculated as the inverse sum of the within and between study variance. Heterogeneity among studies was evaluated using the I^2 statistic, which represents the percentage of total variation across all studies due to between-study heterogeneity (Higgins and Thompson 2002). The potential for publication bias was evaluated using Begg and Egger tests and visual inspection of a Begg funnel plot (Begg and Mazumdar 1994; Egger et al. 1997). We also conducted independent meta-regressions to estimate the contribution of study characteristics (mean age in years from the age range and year of publication in each study) to heterogeneity among the studies. The scoring standard for the Combined Raven's Test – The Rural edition in China (CRT-RC) test classifies a score of ≤69 and 70-79 as low and marginal intelligence, respectively (Wang et al. 1989). We also used the random effects models to estimate risk ratios for the association between fluoride exposure and a low/marginal versus normal Raven's test score among children in studies that used the Combined Raven's Test –Rural in China (CRT-RC) test (Wang et al. 1989). Scores indicating low and marginal intelligence ($\leq$69 and 70-79, respectively) were combined as a single outcome due to small numbers of children in each outcome subgroup.

Results

Six of the 34 studies identified were excluded due to missing information on the number of subjects or the mean and variance of the outcome (see Figure 1 for a study selection flow chart and Supplemental Material, Table S1 for additional information on studies that were excluded from the analysis). Another study (Trivedi et al. 2007) was excluded because SDs reported for the outcome parameter were questionably small (1.13 for high fluoride group, and 1.23 for low fluoride group) and the SMD (-10.8, 95% CI -11.9, -9.6) was more than 10-times lower than the second smallest SMD (-0.95, 95% CI -1.16, -0.75) and 150-times lower than the largest SMD (0.07, 95% CI -0.083, 0.22) reported for the other studies, which had relatively consistent SMD estimates. Inclusion of this study in the meta-analysis resulted with a much smaller pooled random-effects SMD estimate and a much larger I^2 (-0.63 (95% CI -0.83, -0.44), I^2 94.1%) compared to the estimates that excluded this study (-0.45, 95% CI -0.56, -0.34), I^2 80%) (see Supplemental Material, Figure S1). Characteristics of the 27 studies included are shown in Table 1(An et al. 1992; Chen et al. 1991; Fan et al. 2007; Guo et al. 1991; Hong et al. 2001; Li et al. 2003; Li et al. 2009; Li et al. 2010; Lin et al. 1991; Lin et al. 1994; Lin et al. 1995; Lu et al. 2000; Poureslami et al. 2011; Ren et al. 1989; Seraj et al. 2006; Sun et al. 1991; Wang et al.

1996; Wang et al. 2001; Wang et al. 2006; Wang et al. 2007; Xiang et al. 2003; Xu et al. 1994; Yang et al. 1994; Yao et al. 1996; Yao et al. 1997; Zhang et al. 1998; Zhao et al. 1996). Two of the studies included in the analysis were conducted in Iran (Poureslami et al. 2011; Seraj et al. 2006), otherwise the study cohorts were populations from China. Two cohorts were exposed to fluoride from coal burning (Guo et al. 1991; Li et al. 2010), otherwise populations were exposed to fluoride through drinking water. The CRT-RC was used to measure the children's intelligence in 16 studies. Other intelligence measures included the Weschler Intelligence tests (3 studies), Binet IQ test (2 studies), Raven's test (2 studies), Japan IQ test (2 studies), Chinese comparative intelligence test (1 study), and the mental work capacity index (1 study). As each of the intelligence tests used are designed to measure general intelligence, we used data from all eligible studies to estimate the possible effects of fluoride exposure on general intelligence. In addition, we conducted a sensitivity analysis restricted to studies that used similar tests to measure the outcome (specifically, the CRT-RC, Weschler Intelligence test, Binet IQ test, or Raven's test), and an analysis restricted to studies that used the CRT-RC. We also performed an analysis that excluded studies with co-exposures including iodine and arsenic, or with non-drinking water fluoride exposure from coal burning.

Pooled SMD estimates

Among the 27 studies, all but one study showed random-effect SMD estimates that indicated an inverse association, ranging from -0.95 (95% CI: -1.16, -0.75) to -0.10 (95% CI: -0.25, 0.04) (Figure 2). The study with a positive association reported a SMD estimate of 0.07 (95% CI: -0.8, 0.22). Similar results were found with the fixed-effect SMD estimates. The fixed-effects pooled SMD estimate and corresponding 95% CI were -0.40 (-0.44, -0.35), with a p-value <0.001 for the test for homogeneity. The random-effects SMD estimate and 95% CI were -0.45 (95% CI:-0.56, -0.34) with an I^2 of 80% and homogeneity test p-value <0.001 (Figure 2). Because of heterogeneity (excess variability) between study results, we primarily used the random-effects model for subsequent sensitivity analyses, which is generally considered to be the more conservative method (Egger et al. 2001). Among the restricted sets of intelligence tests, the SMD for the model with only CRT-RC tests and drinking-water exposure (and to a lesser extent the model with only CRT-RC tests) was lower than that for all studies combined, although the difference did not appear to be significant. Heterogeneity, however, remained at a similar magnitude when the analyses were restricted (Table 2).

Sources of heterogeneity

We performed meta-regression models to assess study characteristics as potential predictors of effect. Information on the child's gender and parental education were not reported in more than 80% of the studies, and only 7% of the studies reported household income. These variables were therefore not included in the models. Among the two covariates, year of publication (0.02; 95% CI: 0.006, 0.03), but not mean age of the study children (-0.02; 95% CI: -0.094, 0.04), was a significant predictor in the model with all 27 studies included. I^2 residual 68.7%, represented the proportion of residual between-study variation due to heterogeneity. From the adjusted R^2, 39.8% of between-study variance was explained by the two covariates. The overall test of the covariates was significant (p=0.004).

When the model was restricted to the 16 studies that used the CRT-RC, the child's age (but not year of publication) was a significant predictor of the SMD. The R^2 of 65.6% of between-study variance was explained by the two covariates, and only 47.3% of the residual variation was due to heterogeneity. The overall test of both covariates in the model remained significant ($p = 0.0053$). On further restriction of the model to exclude the 7 studies with arsenic and iodine as co-exposures and fluoride originating from coal-burning, thus including only the 9 with fluoride exposure from drinking water, neither age nor year of publication was a significant predictor, and the overall test of covariates was less important ($p = 0.062$), in accordance with the similarity of intelligence test outcomes and the source of exposure in the studies included. Although official reports of lead concentrations in the study villages in China were not available, some studies reported high percentage (95 to 100%) of low lead exposure (less than the standard of 0.01 mg/L) in drinking water samples in villages from several study provinces (Bi et al. 2010; Peng et al. 2008; Sun 2010).

Publication bias

A Begg's funnel plot with the SE of SMD from each study plotted against its corresponding SMD did not show clear evidence of asymmetry, though two studies with a large SE also reported relatively large effect estimates, which may be consistent with publication bias or heterogeneity (Figure 3). The plot appears symmetrical for studies with larger SE, but with substantial variation in SMD among the more precise studies, consistent with the heterogeneity observed among the studies included in the analysis. Begg ($p = 0.22$) and Egger ($p = 0.11$) tests did not indicate significant ($p < 0.05$) departures from symmetry.

Pooled risk ratios

The relative risk of a low/marginal score on the CRT-RC test (<80) among children with high fluoride exposure compared to those with low exposure (16 studies total) was 1.93 (95% CI:

1.46, 2.55; I^2 58.5%). When the model was restricted to 9 studies that used the CRT-RC and included only drinking water fluoride exposure (Chen et al. 1991; Fan et al. 2007; Li et al. 1995; Li et al. 2003; Li et al. 2010; Lu et al.2000; Wang et al. 2006; Yao et al. 1996, 1997), the estimate was similar (RR 1.75; 95% CI: 1.16, 2.65; I^2 70.6%). Although fluoride exposure showed inverse associations with test scores, the available exposure information did not allow a formal dose-response analysis. However, dose-related differences in test scores occurred at a wide range of water-fluoride concentrations.

Discussion

Findings from our meta-analyses of 27 studies published over 22 years suggest an inverse association between high fluoride exposure and children's intelligence. Children who lived in areas with high fluoride exposure had lower IQ scores than those who lived in low exposure or control areas. Our findings are consistent with an earlier review (Tang et al. 2008), although ours more systematically addressed study selection and exclusion information, and more comprehensive in 1) including nine additional studies, 2) performing meta-regression to estimate the contribution of study characteristics as sources of heterogeneity, and 3) estimating pooled risk ratios for the association between fluoride exposure and a low/marginal Raven's test score.

As noted by the NRC committee (NRC 2006), assessments of fluoride safety have relied on incomplete information on potential risks. In regard to developmental neurotoxicity, much information has in fact been published, although mainly as short reports in Chinese that have not been available to most expert committees. We carried out an extensive review that includes epidemiological studies carried out in China. While most reports were fairly brief and complete information on covariates was not available, the results tended to support the potential for fluoride-mediated developmental neurotoxicity at relatively high levels of exposure in some studies. We did not find conclusive evidence of publication bias, though there was substantial heterogeneity among studies. Drinking-water may contain other neurotoxicants, such as arsenic, but exclusion of studies including arsenic and iodine as co-exposures in a sensitivity analysis resulted in a lower estimate, although the difference was not significant. The exposed groups had access to drinking-water with fluoride concentrations up to 11.5 mg/L (Wang et al. 2007), thus in many cases concentrations were above the levels of 0.7-1.2 mg/L (HHS) and 4.0 mg/L (US EPA) considered acceptable in the US. A recent cross-sectional study based on individual-level measure of exposures suggested that low levels of water fluoride (range 0.24 to 2.84 mg/L) had significant negative associations with child's intelligence (Ding et al. 2011). This study was not included in our meta-analysis, which focused only on studies with exposed and reference groups, thereby precluding estimation of dose-related effects.

The results suggest that fluoride may be a developmental neurotoxicant that affects brain development at exposures much below those that can cause toxicity in adults (Grandjean 1982). For neurotoxicants, such as lead and methylmercury, adverse effects are associated with blood concentrations as low as 10 nmol/L. Serum-fluoride concentrations associated with high intakes from drinking-water may exceed 1 mg/L, or 50 μmol/L, thus more than 1000-times the levels of some other neurotoxicants that cause neurodevelopmental damage. Supporting the plausibility of our findings, rats exposed to 1 ppm (50 μmol/L) of water-fluoride for one year showed morphological alterations in the brain and increased levels of aluminum in brain tissue compared with controls (Varner et al. 1998).

The estimated decrease in average IQ associated with fluoride exposure based on our analysis may seem small and may be within the measurement error of IQ testing. However, as research on other neurotoxicants has shown, a shift to the left of IQ distributions in a population will have substantial impacts, especially among those in the high and low ranges of the IQ distribution (Bellinger 2007).

The present study cannot be used to derive an exposure limit, as the actual exposures of the individual children are not known. Misclassification of children in both high- and low-exposure groups may have occurred if the children were drinking water from other sources (e.g., at school or in the field).

The published reports clearly represent independent studies and are not the result of duplicate publication of the same studies (we removed two duplicates). Several studies (Hong et al. 2001; Lin et al. 1991; Wang et al. 2001; Wang et al. 2007; Xiang et al. 2003; Zhao et al. 1996) report other exposures, such as iodine, and arsenic, a neurotoxicant, but our sensitivity analyses showed similar associations between high fluoride exposure and the outcomes even after these studies were excluded. Large tracts of China have superficial fluoride-rich minerals with little, if any, likelihood of contamination by other neurotoxicants that would be associated with fluoride concentrations in drinking water. From the geographical distribution of the studies, it seems unlikely that fluoride-attributed neurotoxicity could be due to other water contaminants.

Still, each of the articles reviewed had deficiencies, in some cases rather serious, which limit the conclusions that can be drawn. However, most deficiencies relate to the reporting, where key information was missing. The fact that some aspects of the study were not reported limits the extent to which the available reports allow a firm conclusion. Some methodological limitations were also noted. Most studies were cross-sectional, but this study design would seem appropriate in a stable population where water supplies and fluoride concentrations have remained unchanged for many years. The current water-fluoride level likely also reflects past developmental exposures. In regard to the outcomes, the inverse association persisted between studies using different intelligence tests, although most studies did not report age adjustment of the cognitive test scores.

Fluoride has received much attention in China, where widespread dental fluorosis indicates the prevalence of high exposures. In 2008, the Ministry of Health reported that fluorosis was found in 28 provinces with 92 million residents (China News, 2008). Although microbiologically safe, water supplies from small springs or mountain sources created pockets of increased exposures near or within areas of low exposures, thus representing exposure settings close to the ideal, as only the fluoride exposure would differ between nearby neighborhoods. Chinese researchers took advantage of this fact and published their findings, though mainly in Chinese journals, and according to the standards of science at the time. This research dates back to the 1980s, but has not been widely cited at least in part because of limited access to Chinese journals.

In its review of fluoride, the US National Research Council (NRC 2006) emphasized that both the beneficial effects of fluoride on dental health and its adverse effects were incompletely documented. Our comprehensive review substantially extends the scope of research available for evaluation and analysis. Although the studies were generally of insufficient quality, the consistency of their findings adds support to existing evidence of fluoride-associated cognitive deficits, and suggests that potential developmental neurotoxicity of fluoride should be a high research priority. While reports from WHO and national agencies have generally focused on beneficial effects (CDC 1999; Petersen and Lennon 2004), the NRC report emphasized the need to consider potential adverse effects as well as benefits of fluoride exposure (NRC 2006).

In conclusion, our results support the possibility of adverse effects of fluoride exposures on children's neurodevelopment. Future research should formally evaluate dose-response relations based on individual-level measures of exposure over time, including more precise prenatal exposure assessment and more extensive standardized measures of neurobehavioral performance, in addition to improving assessment and control of potential confounders.

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Table 1. Characteristics of epidemiological studies of fluoride exposure and child's cognitive outcomes

Reference	Study Location	No. in high exposure group	No. in reference group	Age range (years)	Fluoride exposure: Assessment	Fluoride exposure: Range	Outcome Measure	Results
Ren et al. 1989	Shandong, China	160	169	8-14	High/low F villages	Not specified	Wechler Intelligence test	Children in highF, region had lower IQ scores
Chen et al. 1991	Shanxi, China	320	320	7-14	Drinking water	4.55 mg/L (high); 0.89 mg/L (reference)	CRT-RC[a]	The average IQ of children from high fluoride area were lower than that of the reference area
Guo et al. 1991	Hunan, China	60	61	7-13	F in coal burning	118.1-1361.7 mg/kg (coal burning area); Control area used wood	Chinese Binet	Average IQ in fluoride coal burning area was lower than that in the reference area
Lin et al. 1991	Xinjiang, China	33	86	7-14	Drinking water	0.88mg/L (high); 0.34 mg/L (reference)	CRT-RC[a]	Children in the high fluoride (low iodine) area had lower IQ scores compared with the children from the reference fluoride (low iodine) areas
Sun et al. 1991	Guiyang, China	196	224	6.5-12	Rate of fluorosis	Fluorosis: 98.36% (high); not specified (reference)	Japan IQ test	Mean IQ was lower in all age groups except ≤7 years old group in the area with high fluoride and aluminum (limited to high fluoride population only)
An et al. 1992	Inner Mongolia, China	121	121	7-16	Drinking water	2.1-7.6mg/L (high) 0.6-1.0 mg/L (reference)	Wechler Intelligence test	IQ scores of children in high fluoride areas were significantly lower than those of children living in reference fluoride area
Li et al. 1994	Sichuan, China	106	49	12-13	Buring of high-fluoride coal to cook grain in high fluoride area	4.7-31.6 mg/kg (high) 0.5 mg/kg (reference)	Child mental work capacity	Early, prolonged high fluoride intake causes a decrease in the child's mental work capacity
Xu et al. 1994	Shandong, China	97	32	8-14	Drinking water	1.8 mg/L (high) 0.8 mg/L (reference)	Binet-Siman	Children had lower IQ scores in high fluoride area than those who lived in the reference area.
Yang et al. 1994	Shandong, China	30	30	8-14	Well water	2.97 mg/L (high); 0.5 mg/L (reference)	Chinese comparative intelligence test	The average IQ scores was lower in children from high fluoride and iodine area than those from the reference area, but the results were not significant
Li et al. 1995	Guizhou, China	681	226	8-13	Urine, Dental Fluorosis Index(DFI)	1.81-2.69 mg/L (high); 1.02 mg/L (reference); DFI 0.8-3.2 (high) DFI <0.4 (reference)	CRT-RC[a]	Children living in fluorosis areas had lower IQ scores than children living in non-fluorosis areas
Wang et al. 1996	Xinjiang, China	147	83	4-7	Drinking water	>1.0-8.6 mg/L (high) 0.58-1.0 mg/L (reference)	Wechsler Intelligence Test	Average IQ score was lower in children in the high fluoride group than those in the reference group
Yao et al. 1996	Liaoning, China	266	270	8-12	Drinking water	2-11mg/L (high) 1 mg/L (reference)	CRT-RC[a]	Average IQ scores of children residing in exposed fluoride areas were lower than those in the reference area
Zhao et al. 1996	Shanxi, China	160	160	7-14	Drinking water	4.12 mg/L (high) 0.91 mg/L (reference)	CRT-RC[a]	Children living in high fluoride and arsenic area had significantly lower IQ scores than those living in the reference fluoride (and no arsenic) area

Reference	Study Location	No. in high exposure group	No. in reference group	Age range (years)	Fluoride exposure: Assessment	Fluoride exposure: Range	Outcome Measure	Results
Yao et al. 1997	Liaoning, China	188	314	7-14	Drinking water	2 mg/L (exposed) 0.4 mg/L (reference)	CRT-RC[a]	IQ scores of children in the high fluoride area were lower than those of children in the reference area
Zhang et al. 1998	Xinjiang, China	51	52	4-10	Drinking water	Not specified	Japan IQ Test	Average IQ scores of children residing in high fluoride and arsenic area were lower than those who resided in the reference area
Lu et al. 2000	Tianjin, China	60	58	10-12	Drinking water	3.15 mg/L (high) 0.37 mg/L (reference)	CRT-RC[a]	Children in the high fluoride area scored significantly lower IQ scores than those in the reference area
Hong et al. 2001	Shandong, China	85	32	8-14	Drinking water	2.90 mg/L (high) 0.75 mg/L (reference)	CRT-RC[a]	Average IQ scores were significantly lower in high fluoride group (and iodine) than the reference group
Wang et al. 2001	Shandong, China	30	30	8-12	Drinking water	2.97 mg/L (high) 0.5 mg/L (reference)	CRT-RC[a]	No significant difference in IQ scores of children in the high fluoride/high iodine and reference fluoride/low iodine areas
Li et al. 2003	Inner Mongolia	720	236	6-13	Fluorosis	Endemic versus control regions defined by the Chinese Geological Office	CRT-RC[a]	Average IQ of children in high fluorosis area was lower than that in the reference area
Xiang et al. 2003	Jiangsu, China	222	290	8-13	Drinking water	0.57-4.5 mg/L (high) 0.18-0.76 mg/L (reference)	CRT-RC[a]	Mean IQ score was significantly lower in children who lived in the high fluoride area than that of children in the reference exposure area (both areas also had arsenic exposure)
Seraj et al. 2006	Tehran, Iran	41	85	Not specified	Drinking water	2.5 mg/L (high) 0.4 mg/L (reference)	Raven	The mean IQ of children in the high fluoride area was significantly lower than that from the reference fluoride area
Wang et al. 2006	Shanxi, China	202	166	8-12	Drinking water	5.54±3.88 mg/L (high) 0.73±0.28 mg/L (reference)	CRT-RC[a]	The IQ scores of children in the high fluoride group were significantly lower than those in the reference group
Fan et al 2007	Shaanxi, China	42	37	7-14	Drinking water	1.14-6.09 mg/L (high) 1.33-2.35 mg/L (reference)	CRT-C_2	The average IQ scores of children residing in the high F area were lower than those of children residing in the reference area
Wang et al. 2007	Shanxi, China	253	196	8-12	Drinking water and urine	3.8-11.5 mg/L (water, high) 1.6-11 mg/L (urine, high) 0.2-1.1 mg/L (water, reference) 0.4-3.9 mg/L (urine, reference)	CRT-RC[a]	Mean IQ scores were significantly lower in the high fluoride group than from the reference group in the fluoride/arsenic areas
Li et al. 2009	Hunan, China	60	20	8-12	Coal burning	1.24-2.34 mg/L (high) 0.962 mg/L (reference)	CRT-C_2	Mean IQ was lower in children in coal-burning areas compared to those in the reference group

Reference	Study Location	No. in high exposure group	No. in reference group	Age range (years)	Fluoride exposure: Assessment	Fluoride exposure: Range	Outcome Measure	Results
Li et al. 2010	Henan, China	347	329	7-10	Drinking water	2.47±0.75mg/L (high)	CRT-RC	No significant difference in IQ scores between children in the exposed and reference groups
Poureslami et al. 2011	Iran	59	60	6-9	Drinking Water	2.38 mg/L (high) 0.41 mg/L (reference)	Raven	Children in the high fluoride group scored significantly lower than those in reference group

[a]CRT-RC denotes Chinese Standardized Raven Test, rural version (Wang et al. 1989)

Table 2. Sensitivity analyses of pooled random-effect standardized weighted mean difference (SMD) estimates of child's intelligence score with high exposure of fluoride

Model	Available studies for analysis	SMD (95% CI)	I^2	p-value test of heterogeneity
1. Exclude non-standardized tests[a]	23	-0.44 (-0.54, -0.33)	77.6%	< 0.001
2. Exclude non CRT-RC Tests[b]	16	-0.36 (-0.48, -0.25)	77.8%	<0.001
3. Exclude studies with other exposures (Iodine, Arsenic)[c] or non-drinking water fluoride exposure[d]	9	-0.29 (-0.44, -0.14)	81.8%	<0.001

[a]Mental work capacity (Li et al. 1994); Japan IQ (Sun et al. 1991; Zhang et al. 1998); Chinese comparative scale of intelligence test (Yang et al. 1994)

[b]Weschler intelligence test (An et al. 1992; Ren et al . 1989; Wang et al. 1996); Chinese Binet IQ (Guo et al. 1991); Raven(Poureslami et al. 2011; Seraj et al. 2006); Binet-Siman (Xu et al. 1994)

[c]Iodine (Hong et al. 2001; Lin et al. 1991; Wang et al. 2001); Arsenic (Wang et al. 2007; Xiang et al. 2003; Zhao et al. 1996; Zhang et al. 1998 - already excluded, see footnote 1)

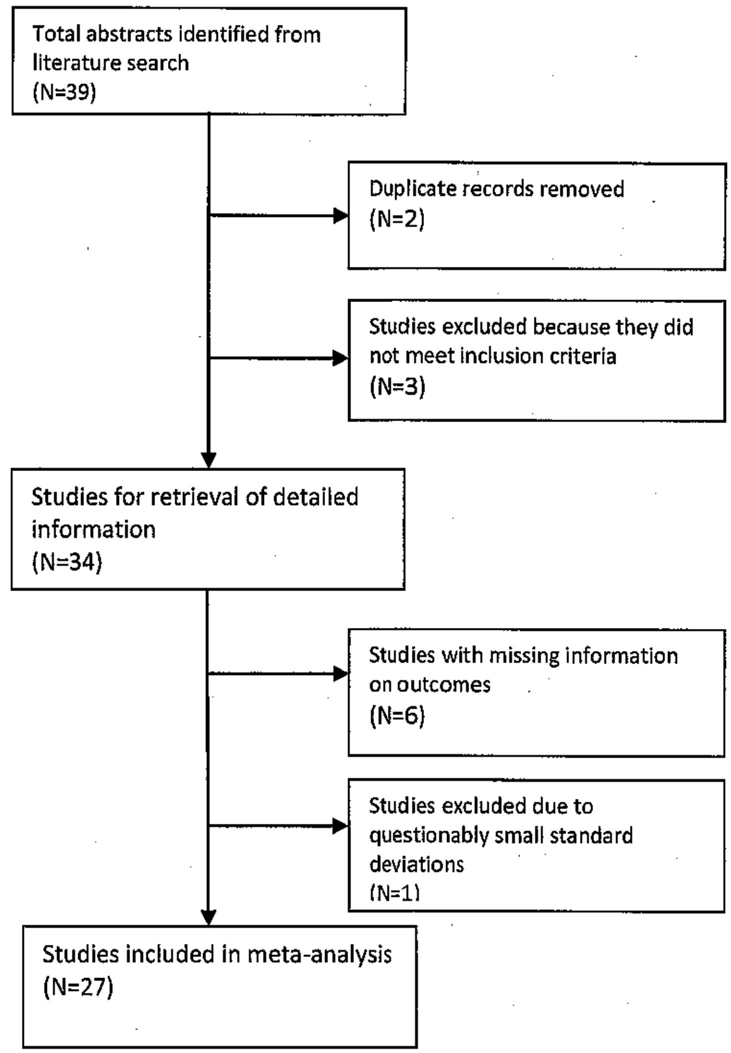
[d]Fluoride from coal-burning (Li et al. 2009; Guo et al. 1991; Li et al 1994 (already excluded, see footnotes a and b)

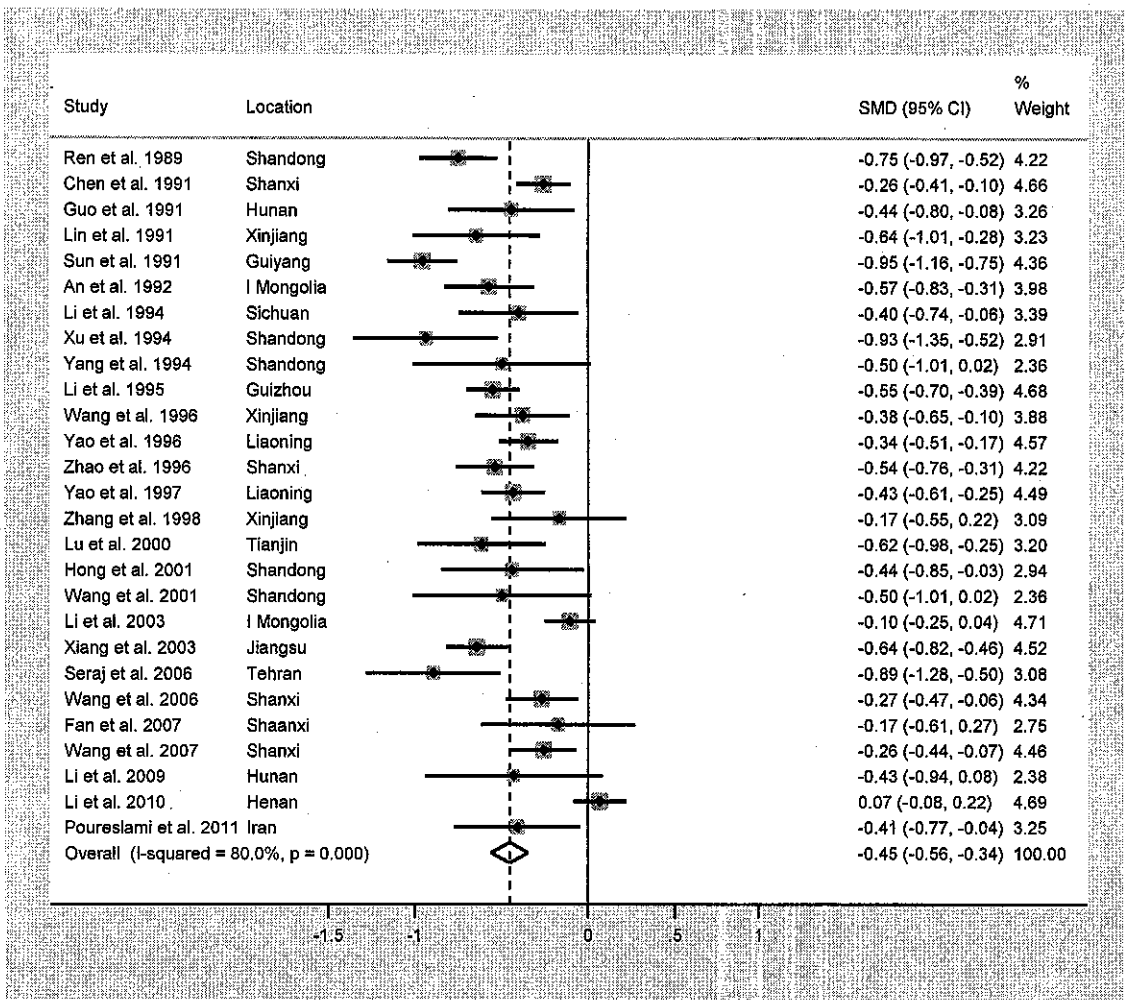
Figure Legend

Figure 1. Flow diagram of the meta-analysis

Figure 2. Random-effect standardized weighted mean difference (SMD) estimates and 95% CIs of child's intelligence score associated with high exposure to fluoride. SMs for individual studies are shown as solid diamonds (♦), and the pooled SMD is shown as a non-filled diamond (◊). Horizontal lines represent 95% CIs for the study-specific SMDs.

Figure 3. Begg's funnel plot showing individual studies included in the analysis according to random-effect standardized weighted mean difference (SMD) estimates (x-axis) and the standard error (se) of each study-specific SMD (y-axis). The solid vertical line indicates the pooled SMD estimate for all studies combined and the dashed lines indicated pseudo 95% confidence limits around the pooled SMD estimate


Total abstracts identified from literature search (N=39)
Duplicate records removed (N=2)
Studies excluded because they did not meet inclusion criteria (N=3)
Studies for retrieval of detailed information (N=34)
Studies with missing information on outcomes (N=6)
Studies excluded due to questionably small standard deviations (N=1)
Studies included in meta-analysis (N=27)



Study
Location
SMD (95% CI)
% Weight
Ren et al. 1989 Shandong -0.75 (-0.97, -0.52) 4.22
Chen et al. 1991 Shanxi -0.26 (-0.41, -0.10) 4.66
Guo et al. 1991 Hunan -0.44 (-0.80, -0.08) 3.26
Lin et al. 1991 Xinjiang -0.64 (-1.01, -0.28) 3.23
Sun et al. 1991 Guiyang -0.95 (-1.16, -0.75) 4.36
An et al. 1992 I Mongolia -0.57 (-0.83, -0.31) 3.98
Li et al. 1994 Sichuan -0.40 (-0.74, -0.06) 3.39
Xu et al. 1994 Shandong -0.93 (-1.35, -0.52) 2.91
Yang et al. 1994 Shandong -0.50 (-1.01, 0.02) 2.36
Li et al. 1995 Guizhou -0.55 (-0.70, -0.39) 4.68
Wang et al. 1996 Xinjiang -0.38 (-0.65, -0.10) 3.88
Yao et al. 1996 Liaoning -0.34 (-0.51, -0.17) 4.57
Zhao et al. 1996 Shanxi -0.54 (-0.76, -0.31) 4.22
Yao et al. 1997 Liaoning -0.43 (-0.61, -0.25) 4.49
Zhang et al. 1998 Xinjiang -0.17 (-0.55, 0.22) 3.09
Lu et al. 2000 Tianjin -0.62 (-0.98, -0.25) 3.20
Hong et al. 2001 Shandong -0.44 (-0.85, -0.03) 2.94
Wang et al. 2001 Shandong -0.50 (-1.01, 0.02) 2.36
Li et al. 2003 I Mongolia -0.10 (-0.25, 0.04) 4.71
Xiang et al. 2003 Jiangsu -0.64 (-0.82, -0.46) 4.52
Seraj et al. 2006 Tehran -0.89 (-1.28, -0.50) 3.08
Wang et al. 2006 Shanxi -0.27 (-0.47, -0.06) 4.34
Fan et al. 2007 Shaanxi -0.17 (-0.61, 0.27) 2.75
Wang et al. 2007 Shanxi -0.26 (-0.44, -0.07) 4.46
Li et al. 2009 Hunan -0.43 (-0.94, 0.08) 2.38
Li et al. 2010 Henan 0.07 (-0.08, 0.22) 4.69
Poureslami et al. 2011 Iran -0.41 (-0.77, -0.04) 3.25
Overall (I-squared = 80.0%, p = 0.000) -0.45 (-0.56, -0.34) 100.00
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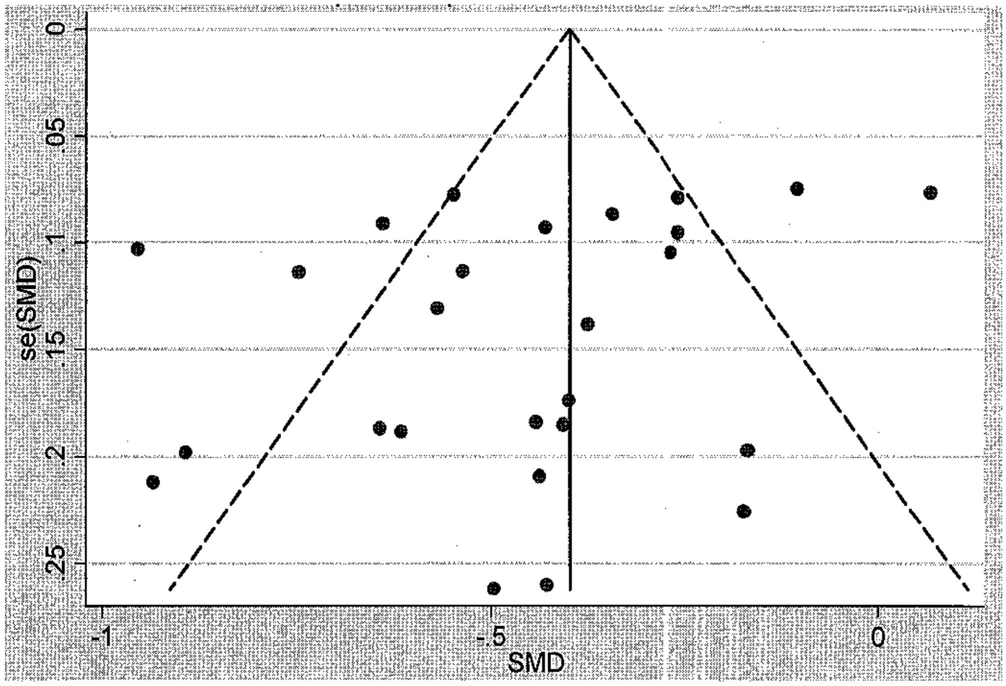
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News at HSPH (HARVARD School of Public Health)

Features

Impact of Fluoride on Neurological Development in Children

July 25, 2012

For years health experts have been unable to agree on whether fluoride in the drinking water may be toxic to the developing human brain. Extremely high levels of fluoride are known to cause neurotoxicity in adults, and negative impacts on memory and learning have been reported in rodent studies, but little is known about the substance's impact on children's neurodevelopment. In a meta-analysis, researchers from Harvard School of Public Health (HSPH) and China Medical University in Shenyang for the first time combined 27 studies and found strong indications that fluoride may adversely affect cognitive development in children. Based on the findings, the authors say that this risk should not be ignored, and that more research on fluoride's impact on the developing brain is warranted.

The study was published online in *Environmental Health Perspectives* on July 20, 2012.

The researchers conducted a systematic review of studies, almost all of which are from China where risks from fluoride are well-established. Fluoride is a naturally occurring substance in groundwater, and exposures to the chemical are increased in some parts of China. Virtually no human studies in this field have been conducted in the U.S., said lead author Anna Choi, research scientist in the Department of Environmental Health at HSPH.

Even though many of the studies on children in China differed in many ways or were incomplete, the authors consider the data compilation and joint analysis an important first step in evaluating the potential risk. "For the first time we have been able to do a comprehensive meta-analysis that has the potential for helping us plan better studies. We want to make sure that cognitive development is considered as a possible target for fluoride toxicity," Choi said.

Choi and senior author Philippe Grandjean, adjunct professor of environmental health at HSPH, and their colleagues collated the epidemiological studies of children exposed to fluoride from drinking water. The China National Knowledge Infrastructure database also was included to locate studies published in Chinese journals. They then analyzed possible associations with IQ measures in more than 8,000 children of school age; all but one study suggested that high fluoride content in water may negatively affect cognitive development.

The average loss in IQ was reported as a standardized weighted mean difference of 0.45, which would be approximately equivalent to seven IQ points for commonly used IQ scores with a standard deviation of 15.* Some studies suggested that even slightly increased fluoride exposure could be toxic to the brain. Thus, children in high-fluoride areas had significantly lower IQ scores than those who lived in low-fluoride areas. The children studied were up to 14 years of age, but the investigators speculate that any toxic effect on brain development may have happened earlier, and that the brain may not be fully capable of compensating for the toxicity.

"Fluoride seems to fit in with lead, mercury, and other poisons that cause chemical brain drain," Grandjean says. "The effect of each toxicant may seem small, but the combined damage on a population scale can be serious, especially because the brain power of the next generation is crucial to all of us."

** This sentence was updated on September 5, 2012.*

--Marge Dwyer

Cancer Epidemiology 36 (2012) e83–e88

Contents lists available at SciVerse ScienceDirect

Cancer Epidemiology

The International Journal of Cancer Epidemiology, Detection, and Prevention

journal homepage: www.cancerepidemiology.net

Fluoride in drinking water and osteosarcoma incidence rates in the continental United States among children and adolescents

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ARTICLE INFO

Article history:
Received 15 July 2011
Received in revised form 24 November 2011
Accepted 26 November 2011
Available online 19 December 2011

Keywords:
Osteosarcoma
Fluoridation
Fluorides
Preventive dentistry
Public health dentistry
Water

ABSTRACT

Introduction: It has been suggested that fluoride in drinking water may increase the risk of osteosarcoma in children and adolescents, although the evidence is inconclusive. We investigated the association between community water fluoridation (CWF) and osteosarcoma in childhood and adolescence in the continental U.S.

Methods: We used the cumulative osteosarcoma incidence rate data from the CDC Wonder database for 1999–2006, categorized by age group, sex and states. States were categorized as low (≤30%) or high (≥85%) according to the percentage of the population receiving CWF between 1992 and 2006. Confidence intervals for the incidence rates were calculated using the Gamma distribution and the incidence rates were compared between groups using Poisson regression models.

Results: We found no sex-specific statistical differences in the national incidence rates in the younger groups (5–9, 10–14), although 15–19 males were at higher risk to osteosarcoma than females in the same age group ($p < 0.001$). Sex and age group specific incidence rates were similar in both CWF state categories. The higher incidence rates among 15–19 year old males vs females was not associated with the state fluoridation status. We also compared sex and age specific osteosarcoma incidence rates cumulated from 1973 to 2007 from the SEER 9 Cancer Registries for single age groups from 5 to 19. There were no statistical differences between sexes for 5–14 year old children although incidence rates for single age groups for 15–19 year old males were significantly higher than for females.

Conclusion: Our ecological analysis suggests that the water fluoridation status in the continental U.S. has no influence on osteosarcoma incidence rates during childhood and adolescence.

1. Introduction

Osteosarcoma, a rare primary malignant bone tumour, is the sixth leading cancer in children under age 15 [1]. It is slightly more common in males, with an annual incidence rate in the United States of 5.4 cases per million for males under 20 years of age and 4.0 per million for females of the same age group [2]. The aetiology of osteosarcoma is largely unknown although it has been suggested that fluoride intake may be linked to an increased incidence of osteosarcoma in children and adolescents.

The epidemiological evidence on the relationship between fluoride exposure and osteosarcoma has been reviewed extensively by various scientific organizations [3–8]. Overall, animal and human studies are inconclusive and contradictory, showing a positive association in some (more cancers), negative in others (less cancers) while others showed no association [5]. Several studies have examined bone cancer incidence rates and time trends in fluoridated and non-fluoridated water in various geographical areas [9–15]. In general, these studies highlight the scarcity of information regarding the relationship between fluoride and osteosarcoma during childhood and adolescence, and some did not differentiate between other types of bone cancers and osteosarcoma [11,14,15].

A more recent population-based study has compared age-specific incidence rates of osteosarcoma between the Republic of Ireland, where approximately 70% of the population receives fluoridated water, and Northern Ireland, where water fluoridation is not implemented, to establish if differences in incidence between the two regions could be related to their different drinking water fluoridation policies. No significant differences

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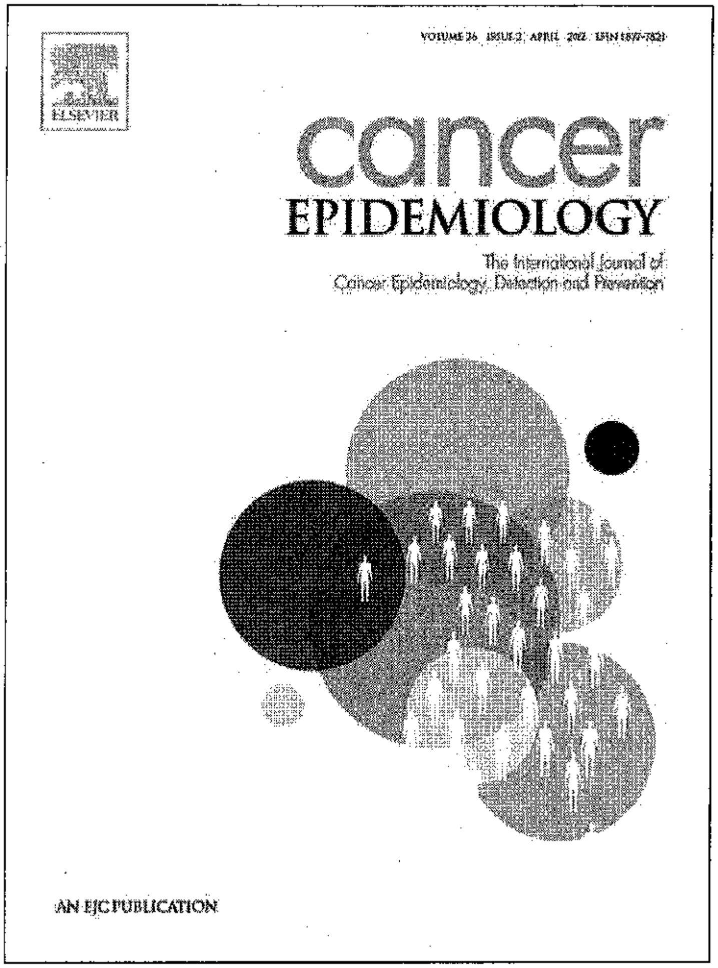
doi:10.1016/j.canep.2011.11.008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were observed in incidence rates between fluoridated and non-fluoridated regions [16]. However, this study did not provide any statistical analysis for specific age groups under 25 years.

Interest in the potential carcinogenicity of fluoride has resurfaced following the findings of a case–control study which explored the association between fluoride exposure in drinking water and osteosarcoma diagnosed before the age of 20 years [17]. This study reported that males with osteosarcoma were significantly more likely to have been exposed to fluoride in drinking water compared to matched controls, demonstrating a peak in the odds ratios from 6 to 8 years of age. The association was not apparent among females [17]. The authors of the paper as well as other researchers cautioned that the study's many methodological deficits limit the conclusions that can be drawn from it [7,18]. More recently, results of a second set of cases recruited from the same source have been published [19]. No significant difference in bone fluoride content between incident osteosarcoma cases and non-osteosarcoma bone tumour controls was found in this case–control study, albeit without specificity to those less than 20 years of age.

An important source of dietary fluoride in U.S. children comes from drinking water [20]. According to the U.S. Environmental Protection Agency analysis, the relative source contribution values for drinking water range from 40% to 60% among 1 to 14 year olds [20]. The percentage of the U.S. population which received fluoridated water from public water systems whether artificially adjusted by the community at levels of 0.7–1.2 mg/L, or naturally occurring at 0.7 mg/L or higher, increased from 48.8% in 1975 to 61.5% in 2006 [21]. Levels of 0.7–1.2 mg/L are considered optimal for dental benefits, depending on the average maximum daily air temperature of the area [22]. Of the population receiving fluoridated water, in 1992, 93% received water with artificially adjusted fluoride levels, the rest had naturally fluoridated water at varying concentrations [7]. Approximately 4.7% of those receiving fluoridated water were served by naturally fluoridated water at concentrations lower or equal to 1.2 mg/L, 1% had natural concentrations between 1.3 and 1.9 mg/L and another 1% had between 2.0 and 3.9 mg/L [7]. Barely 0.14% had natural concentrations equal to or exceeding 4.0 mg/L [7]. In addition to the aforementioned populations that receive fluoridated water from public systems, an estimated 14% of the U.S. population obtained their domestic water from private wells in 2005 [23]. Little information is available on the fluoride content of private wells but the variability is expected to be high and dependant on the region of the U.S. [7]. Other important contributors to total daily dietary fluoride intakes are commercial beverages prepared with water from fluoridated water systems, which are available in both fluoridated and non-fluoridated areas, and foods, to varying degrees [23]. Toothpaste ingestion also contributes to total fluoride intake, especially in children under 4 years old who do not have the ability to spit it out properly [24].

Conducting observational epidemiologic studies (with a cohort or case–control design) to test the hypothesis of an association between fluoride in drinking water and osteosarcoma implies major challenges, such as very few cases and the correspondingly wide confidence intervals of the relative risk estimates, difficulty in characterizing biologic doses because of the ubiquitous presence of fluoride, assessment of exposure, and obtaining bone samples to assess exposure. Therefore, ecological studies like the present one can add useful information to the existing body of knowledge.

While more well-designed observational studies with adequate exposure assessment will eventually be developed, the availability of administrative data permits us to realize this ecological study. The intention of this paper is to explore the hypotheses: that the percentage of the population on public water systems receiving fluoridated water correlates with sex, age and state-specific rates of osteosarcoma incidence in continental U.S. children and adolescents; that young males are more at risk to osteosarcoma than females.

2. Materials and methods

Population-based cumulative incidence data of osteosarcoma was obtained for 1999–2006 from the U.S. Cancer Statistics available through the Centers for Disease Control and Prevention (CDC) Wonder public health information system [25]. This on-line programme provides incidence data for the vast majority of the U.S. population with the exception of the District of Columbia, 1999–2006; Maryland 2000–2001, 2004–2005; Mississippi 1999–2002; South Dakota 1999–2000; Tennessee 1999–2003; Virginia 1999–2002; and Wisconsin 1999–2006. Sex-specific data were obtained for three age groups: 5–9, 10–14, and 15–19 year olds. Data for 0–4 year old children were unavailable, being very low and suppressed from the CDC Wonder database for confidentiality reasons. We expressly excluded Hawaii data from our analysis because of potential bias from environmental, geological, and hereditary factors particular to this state.

We first identified osteosarcoma incidence rates occurring in the continental U.S. between 1999 and 2006, categorized by age group and sex. The osteosarcoma incidence rate is the number of new osteosarcoma cancers occurring in a specified population during a year, usually expressed as the number of cancers per 100,000 population at risk. In a second step, we dichotomized the states according to the percentage of the population receiving community water fluoridation (CWF): states in which 30% or less of the population received fluoridated water between 1992 and 2006 consistently (low CWF states) and those in which 85% or more of the population met the same criteria (high CWF states). These cut-off points provided the greatest spread between the two categories while maximizing the available populational data. The age and sex specific incidence of osteosarcoma was recorded in each CFW category. Data regarding estimated percentage of the population served by community water systems who received naturally occurring or adjusted fluoridated water by state in 1992, 2000, 2002, 2004, 2005 and 2006 were obtained from the National Oral Health Surveillance System, developed with the collaboration of the CDC and the Association of State and Territorial Dental Directors [26,27]. Montana was included among the four states in which 30% or less of the population received fluoridated water between 1992 and 2006 (California, New Jersey, Oregon and Montana) because the fluoride level remained under 30% from 1992 to 2005 but briefly increased from 29.4% to 31.3% between 2005 and 2006. The states corresponding to each of the CWF categories are indicated under Table 1.

Confidence intervals for the US Cancer Statistics' incidence rates were calculated using the Gamma distribution method. The incidence rates were then compared between groups using Poisson regression models taking into account age, sex and community water fluoridation status, as the case may be. All data analyses were performed using SAS statistical software. All 5% or lower p-values are considered to be statistically significant.

As a complementary analysis, we also compared sex and age specific incidence rates for ages 5–19 extracted from the surveillance, epidemiology and end results (SEER) public-access database of the National Cancer Institute [28] to test the hypothesis that young males are more at risk to osteosarcoma than females. Unlike the nationwide CDC Wonder database, SEER 9 Registries Database represents only about 9.5% of the U.S. population, but provides data compiled from cases diagnosed from 1973 through 2007 as compared with 1999 through 2006 for CDC Wonder [29]. SEER*Stat software (version 6.6.2) was used to calculate the sex-specific incidence rates for each single age category [30]. Osteosarcoma cases were selected according to the

Table 1
Child and adolescent incidence of osteosarcoma in the continental United States and by state community water fluoridation (CWF) status,[a] cases diagnosed from 1999 to 2006, CDC Wonder database.

Sex	Age	Rate/10^6	(95% C.I.)	p-Value[b]	Risk ratio (95% C.I.)
Overall incidence in the continental United States					
Females	5–9	3.0	(2.6–3.4)		Ref.
Males	5–9	2.9	(2.5–3.3)	0.59 (NS)	0.95 (0.79–1.14)
Females	10–14	8.5	(7.8–9.2)		Ref.
Males	10–14	7.8	(7.2–8.5)	0.16 (NS)	0.92 (0.82–1.03)
Females	15–19	6.2	(5.6–6.8)		Ref.
Males	15–19	11.2	(10.5–12.0)	<0.001	1.81(1.62–2.03)
States with low CWF status (<30% population receive fluoridated water)[c]					
Females	5–9	2.9	(2.1–4.0)		Ref.
Males	5–9	3.1	(2.2–4.1)	0.33 (NS)	1.05 (0.69–1.60)
Females	10–14	9.9	(8.3–11.7)		Ref.
Males	10–14	8.2	(6.8–9.8)	0.13 (NS)	0.83 (0.65–1.06)
Females	15–19	5.9	(4.6–7.3)		Ref.
Males	15–19	11.5	(9.8–13.4)	<0.001	1.95 (1.49–2.56)
States with high CWF status (>85% population receive fluoridated water)[d]					
Females	5–9	3.1	(2.4–4.0)		Ref.
Males	5–9	3.0	(2.3–3.9)	0.93 (NS)	0.99 (0.69–1.40)
Females	10–14	8.4	(7.2–9.7)		Ref.
Males	10–14	7.8	(6.7–9.1)	0.50 (NS)	0.93 (0.75–1.15)
Females	15–19	6.3	(5.3–7.5)		Ref.
Males	15–19	11.6	(10.2–13.1)	<0.001	1.83 (1.48–2.26)

[a] State community water fluoridation (CWF) status from 1992–2006, National Oral Health Surveillance System, CDC.
[b] p-Value for the comparison between sexes from Poisson regression within the same age group and in the same geographic category.
[c] California, Oregon, New Jersey, and Montana.
[d] Connecticut, Georgia, Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, North Dakota, Ohio, Rhode Island, South Carolina, South Dakota, and Tennessee.

International Classification of Disease for Oncology (ICD-O-3) [31]. Seven of the nine SEER 9 registries were included in our analyses: Atlanta, Connecticut, Detroit, Iowa, New Mexico, San Francisco-Oakland, and Seattle-Puget Sound. The Seattle-Puget Sound and Atlanta registries contributed data beginning in 1974 and 1975, respectively. For regions represented in these SEER 9 registries, major cities started receiving fluoridated water during the 1960–1970's period [32] although precise quantitative measurement of fluoride in drinking water of all counties and municipalities included in these registries is beyond the scope of this study. The Hawaii and Utah registries were excluded from our analysis because these two states had the lowest fluoridation status in the United States with the exception of Nevada, which is not represented in the SEER 9 registries. In Hawaii, the percentage of the population having access to water fluoridation has been consistently low over the past 40 years, reaching approximately 16% at its highest level around 1985, dropping to 8% by 2006 [21,26,33,34]. In Utah, only 3% or less of citizens have been serviced by community water systems with optimal levels of fluoride, until Utah started to add fluoride in drinking water in 2002 and this percentage reached 54.3% in 2006 [21,26,33].

3. Results

Table 1 shows the 1999–2006 osteosarcoma incidence rates between sexes by age group in the continental United States. The results indicate that there are no statistical differences ($p > 0.05$) between 5–9 year old males and females (2.9 cases/million vs 3.0 cases/million, respectively) as well as among 10–14 year olds (7.8 cases/million (males) vs 8.5 cases/million (females)). By contrast, the incidence rate of osteosarcoma among 15–19 year olds is statistically ($p < 0.0001$) higher in males than in females (11.2 cases/million vs 6.2 cases/million, respectively), which is reflected by a risk ratio of 1.81, 95% C.I. 1.62–2.03.

Also shown in Table 1 are the incidence rates according to the state community water fluoridation status (<30% vs >85% of the state population receiving water fluoridation). Comparisons of sex-specific incidence rates among 5–9 and 10–14 year olds in either state CWF category do not reveal any statistical difference, although once again 15–19 year old males had a significantly higher osteosarcoma incidence rate than females in both high and low CWF state categories. In Table 2, incidence rates between CWF state categories do not reveal any statistical difference for all age groups of the same sex, including 15–19 year olds.

As demonstrated in Table 3 and Fig. 1, the 1973–2007 osteosarcoma incidence rates do not show any statistically significant difference between sexes from ages 5 to 14. From age 15 to 19, rates among males are statistically higher than for females. By contrast, higher incidence rates can be observed among 11 to 13 year old females, although these are not statistically different from males of the same age range.

4. Discussion

From data extracted from the CDC Wonder database, our results show relatively similar osteosarcoma incidence rates between males and females in the 5–9 and 10–14 year old groups in the continental United States. Likewise, we found similar incidence rates between males and females in the 5–9 and 10–14 year old groups in either high CWF or low CWF states. In fact, female incidence rates were slightly higher in both groups but not significantly. Statistically higher incidence rates found among

Table 2
Comparison between states with high (>85%) and low (<30%) community water fluoridation (CWF) status,[a] CDC Wonder database.

Sex	Age	CWF status		p-Value[b]	Risk ratio (95% C.I.)
		High	Low		
Females	5–9	3.1	2.9	0.81 (NS)	1.05 (0.71–1.56)
Males	5–9	3.0	3.1	0.95 (NS)	0.99 (0.67–1.45)
Females	10–14	8.4	9.9	0.15 (NS)	0.85 (0.68–1.06)
Males	10–14	7.8	8.2	0.70 (NS)	0.96 (0.76–1.21)
Females	15–19	6.3	5.9	0.60 (NS)	1.08 (0.82–1.43)
Males	15–19	11.6	11.5	0.93 (NS)	1.01 (0.83–1.23)

[a] State community water fluoridation (CWF) status from 1992 to 2006, National Oral Health Surveillance System, CDC.
[b] p-Value for the comparison between the two State CWF categories from Poisson regression within the same age group and the same sex.

Table 3
Child and adolescent incidence of osteosarcoma in the continental United States by individual age, cases diagnosed from 1973 to 2007, SEER 9 Database.

Age (year)	Male		Female		p-Value	Risk ratio (95% C.I.)
	Rate/10^6	Count	Rate/10^6	Count		
5	1.14	6	0.79	4	0.58 (NS)	1.44 (0.41–5.09)
6	1.13	6	0.60	3	0.36 (NS)	1.91 (0.48–7.63)
7	1.89	10	1.97	10	0.92 (NS)	0.96 (0.40–2.30)
8	2.11	11	3.20	16	0.29 (NS)	0.66 (0.31–1.42)
9	4.60	25	4.63	24	0.98 (NS)	0.99 (0.57–1.74)
10	5.98	33	4.38	23	0.25 (NS)	1.37 (0.80–2.33)
11	4.21	23	5.55	29	0.32 (NS)	0.76 (0.44–1.31)
12	7.49	41	8.39	44	0.60 (NS)	0.89 (0.58–1.37)
13	9.07	50	9.47	50	0.83 (NS)	0.96 (0.65–1.42)
14	10.25	57	8.83	47	0.45 (NS)	1.16 (0.79–1.71)
15	12.44	70	8.56	46	0.05	1.45 (1.00–2.11)
16	12.22	69	7.78	42	0.02	1.57 (1.07–2.31)
17	9.37	53	4.97	27	0.007	1.88 (1.19–3.00)
18	8.84	48	3.66	19	0.001	2.42 (1.42–4.11)
19	5.83	32	3.20	17	0.046	1.82 (1.01–3.28)

Hawaii and Utah registries were excluded.

15–19 year old males were not associated with the state fluoridation status. Comparing incidence rates between high CWF and low CWF states of children of the same sex and of all age groups also yielded markedly similar results, with non-statistically significant differences. We performed a sensitivity analysis by using various cut-off points to dichotomize state according to CWF status (e.g.: 25% vs 75%) but in all cases, the results we obtained were similar (unpublished data).

Similarly, our analysis from SEER 9 data shows similar incidence rates between males and females between 5 and 14 years of age. We observed no evidence of "peaking" in male incidence rates or risk ratio between ages 5 and 8 as was reported in Bassin's 2006 study [17]. By contrast, incidence rates among 15–19 year old males were higher than for females, but as was discussed before, this cannot be attributed to CWF status.

One area of concern is whether unequal fluoride intakes between males and females could act as a possible confounder. It could be argued that a lower intake of water by males could act to decrease their osteosarcoma incidence rate if there is indeed a causal relationship between the concentration of fluoride in water and osteosarcoma. However, two nationwide studies have shown that males generally had higher mean water intakes than females during childhood and adolescence [35,36]. This difference was statistically significant for all age groups after adjusting for factors such as race, region, season, body weight, urban residence and age [35]. Other published data show that girls in age groups ranging from 0 to 11 had consistently lower mean fluoride intake from all sources than boys when expressed in either mg/day or mg/kg/day [37]. These data are of significance to the interpretation of our results, which do not support the hypothesis that males in any childhood/adolescent age group are at higher risk of osteosarcoma from fluoride in drinking water despite their apparently greater water intake.

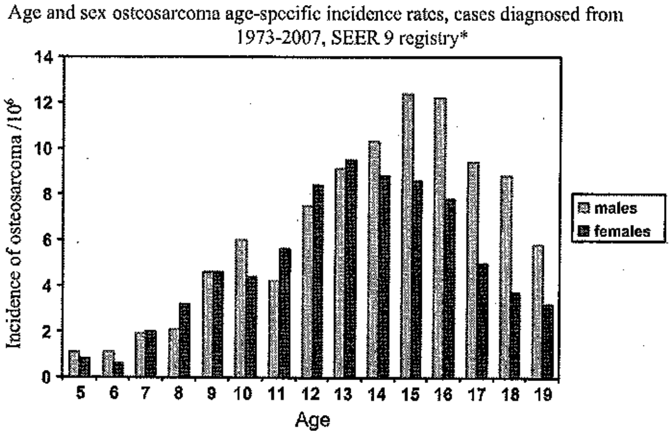

Fig. 1. Age and sex osteosarcoma age-specific incidence rates, cases diagnosed from 1973 to 2007, SEER 9 registry*. *Utah and Hawaii registries were excluded.

We also estimated the potential contribution from systems delivering tap water with naturally occurring levels of fluoride from 1992 CDC data on population sizes receiving naturally fluoridated water by state [38] and corresponding 1990 U.S. Census population estimates by state [39]. Approximately 1.6% of the population in low CFW states received naturally fluoridated water with fluoride levels 0.7–4.0 mg/L as compared to 2.7% of the population in high CFW states. According to our calculations, percentages of the population receiving naturally fluoridated water were higher in high CWF states in all concentration ranges reported (≤1.2 mg/L, 1.3–1.9 mg/L, 2.0–3.9 mg/L, ≥4.0 mg/L). Assuming that these percentages have remained relatively stable from 1992 to 2006, natural fluoride levels from public water systems would be an unlikely source of bias in the examination of the association between fluoride and osteosarcoma between CWF state categories (Table 2) because exposure is more predominant in the (exposed) high CWF category, at all reported concentration ranges. Also, since water consumption according to sex would most likely be unaffected by access to water systems distributing naturally occurring or adjusted fluoride levels, we would also not expect our results to be affected when comparing incidence rates between males and females of different age groups within the same CWF category, between state categories (Tables 1 and 2), or in continental U.S. (Table 3).

There are some typical limitations to our ecological study. Firstly, problems inherent with the "ecological fallacy" are well known. Such studies are based on aggregate population data rather than individual data and lack the ability to control for the effects of confounding factors at the individual level while such factors could explain the observed results. For instance, we did not include the contribution of private wells and bottled water, and their individual fluoride concentrations in our analyses. Also, population estimates of fluoride in drinking water do not reflect actual total consumption by subjects. For example, the use of some home water purifiers can lower fluoride intake. Also, beverages produced in fluoridated and non-fluoridated areas use local tapwater and are distributed throughout the country. Our study did not take into account their contribution to fluoride intake. Nevertheless, despite the limitations of ecological studies, they can provide useful explanatory information when the outcome of interest is rare and that routinely collected data are available from a very broad database.

5. Conclusions

We compared age and sex-adjusted osteosarcoma incidence data among youth aged 5–19 years on a nationwide basis and according to the state water fluoridation level. The results of our study provide no evidence that young males are at greater risk than females of the same age group to osteosarcoma from fluoride in drinking water. While causality cannot be inferred from this ecological analysis, our findings are consistent with the hypothesis that community water fluoridation has no influence on the development of osteosarcoma for either sex or age group during childhood and adolescence.

Conflict of interest statement

The authors declare that they do not have any potential conflict of interest to report.

Acknowledgements

We would like to thank Dr. Chantal Galarneau for her helpful advice and discussions, Mr. Denis Hamel for his assistance in the statistical analyses and Mr. Bruce C. Bezeau who contributed to the revision of the manuscript.

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