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Review

Early fluoride intake and Molar Incisor Hypomineralisation (MIH) defects: A systematic review and dose-response meta-analysis

Federica Veneri[1,2], Tommaso Filippini[3,4], Marta Cecchini[3], Marco Vinceti[3,5], Ugo Consolo[1], Luigi Generali[1]

[1]Unit of Dentistry & Oral-Maxillo-Facial Surgery, Department of Surgery, Medicine, Dentistry and Morphological Sciences with Transplant Surgery, Oncology and Regenerative Medicine Relevance (CHIMOMO), University of Modena and Reggio Emilia, Modena, Italy; [2]PhD Program in Clinical and Experimental Medicine, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; [3]Environmental, Genetic and Nutritional Epidemiology Research Center (CREAGEN), Section of Public Health, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; [4]School of Public Health, University of California Berkeley, Berkeley, USA; [5]Department of Epidemiology, Boston University School of Public Health, Boston, USA

Abstract. *Background:* Excessive intake of fluoride has been implicated in the complex multifactorial etiology of hypomineralisation (MIH) defects. *Objective:* To study the possible effect of early exposure to fluoride on the risk of molar incisor hypomineralisation, also through a dose-response approach. *Methods:* Observational and clinical studies investigating the relation between fluoride exposure from any source or evaluating exposure biomarkers and MIH defects. PubMed MEDLINE, Embase and Web of Science databases were consulted up to December 1, 2023, using terms related to "fluoride", "enamel defects" and "demarcated opacities". We performed a meta-analysis comparing the highest versus lowest fluoride exposure using a random-effects model, and we quantitively assessed this relation using piece-wise linear meta-regression. *Results:* Thirteen studies were included in the meta-analysis, 12 of which were eligible for the dose-response analysis, all regarding exposure from fluoride in drinking water. Three of them specifically addressed MIH, while the remaining concerned "demarcated opacities", yet with features attributable to MIH. Comparing the highest versus lowest water fluoride exposure categories, virtually no evidence of a fluoride effect was identified, with an overall odds ratio of 0.93 [95% confidence interval 0.60; 1.45]. The dose-response meta-regression showed a decreasing risk for MIH defects exposure up to 1 mg/L, whereas an increase in risk emerged at higher exposure levels. *Conclusions:* This meta-analysis suggests that early systemic exposure to fluoride may affect the occurrence of MIH defects differently depending on fluoride concentration. However, these results need to be evaluated with caution due to potential methodological limitations of the studies included. (www.actabiomedica.it)

Key words: demarcated opacities, fluoride, hypomineralisation, MIH, molar-incisor, water fluoridation

Introduction

Molar Incisor Hypomineralisation (MIH) was defined in 2001 to describe a specific pattern of hypomineralisation of systemic origin, affecting at least one permanent first molar and frequently associated with affected incisors (1). MIH is addressed with a variety of terms that have been widely used in the recent past, including demarcated opacities, developmental defect of enamel (DDE), molar cheese, mottled enamel

and non-fluorotic opacities. In order to unify clinical practice and research, specific diagnostic criteria have been defined by the European Academy of Paediatric Dentistry (EAPD) (2,3). MIH is a qualitative complex developmental defect of the enamel, which can manifest as demarcated white, yellow or brown opacities caused by changes in enamel mineral and protein composition. Because of the enamel anomalies, the clinical management of MIH patients is challenging due to possible esthetic concerns, hypersensitivity, difficulties in obtaining anesthesia during dental treatment as a consequence of chronic inflammation, posteruptive breakdown, rapid caries progression and the need for recurrent reinterventions (2). MIH is recognized as a worldwide clinical concern and a recent systematic review estimated a global prevalence of 13.5% (95% CI 12.0–15.1), with 36.3% of moderate to severe cases (4).

The etiology of MIH is still unclear, but similar to other common oral conditions, such as dental caries and malocclusion, a complex combination of factors has been considered (5,6). A multifactorial approach involving genetics, iatrogenic causes, perinatal and pregnancy-related conditions, diseases, and exposure to environmental contaminants have all been investigated as possible causes of MIH defects (1,7). Pathogenesis is likely to be related to ameloblast damage during the delicate early maturation phase of the first permanent molars and incisors, usually beginning right before or shortly after birth and completing at 4-5 years of age (7). Within the complex etiology of MIH, the trace element fluoride is unlikely to be one of the main etiologic factors (7). However, given that early systemic exposure can lead to other enamel defects and to other developmental health issues in children, such as cognitive and behavioral impairment, a possible role of fluoride in the etiology of MIH defects has been investigated (8–12).

Fluoride can affect amelogenesis and its relation with the development of other defects of the enamel, such as dental fluorosis (DF), is well documented (13,14). Some authors have hypothesized that environmental fluoride can somewhat influence MIH development and presentation, whether by lowering the threshold at which MIH occurs or by strengthening the mineral component of the enamel, thus counteracting MIH (8,9,13,15). Despite the similar prevalence of dental fluorosis (DF) and MIH in children exposed to different level of water fluoride reported by some studies, MIH lesions were found to be more severe in children exposed to water at high fluoride contents (9,13). A higher prevalence of non-fluoride developmental defects has also been reported in areas with lower fluoride content in water (11).

Despite these indications of a possible involvement of fluoride in MIH etiology, such relation is still substantially unclear and to date, to the best of our knowledge, it has not been specifically addressed by any systematic review and meta-analysis. Therefore, the aim of this systematic review and meta-analysis is to assess whether fluoride may be a protective or a risk factor for MIH defects, and to characterize the dose-response relation between fluoride exposure and MIH risk.

Methods

This systematic review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 statement (16). The protocol was defined by the authors and registered at the National Institute for Health Research PROSPERO, International Prospective Register of Systematic Review (registration no. CRD42022321897).

Search strategy and study identification

We performed an online literature search in PubMed/MEDLINE, Web of Science and Embase databases, from inception until December 1, 2023 and with no language or date restrictions. According to the PECOS statement (Population, Exposure, Comparator, Outcomes, and Study design), the research question was "In children, what is the effect of different levels of fluoride exposure on the risk of molar incisor hypomineralisation based on clinical studies?". The research question led us to select any observational studies and clinical trials investigating the relation between fluoride exposure from any source (e.g. water, dietary and supplemental intake, topical dental products) or evaluating a biomarker of exposure (e.g. urinary,

Table 1. Database detailed search strategy.

Database	Search string
Pubmed	(fluoride[MH] OR fluoride[tiab]) AND ("Dental Enamel Hypoplasia"[Mesh] OR "molar incisor hypomineralization"[tiab] OR "demarcated opacities"[tiab] OR "cheese molars"[tiab]) AND humans[MH]
Embase	('fluoride'/exp OR 'fluoride') AND ('molar incisor hypomineralization' OR 'demarcated opacities' OR 'cheese molars' OR 'developmental opacities') AND 'human'/exp
Web of Science	TS=(fluoride) AND (TS=(molar incisor hypomineralization) OR TS=(demarcated opacities) OR TS=(cheese molars) OR TS=(developmental opacities) OR TS=(idiopathic opacities))

skeletal, hair fluoride) and MIH. Only research articles were included, while conference proceedings, letters to the editor, commentaries, case reports, reviews and meta-analysis were not considered. The literature search was performed with combinations of terms related to "fluoride" as exposure and to "molar incisor hypomineralisation" (e.g. "demarcated opacities", "developmental opacities") as outcomes, by using related MeSH terms, topic terms and exploded terms on the three databases, respectively. The detailed search strategy is reported in Table 1. Backward citation chasing was manually conducted by screening the references of the studies included, in order to identify possible additional eligible articles.

We included only studies reporting (i) type and dose/concentration of fluoride exposure (dose, mean/median level or category boundaries); (ii) outcome assessment according to validated diagnostic criteria including (but not limited to) European Academy of Pediatric Dentistry MIH criteria, or the developmental defects of enamel index (DDE index) (3,17); (iii) estimates of the outcome in relation to fluoride exposure, i.e. prevalence, mean difference, relative risk estimates such as odds ratio (OR) along with the 95% confidence interval (CI) or enough data allowing their calculation. Papers were excluded that concerned (i) enamel defects affecting primary dentition; (ii) non-MIH enamel defects referred to with terms such as: diffuse opacities, hypoplasia (pits, grooves/linear, areas), amelogenesis imperfecta, white spots, and non-MIH hypomineralisation defects. We also excluded articles based on highly specific populations, including preterm children, institutionalized populations, and specific occupations. If multiple studies addressed an overlapping population, only the most complete report was included, i.e. reporting on the larger population or with detailed information about fluoride exposure. Two authors (FV and MC) independently performed the screening of titles, abstracts and full texts for inclusion in the review. Possible disagreements were resolved through consensus-based discussion with a third author (TF).

Data extraction

The data extraction from the included studies was conducted by one author (FV) and double checked by another author (MC). From each eligible study, we extracted location and year, study design, total study population, population age and sex, type of main exposure and possible additional exposures, type and method of outcome assessment, doses of fluoride exposure, and risk estimates with 95% confidence intervals (CI). Moreover, MIH severity was registered whenever studies reported it. Whenever present, we also recorded details of confounding factors or possible adjustments.

Risk of bias assessment

Risk of bias (RoB) assessment of the included studies was performed using the Risk of Bias in Non-Randomized Studies of Exposure (ROBINS-E) tool (18). Two authors [FV and MC] conducted the evaluation. Any discrepancy was resolved by a third author [TF]. Details and criteria for RoB assessment are reported in Table 2. Studies were considered "low RoB" if all domains were rated as low risk; they were considered "moderate" or "high" RoB if one or more domains were at moderate or high RoB, respectively.

Data analysis

We performed a meta-analysis comparing the highest versus the lowest fluoride exposure using

Table 2. Criteria adopted for Risk of Bias assessment using the Risk of Bias for Non-randomized Studies of Exposures (ROBINS-E) tool.

Domains	Criteria
Bias due to confounding	Studies are considered at **low risk** of bias if they consider age and socioeconomic level in the adjustment factors. Studies are considered at **moderate risk** of bias if they consider only one of the two. Studies are considered at **high risk** of bias if adjusting factors are not reported. *Age matching is accounted for as "adjustment" only if a range of 5 years is considered or if similar mean age and standard deviation among groups are reported. **Management of confounders only reported in plain text (without displaying supporting data) was not considered sufficient to be accounted for as "adjustment"
Bias in selecting participants in the study	Studies are considered at **low risk** of bias if selection of eligible participants is independent of fluoride exposure. Studies are considered at **moderate risk** of bias if participant selection is from fluoride contaminated areas. Studies are considered at **high risk** of bias if the criteria of participant selection is not specified.
Bias in exposure classification	Studies are considered at **low risk** of bias if fluoride exposure is assessed through urine or serum analysis and/or personal intake. Studies are considered at **moderate risk** of bias if they analyze fluoride concentration in drinking water. Studies are considered at **high risk** of bias if the assessment of fluoride exposure is not specified.
Bias in departure from intended exposure	Studies are considered at **low risk** of bias if exposure dose is reported; at **high risk** if exposure dose is not reported.
Bias due to missing data	Studies are considered at **low risk** of bias if less than 10% of participants are excluded due to missing data; at **moderate risk** of bias if less than 20% of participants are excluded due to missing data. Studies with a higher proportion (≥20%) are considered at **high risk** of bias.
Bias in outcome measurement	Studies are considered at **low risk** of bias if outcome assessment is based on MIH-specific validated criteria. Studies are considered at **moderate risk** of bias if outcome assessment refers to "demarcated opacities" according to other validated diagnostic classifications (e.g. DDE); studies are considered at **high risk** of bias if outcome assessment is not specified.
Bias in selection of reported results	Studies are considered at **low risk** of bias if they report prior publication of the protocol or data are made available in a public and accessible repository. Studies are considered at **moderate risk** of bias if they present outcome measures and analyses consistent with an a priori plan outlined in the manuscript. Studies are considered at **high risk** of bias if no protocol is available and no a priori plan is outlined.
Overall risk of bias	If at least one domain was found at **high risk** of bias, the overall risk was considered high. If at least one domain was found at **moderate risk** of bias, the overall risk was considered moderate. If all domains were at **low risk** of bias, the overall risk was considered low.

the restricted maximum likelihood random-effects model and presented data through forest plots. We also quantitively assessed the relation between exposure and MIH risk using a meta-regression approach according to increasing levels of fluoride exposure. When unavailable, we extracted the number of MIH cases and non-cases for these two exposure categories and calculated the OR with a 95% CI. When a mean or median dose was not reported, we either calculated the central value of the dose range or considered a dose value 20% higher or lower than the reported boundary, based on the indications from other studies with complete data (19). During the meta-regression analysis, we stratified the studies according to water fluoride exposure, namely above and below 1.0 mg/L, which was accounted for as the optimal water fluoride level by the World Health Organization (20).

We assessed the publication bias through visual inspection of symmetry in funnel plots and by performing Egger's test. Finally, we assessed heterogeneity using the I^2 statistics. We used Stata software with 'meta' routine (v17.0, Stata Corp., College Station, TX, 2021) for all data analyses.

Results

Study selection

A total of 327 potentially relevant records was retrieved from the database search. After duplicate removal (n=108), we discarded 177 records through the screening of titles and abstracts, while the remaining 42 articles underwent full text evaluation. Thirty-two papers did not meet the eligibility criteria and were therefore excluded for the following reasons: 14 studies reported clinical assessment criteria not consistent with MIH diagnosis, 11 studies did not allow for a correlation between fluoride exposure and MIH, 3 studies did not report exposure doses, 3 full texts were not available, and 1 study addressed a duplicate cohort. We also added 3 articles retrieved after manually searching the reference lists of the included studies. Overall, 13 studies were eventually included for the systematic review and meta-analysis, 12 of which were also eligible for dose-response meta-analysis. The PRISMA flowchart reporting the details of the study selection process is shown in Figure 1.

Study characteristics

The main characteristics of the included studies are shown in Table 3. A total of 8325 participants

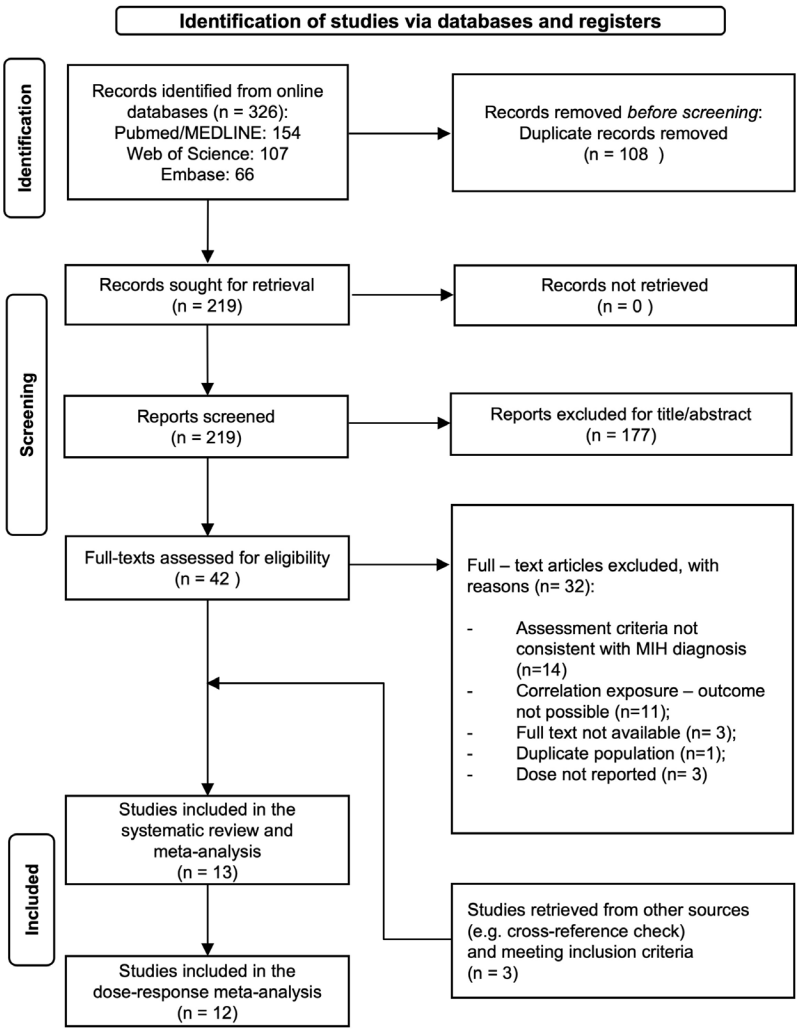

Figure 1. PRISMA Flow-chart of the study selection process.

Table 3. Characteristics of the included studies.

Author and year	Study design	Country and year	Number of participants	Age (years)	Exposure assessment	Unit	Fluoride dose	Other fluorides	Outcome	Outcome assessment	Fluoride in water assessment method	Main findings
Angelillo 1990 (22)	cross-sectional	Italy; 1990	643	11 to 13	Water fluoride	mg/L	0.30; 1.00; 4.00	no	Demarcated opacities	DDE	NR	Enamel defects were significantly higher in higher fluoride areas. Demarcated opacities were the most common defects
Balmer 2005 (13)	cross-sectional	England Australia	50 (M/F=16/34)	11.3±2.41 11.9±2.31	Water fluoride	mg/L	0.08; 1.06	no	MIH	mDDE	NR	Higher prevalence of enamel defects in fluoridated than in non-fluoridated areas, but similar prevalence of MIH
Balmer 2015 (8)	cross-sectional	England; 2008-2009	3233	12	Water fluoride	mg/L	0.00; 1.00	NR	MIH	mDDE	NR	Higher prevalence of MIH in non-fluoridated than in fluoridated areas. Living in a fluoridated area increased the chance of demarcated defects in incisors and molars of MIH children
Cutress 1985 (27)	cross-sectional	New Zealand	176	9	Water fluoride	mg/L	0.24; 1.00	No/ occasional/ regular tablets assumption	Demarcated opacities	DDE	NR	Prevalence of demarcated opacities was similar in non-fluoridated area and fluoridated areas
De Liefde 1985 (26)	cross-sectional	New Zealand; 1982	428	9±0.7	Water fluoride	mg/L	0.20; 1.00	no	Demarcated opacities	DDE	NR	Slightly higher prevalence of demarcated opacities in fluoridated areas

Ekanayake 2003 (23)	cross-sectional	Sri Lanka	486 (M/F=236/250)	14	Water fluoride	mg/L	0.24; 0.40; 0.60; 0.84	Fluoride toothpaste (75% cases)	Demarcated opacities	mDDE	NR	The overall prevalence of demarcated opacities was low and almost similar across fluoride groups
Fernandes 2021 (9)	cross-sectional	Brazil; 2019	610 (M/F=329/281)	6 to 12	Water fluoride	mg/L	0.56; 0.84	toothpaste 1450 ppm	MIH	EAPD criteria	Ion selective electrode	Exposure to a higher fluoride concentration in the drinking water did not increase the likelihood of developing MIH. The severity of MIH was associated with dental fluorosis in areas with moderate to high fluoride levels in the drinking water
Grahnén 1974 (28)	cross-sectional	Sweden; 1966	515	6 to 9	Water fluoride	mg/L	0.08; 0.40; 0.72; 0.75; 1.00; 1.10; 1.65	NR	Demarcated opacities	Zimmermann; Grahnen and Selander	NR	Prevalence of non-fluoride opacities was similar among groups with different fluoride levels in drinking water, showing a different trend from fluorosis
Hiller 1998 (21)	controlled clinical trial	Germany; 1992	316	8.5 to 10	Water fluoride mg/L <0.2 (control) no				Demarcated opacities	mDDE	NR	Higher prevalence of demarcated opacities in children undergoing fluoride supplementation programs than in the control group
					Fluoride tablets	mg/day	0.25; 0.50; 0.75 (from birth to 5 yrs old) 0.25; 0.50; 0.75 (from 7 months old to 5 yrs old) 0.25 (from birth to 3 yrs old)	Water fluoride <0.1 mg/l				

Table 3 (*Continued*)

Author and year	Study design	Country and year	Number of participants	Age (years)	Exposure assessment	Unit	Fluoride dose	Other fluorides	Outcome	Outcome assessment	Fluoride in water assessment method	Main findings
Machiulskiene 2009 (11)	cross-sectional	Lithuania; 2004	300 (M/F=158/142)	13.1±0.6 13.2±0.7	Water fluoride	mg/L	0.30; 1.10	NR	Demarcated opacities	Fejerskov et al.	NR	Higher prevalence of non-fluoride opacities in the low-level fluoride group
Milsom 1990 (24)	cross-sectional	England	222 (M/F=112/110)	8	Water fluoride	mg/L	0.16; 1.00	no	Demarcated opacities	DDE	NR	Similar prevalence of demarcated opacities in children living in fluoridated and non-fluoridated areas
Nunn 1994 (25)	cross-sectional	Sri Lanka, England; 1990-1991	607 (M/F=285/322)	12	Water fluoride	mg/L	0.10; 0.50; 1.00; 0.10; 0.50; 1.00	NR	Demarcated opacities	DDE	NR	Similar prevalence of demarcated opacities in fluoridated and non-fluoridated areas
Ramesh 2011 (15)	cross-sectional	India	739 (M/F=406/333)	13	Water fluoride	mg/L	0.56; 0.95; 1.44	no	Demarcated opacities	mDDE	ion selective electrode	Lower prevalence of demarcated opacities in higher fluoridated areas.

Abbreviations: DDE developmental defects of enamel index; EAPD: European Academy of Paediatric Dentistry; mDDE: modified developmental defects of enamel index; MIH: Molar Incisor Hypomineralisation; NR: not reported.

across ten countries (Sweden, New Zealand, Italy, England, Sri Lanka, Germany, Australia, Lithuania, India, Brazil) were included in this review, with a publication year ranging from 1974 to 2021. The age of participants ranged from 6 to 13 years. Twelve of the 13 included articles had a cross-sectional design and investigated fluoride exposure from drinking water. Only one article (21) was designed as an experimental study on fluoride supplementation and was therefore excluded from the dose-response meta-analysis. In this study, children from experimental groups were assigned to different programs of fluoride supplementation (tablets containing 0.25 mg/day, 0.5 mg/day and 0.75 mg/day) varying from 0 to 5 years old. In addition, an overall higher prevalence of demarcated opacities was reported in the fluoride groups compared to the non-fluoride control group (24.7% vs. 16.5%). One study also reported the use of 1450 ppm fluoride toothpaste as possible additional exposure associated with drinking water, but no data on its effective use were available (9). The water fluoride concentration reached 4 mg/L over the included studies, though all but one study (22) assessed concentrations up to 1.65 mg/L. MIH was specifically considered as the study outcome in three studies (8,9,13). Due to such scarcity of specific MIH data, we also included the other ten studies that addressed "demarcated opacities", with features attributable to MIH, according to the adopted clinical assessment (e.g., index teeth examined). Fernandes et al. (9) based MIH diagnosis on EAPD MIH criteria, while Balmer et al. referred to the "demarcated opacities" aspects of the mDDE index (8,13). Three more studies referred to the mDDE index (15,21,23), while the remaining studies based their diagnosis on the standard DDE index (22,24–27), or on the criteria established by Zimmermann, Grahnen, Selander (28) and by Fejerskov et al., respectively (11).

Risk of Bias analysis

Details of RoB assessment are reported in Table 4. All of the 13 included studies yielded an overall "moderate risk of bias". The risk of bias arising from not appropriately considering or adjusting for possible confounders, such as age and socioeconomic status, was found to be moderate in 11 studies and low in the remaining 2. All the included studies were at low RoB concerning the reporting exposure dose domain. Exposure classification was found to be a source of moderate risk of bias across the studies, as only exposure to fluoride from drinking water was considered. Only one study (21) assessed exposure from tablets. Nevertheless, this was considered at moderate risk of bias, as it neither measured exposure through a biomarker nor estimated the total intake. Similarly, all the studies were at low risk of bias with regard to missing data, since results from more than 90% of the participants were reported. Ten of 13 studies were considered at moderate risk of bias with regard to outcome assessment, because they evaluated demarcated opacities through validated (yet not MIH-specific) diagnostic criteria. The remaining three studies specifically evaluated MIH: referring their diagnosis to validated criteria, they were therefore at low risk of bias. In all studies but one, the selection of participants was found to be at moderate risk of bias, since it was not independent from fluoride exposure (e.g., areas with different fluoride concentration in drinking water). Five studies were conducted according to a previously published protocol, whereas 8 studies outlined an a priori protocol in the manuscript. Thus, they could be respectively considered at low and moderate risk of bias for the selection of reported results.

Quantitative analysis

The forest plots displaying the study-specific and summary ORs for MIH, comparing the highest versus lowest fluoride categories, are reported in Figure 2. The overall OR for the association of fluoride exposure with MIH prevalence, including the only study investigating fluoride supplementation programs, was 0.93 (95% CI 0.60; 1.45). When evaluating only exposure from fluoride in drinking water, the OR was 0.89 (95% CI 0.55; 1.42).

Similarly, when stratifying the analysis by outcome, we obtained an OR of 0.78 (95% CI 0.47; 1.29) for MIH and 0.95 (95% CI 0.54; 1.66) for demarcated opacities attributable to MIH (Figure 3). Meta-regression according to fluoride levels in drinking water (Figure 4) showed a decreasing MIH risk for exposure up to 1 mg/L and based on 11 studies.

Table 4. Risk of Bias (RoB) for the selected studies.

Studies	Bias due to confounding	Bias in selecting participants in the study	Bias in exposure classification	Bias in departure from intended exposure	Bias due to missing data	Bias in outcome measurement	Bias in selection of reported results	Overall RoB
Angelillo 1990 (22)	Moderate	Moderate	Moderate	Low	Low	Moderate	Moderate	Moderate
Balmer 2005 (13)	Moderate	Moderate	Moderate	Low	Low	Low	Moderate	Moderate
Balmer 2015 (8)	Low	Moderate	Moderate	Low	Low	Low	Low	Moderate
Cutress 1985 (27)	Moderate	Moderate	Moderate	Low	Low	Moderate	Moderate	Moderate
De Liefde 1985 (26)	Moderate	Moderate	Moderate	Low	Low	Moderate	Moderate	Moderate
Ekanayake 2003 (23)	Moderate	Moderate	Moderate	Low	Low	Moderate	Moderate	Moderate
Fernandes 2021 (9)	Moderate	Moderate	Moderate	Low	Low	Low	Low	Moderate
Grahnén 1974 (28)	Moderate	Moderate	Moderate	Low	Low	Moderate	Moderate	Moderate
Hiller 1998 (21)	Moderate	Moderate	Moderate	Low	Low	Moderate	Low	Moderate
Machiulskiene 2009 (11)	Moderate	Moderate	Moderate	Low	Low	Moderate	Moderate	Moderate
Milsom 1990 (24)	Low	Moderate	Moderate	Low	Low	Moderate	Moderate	Moderate
Nunn 1994 (25)	Moderate	Moderate	Moderate	Low	Low	Moderate	Low	Moderate
Ramesh 2011 (15)	Moderate	Low	Moderate	Low	Low	Moderate	Low	Moderate

Conversely, a positive and substantially linear association between exposure through water and MIH risk emerged above 1 mg/L, mainly for the effect of a single study assessing MIH risk at very high exposure levels, i.e. 4 mg/L (22). The publication bias of the studies included in this review was considered low, as by the almost symmetrical shape of the funnel plot and Egger's test slope value (Figure 5).

Discussion

To the best of our knowledge, this is the first review and meta-analysis investigating a dose-response relation between fluoride exposure and MIH defects risk. Unfortunately, the limited number of relevant studies considerably reduced the possibility of computing statistically precise risk estimates. In our review, we retrieved only three eligible studies specifically addressing MIH as an outcome (8,9,13). However, MIH is a relatively recent term and so is the introduction of specific diagnostic criteria (2001), therefore we assumed that 'older' studies most likely referred to MIH with a variety of terms such as "demarcated opacities", "developmental defect of enamel" or "non-fluorotic opacities". Conversely, we excluded studies not explicitly indicating first permanent molars (FPMs) among index teeth or not reporting which teeth were examined (1). Thereby, consistent with what other authors did, we decided to include less recent and contemporary studies investigating MIH-attributable lesions referred to as "demarcated opacities" on the basis of

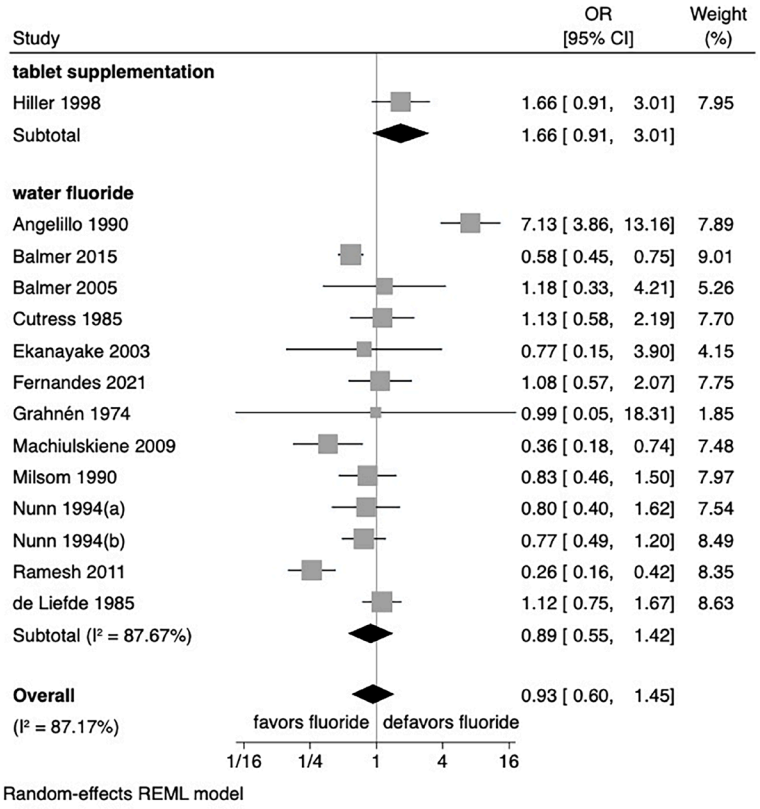

Figure 2. Forest plot of the included studies: Odds ratio (OR) with 95% confidence interval (CI) between exposure to fluoride and MIH occurrence, stratified for type of exposure (water fluoride and supplementation program). The squares represent risk estimate and horizontal lines represent their 95% CI. The area of each square is proportional to the weight of the study in the meta-analysis. The diamonds represent the combined risk for each type of exposure, and the solid line represents a null value. The inverse-variance estimation method was used for study weighting.

validated criteria and including in the clinical diagnostic examination the FPMs, which are indicated as index teeth in MIH diagnostic criteria (1,2,8). We acknowledge that this assumption may be a source of bias to be considered when evaluating our findings. However, we consider it unlikely that the use of such inclusion criteria may have caused a substantial alteration to the assessment of the correlation between fluoride and occurrence of MIH defects.

Overall, there is virtually no evidence of an effect of fluoride on MIH occurrence when comparing the highest versus lowest categories of water fluoride exposure. The analysis by outcome confirmed this trend and yielded similar results in relation to MIH-specific studies. Subgroup analysis by sex could not be performed due to a lack of data. However, many studies in the literature reported that sex was not associated with different prevalence of MIH defects (4,9). As for the meta-regression analysis for fluoride in drinking water, there seems to be a neutral, if not slightly beneficial effect on MIH risk under 1 mg/L, while for higher exposures, there was an indication of an increased risk for increasing exposure levels. These findings are in line with international guidelines, but should be assessed when choosing water fluoridation policies within the concentration range previously deemed safe, i.e. 0.7 to 1.2 mg/L (29,30). In 2015, the Center for Disease Control and Prevention (CDC) updated its water

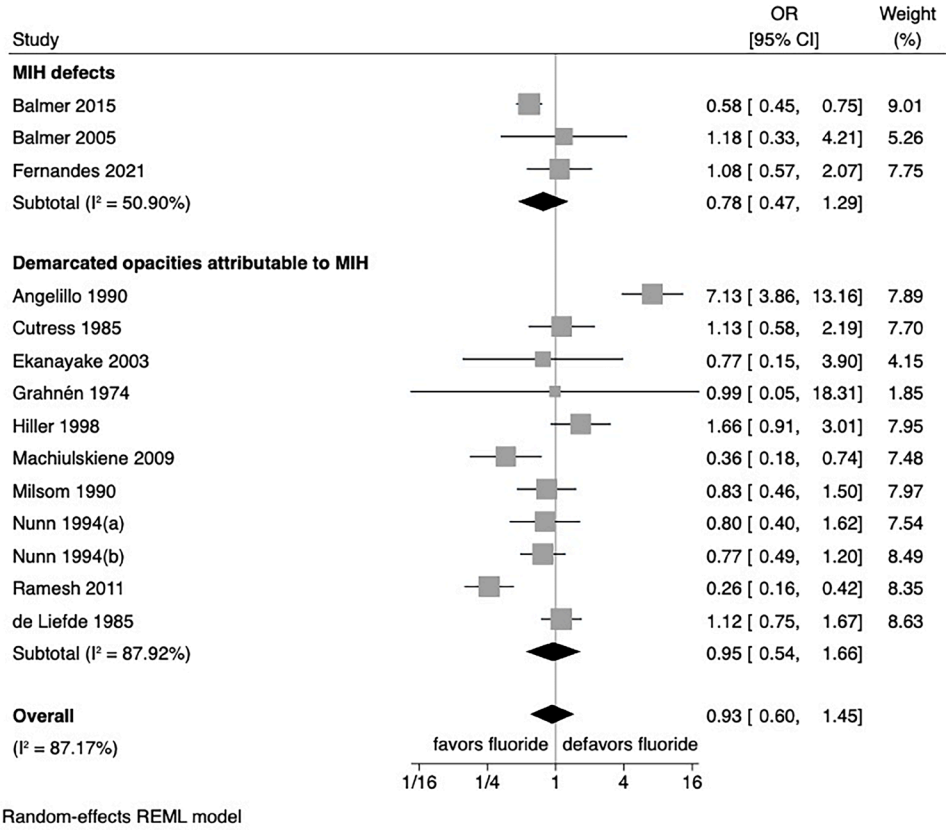

Figure 3. Forest plot of the included studies: Odds ratio (OR) with 95% confidence interval (CI) between exposure to fluoride and MIH occurrence, stratified for type of outcome (MIH and demarcated opacities attributable to MIH). The squares represent risk estimate and horizontal lines represent their 95% CI. The area of each square is proportional to the weight of the study in the meta-analysis. The diamonds represent the combined risk for each type of exposure, and the solid line represents a null value. The inverse-variance estimation method was used for study weighting.

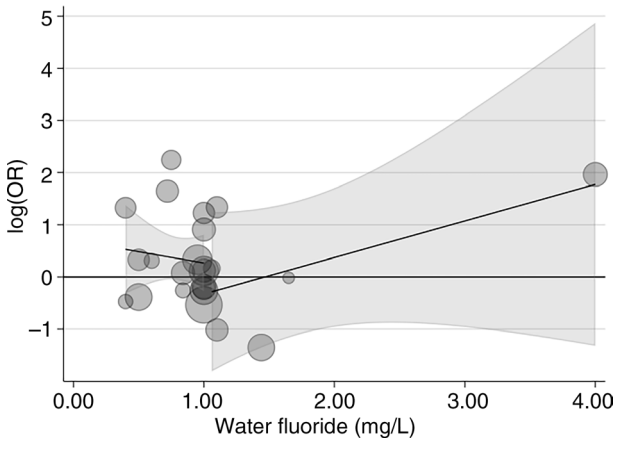

Figure 4. Meta regression showing the dose-response relation between exposure to fluoride in drinking water and risk of MIH defects. MIH: Molar-incisor hypomineralisation; OR: Odds ratio.

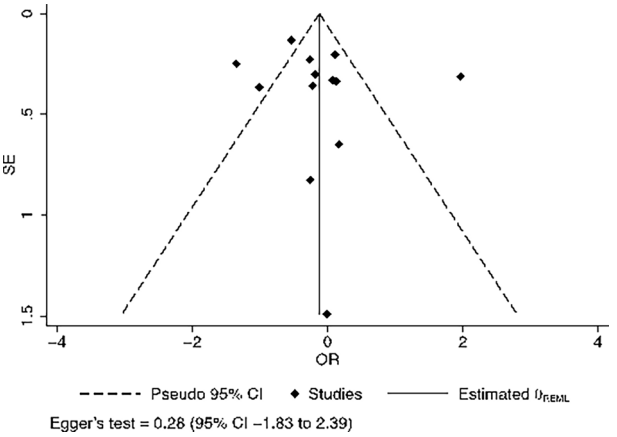

Figure 5. Funnel plot and Egger's test of the included studies.

fluoridation guidelines setting such level at 0.7 mg/L for the US (30).

We acknowledge that this complex and conflicting relation, which depends on exposure ranges, mainly emerged for the upward trend from a single influential study conducted before MIH criteria were identified (22). This suggested a higher prevalence of hypomineralisation defects at unusually high exposure levels, i.e. 4 mg/L fluoride in drinking water. Consequently, while an inverse and potentially beneficial association between water fluoride and MIH risk is supported by a number of studies, the exact relation above 1 mg/L of water fluoride concentrations remains statistically very unstable. Nor is it possible to conclusively determine at which cut point of exposure the risk starts to increase.

The evidence generated by our meta-analysis has, however, biological plausibility, being consistent with both pre- and post-eruptive fluoride effect on the enamel. MIH-affected enamel has carbonated apatite contents abnormally higher than sound enamel, which leads to increased solubility of the mineral component. Therefore, fluoride presence during amelogenesis can compensate by strengthening the mineral phase through the formation of more stable fluorapatite crystals, which can counteract the clinical presentation of mineralisation defects (31). In addition, it has been demonstrated that fluoride can induce a significant post-eruptive natural repair or "maturation" of MIH-affected enamel, improving its structural properties (31). Conversely, excessive amounts of fluoride as well as other contaminants, drugs, traumas or diseases have been demonstrated to adversely affect ameloblasts during the delicate enamel formation and maturation phase, as it occurs in other conditions such as fluorosis or hypoplasia (7).

Additionally, it is worth mentioning an interesting pathogenetic hypothesis on the synergistic detrimental effect of the simultaneous exposure to fluoride and other toxicants (e.g. 2,3,7,8-tetrachlorodibenzo-p-dioxin). This has been investigated *in vitro* by Salmela et al., who observed a clear effect of the two combined toxicants at concentrations at which they otherwise had no or barely detectable effects alone (32).

In assessing these results, it should be considered that potential confounders such as age and socioeconomic status have rarely been addressed across the retrieved studies, therefore limiting the internal validity of such studies and, as a consequence, of the evidence generated by this review. The recommended age for clinical examination to detect MIH is 8 years, when all four permanent molars and most incisors are likely to have erupted in most children and signs of MIH should still be present, before other overlapping conditions may interfere with diagnosis (2). However, age at diagnosis ranged from 6 to 13 years old across the included studies, and subgroup or adjusted analyses by age could not be performed due to a scarcity of data. With regard to exposure, all studies assessed fluoride exposure from drinking water in children, without evaluating biomarkers or reporting total daily intake. Therefore, the assessment of fluoride exposure was somehow imprecise in these studies, which were unable to take into account other sources of fluoride. Fluoride in drinking water and water-based beverages is the main source of fluoride in the general population, accounting for up to 90-95% of the total intake in adults and 52% in infants, but only 22% in children in fluoridated areas (14,33). Nonetheless, the estimated daily water and water-based beverage consumption in children below 14 years old is likely not to exceed 0.6 L, which results in fluoride exposure of approximately of 0.06 to 0.12 mg/day with water fluoride concentration of 0.1 mg/L, 0.7 to 0.9 mg/day with water fluoride concentration of 1.5 mg/L, and 1.4 to 1.75 mg/day with water fluoride concentration of 3.0 mg/L (29,34). Other important sources of fluoride may be toothpaste (52-63%), fluoride supplements (0-14%) and foods (9-10%), which shows considerable variability in individual exposure (14,35). This is probably underestimated due to a lack of specific data. In addition, water consumption varies widely, along with environmental and seasonal temperatures. As a result, the recommended fluoride daily adequate intake (AI) established by EFSA as 0.05 mg/Kg can easily be exceeded, possibly causing dental anomalies as well as other health issues (34,36,37).

Taken together, these results suggest that fluoride in drinking water at concentrations up to approximately 1 mg/L have a neutral, if not slightly protective, effect on MIH risk. On the other hand, higher levels may increase the risk of developing MIH-related hypomineralisation defects. However, these findings

should be carefully assessed due to some potential methodological limitations of the included studies, such as outcome misclassification, lack of adequate consideration of potential confounders and statistical imprecision of the estimates. Further high-quality studies with MIH-specific evaluation and a more homogeneous distribution of fluoride levels are required to overcome possible bias and achieve more reliable and precise estimates.

Ethic Committee: Not applicable. This is a systematic review; thus, no ethical approval was needed.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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Correspondence:
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Federica Veneri, DDS, MSc
Unit of Dentistry & Oral-Maxillo-Facial Surgery, Department of Surgery, Medicine, Dentistry and Morphological Sciences with Transplant Surgery, Oncology and Regenerative Medicine Relevance (CHIMOMO); University of Modena and Reggio Emilia
Largo del Pozzo, 71, 41124 Modena, Italy
Phone: +39 0594224324
E-mail: federica.veneri@unimore.it
ORCID: 0000-0002-5903-3830