Environmental Research 242 (2024) 117759

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Review article

Does fluoride exposure affect thyroid function? A systematic review and dose-response meta-analysis

Inga Iamandii [a], Lisa De Pasquale [b], Maria Edvige Giannone [a], Federica Veneri [c,d], Luigi Generali [c], Ugo Consolo [c], Linda S. Birnbaum [e], Jacqueline Castenmiller [f], Thorhallur I. Halldorsson [g,h], Tommaso Filippini [a,i], Marco Vinceti [a,j,*]

[a] 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
[b] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy
[c] Department of Surgery, Medicine, Dentistry and Morphological Sciences with Transplant Surgery, Oncology and Regenerative Medicine Relevance (CHIMOMO), Unit of Dentistry & Oral-Maxillo-Facial Surgery, University of Modena and Reggio Emilia, Modena, Italy
[d] PhD Program in Clinical and Experimental Medicine, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy
[e] Nicholas School of the Environment, Duke University, Durham, NC, USA
[f] Office for Risk Assessment & Research, Netherlands Food and Consumer Product Safety Authority, Utrecht, the Netherlands
[g] Department of Epidemiology Research, Centre for Fetal Programming, Copenhagen, Denmark
[h] Faculty of Food Science and Nutrition, University of Iceland, Reykjavík, Iceland
[i] School of Public Health, University of California Berkeley, Berkeley, CA, USA
[j] Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA

ARTICLE INFO

Handling Editor: Jose L Domingo

Keywords:
Fluoride
Water
Thyroid
Thyroid function
Thyroid disease
Risk assessment

ABSTRACT

Introduction: Fluoride exposure may have various adverse health effects, including affecting thyroid function and disease risk, but the pattern of such relation is still uncertain.
Methods: We systematically searched human studies assessing the relation between fluoride exposure and thyroid function and disease. We compared the highest versus the lowest fluoride category across these studies, and we performed a one-stage dose-response meta-analysis for aggregated data to explore the shape of the association.
Results: Most retrieved studies (27 of which with a cross-sectional design) were conducted in Asia and in children, assessing fluoride exposure through its concentrations in drinking water, urine, serum, or dietary intake. Twenty-four studies reported data on thyroid function by measuring thyroid-related hormones in blood (mainly thyroid-stimulating-hormone - TSH), 9 reported data on thyroid disease, and 4 on thyroid volume. By comparing the highest versus the lowest fluoride categories, overall mean TSH difference was 1.05 μIU/mL. Dose-response curve showed no change in TSH concentrations in the lowest water fluoride exposure range, while the hormone levels started to linearly increase around 2.5 mg/L, also dependending on the risk of bias of the included studies. The association between biomarkers of fluoride exposure and TSH was also positive, with little evidence of a threshold. Evidence for an association between fluoride exposure and blood concentrations of thyroid hormones was less evident, though there was an indication of inverse association with triiodothyronine. For thyroid disease, the few available studies suggested a positive association with goiter and with hypothyroidism in both children and adults.
Conclusions: Overall, exposure to high-fluoride drinking water appears to non-linearly affect thyroid function and increase TSH release in children, starting above a threshold of exposure, and to increase the risk of some thyroid diseases.

* Corresponding author. Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Via Campi 287, 41125, Modena, Italy.
E-mail address: marco.vinceti@unimore.it (M. Vinceti).

https://doi.org/10.1016/j.envres.2023.117759
Received 4 November 2023; Received in revised form 20 November 2023; Accepted 21 November 2023
Available online 28 November 2023

1. Introduction

Fluoride is the 13th most abundant element on Earth (Ghosh et al., 2013), naturally present in different amounts in certain foods, plants, and water (Singh et al., 2018; Ullah et al., 2017). Even though a small amount of exposure can occur from accidental ingestion of topical dental products, fluoridated water and fluoride-rich and enriched foods and beverages represent the main sources of fluoride intake (CDC, 2020; Lubojanski et al., 2023; Peckham and Awofeso, 2014). A too high fluoride exposure may adversely affect human health (Ahmad et al., 2022; Lubojanski et al., 2023; Shahab et al., 2017), with particular reference to dental and skeletal fluorosis (Fewtrell et al., 2007; Veneri et al., 2023a), and more recently growing body of evidence suggesting neurodevelopmental effects in children (Duan et al., 2018; Fiore et al., 2023; Veneri et al., 2023b). Moreover, high fluoride exposure has been associated with abnormal thyroid function, such as increased release and concentrations of thyroid-stimulating hormone (TSH), and decreased thyroxine (T4) and triiodothyronine (T3) levels (Andezhath et al., 2005; Wang et al., 2020). Despite a number of studies investigating the role of fluoride in thyroid toxicity, dose-response pooled estimates of the association between fluoride exposure and thyroid function and disease are still lacking (Chaitanya et al., 2018; Liu et al., 2014; Peckham et al., 2015). Therefore, we aimed at performing a systematic review and meta-analysis, also taking advantage from a statistical approach made recently available and suitable to characterize the shape of the relation between fluoride exposure and thyroid function, disease, and volume (Crippa et al., 2019).

2. Methods

2.1. Protocol and registration

We conducted this systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (Page et al., 2021), after registering the study protocol in PROSPERO database (registration no. CRD42022321899).

2.2. Search strategy and study selection

The research question was designed according to the PECOS statement (Population, Exposure, Comparator, Outcomes, and Study design) (Morgan et al., 2018) as follows: "What is the effect of fluoride exposure on thyroid, according to a dose-response relation in humans?". Therefore, a study was considered eligible if it met the following inclusion criteria: (P) population of any age; (E) assessment of long-term fluoride exposure through drinking water or diet, and/or assessment of biomarkers of exposure (urinary or serum fluoride); (C) comparison of at least two categories of fluoride exposure; (O) biomarkers of thyroid function (e.g., TSH, T4, T3 hormones), thyroid disease risk (e.g., hypothyroidism, goiter) or thyroid volume as endpoints in (S) both nonexperimental (observational) or experimental (clinical trial) study design. Conversely, we excluded studies that did not present original data (e.g., review articles, editorials, comments, or guidelines) or were written in languages other than English. No restrictions were applied regarding the geographical location or publication date and, when more studies examined the same population, only the most complete one was included.

We performed a literature search through PubMed/MEDLINE, Web of Science, and Embase databases to retrieve studies published from inception up to November 15, 2023, by using the search terms "fluoride" and "thyroid". Details about the search strategies are reported in Supplementary Table S1. The studies identified were imported into the Rayyan web app for systematic reviews (Ouzzani et al., 2016) and their titles, abstracts and full-texts were reviewed independently by three authors (II, LDP and FV), in accordance with the eligibility criteria. Any disagreements between these authors were resolved through the intervention of other two authors (MV and TF). Also, we searched backward and forward citations by manually checking the reference lists of these studies to retrieve additional literature of potential interest. To be further included in the meta-analysis, studies had to report effect/risk estimates according to exposure category, such as relative risk (RR), hazard ratio (HR), odds ratio (OR), mean difference (MD), or data allowing their calculation including the corresponding 95% confidence interval (95% CI), standard error (SE), standard deviation (SD), or interquartile range (IQR).

2.3. Data extraction

Data extraction from the eligible studies was independently performed by two authors (II and LDP) and verified by a third author (TF), using a standardized data collection form. We extracted the following information: (1) first author's name; (2) study characteristics (title, study design, country, publication year); (3) population characteristics (sex, age at baseline and sample size); (4) exposure characteristics (definition, assessment method, categories of exposure); (5) outcome characteristics (definition, assessment method, number of cases); (6) effect estimates (RRs, HRs, ORs, MDs) with their 95% CIs, SE, SD, or data to calculate them according to the Cochrane Handbook for Systematic Reviews of Interventions recommendations (Higgins et al., 2019), from the most-adjusted model; and (7) adjustment variables in the multivariable analysis.

2.4. Risk of bias assessment

The risk of bias of the included studies was assessed independently by three authors (II, LDP, and MEG), using the most recent version of the Risk of Bias tool In Non-randomized Studies of Exposures "ROBINS-E" (Higgins et al., 2023). In case of disagreement between assessors, a decision was taken by involving a fourth author (MV). The tool was adapted to accommodate our specific research question and the characteristics of the studies selected by following seven bias domains (Supplementary Table 2): (1) bias due to confounding; (2) bias in selecting participants in the study; (3) bias in exposure measurement; (4) bias in departure from intended exposure; (5) bias due to missing data; (6) bias in outcome measurement; (7) bias in selection of reported results. For three domains (bias due to confounding, bias in selecting participants in the study, bias in outcome measurement), studies were judged to be at "Low", "Some concerns", "High", or "Very high" risk of bias. Studies were judged to be at "Low", "Some concerns", or "High" risk of bias for other three domains (bias in exposure measurement, bias due to missing data, bias in selection of reported results) and at "Low" or "Very high" risk of bias for bias in departure from intended exposure. Finally, the overall risk of bias of a study was considered "Low" if all domains were at "Low" risk of bias, "Some concerns" if at least one domain was found at "Some concerns" of bias, "High" if at least one domain was found at "High" risk of bias or four or more domains are at "Some concerns" of bias, and "Very high" if at least one domain was found at "Very high" risk of bias, or four or more domains are at "High" risk of bias.

2.5. Data analysis

We performed a meta-analysis by comparing the highest fluoride exposure category versus the lowest one and reporting the results in a forest-plot, using the restricted maximum likelihood random-effects model. When risk estimates for thyroid disease were unavailable, we extracted the number of cases and non-cases for each exposure category and then calculated the risk estimate alongside its 95% CI using the 'csi' routine of Stata software (v17.0, Stata Corp., College Station, TX, 2021). We stratified the analyses according to type of exposure indicator (e.g., water, urinary, serum fluoride, or daily fluoride intake), type of

endpoint (e.g., concentrations of thyroid-related hormones such as TSH, free T4, total T4, free T3, total T3, thyroid disease, and thyroid volume), and age categories (e.g., children, adults, children and adults), whenever data were available. We conducted sensitivity analyses excluding studies judged to be at "High" and/or "Very high" risk of bias. We also explored the shape of the association of fluoride exposure with thyroid-related hormone concentrations through a dose-response meta-analysis using a one-stage approach (Crippa et al., 2019; Orsini et al., 2022), in line with previous studies on other endpoints (Di Federico et al., 2023; Filippini et al., 2021). In particular, we used a restricted cubic spline with 3 knots at fixed cut-points (10th, 50th, and 90th percentiles) through the restricted maximum likelihood random-effects model. For this purpose, we extracted the mean/median fluoride levels or we computed the midpoint of each exposure category depending on data availability. If the highest and the lowest exposure categories were "open", we calculated a value that was 20% higher or lower, respectively, than the closest cutpoint (Filippini et al., 2022; Vinceti et al., 2016). Finally, we assessed publication bias through visual inspection of symmetry in funnel plots and by performing Egger's test when at least five studies were available for analysis (Egger et al., 1997), the heterogeneity of included studies using the I^2 statistics (Higgins et al., 2003), and by providing a graphical overlay of study-specific trends using predicted curves showing the influence of variation across studies (Murad et al., 2023; Orsini et al., 2022). All analyses were performed with Stata software (v17.0, Stata Corp., College Station, TX, 2021).

3. Results

3.1. Study selection

Literature search and study selection process are described in the PRISMA flow-chart (Fig. 1). In the initial search, we identified 1232 articles, of which 187 were duplicate records. After screening for title and abstract, 975 studies were further removed because they were considered not relevant or they were written in languages other than English, leaving 70 studies for full-text assessment. Based on their evaluation, we additionally excluded 42 studies for the following reasons: (1) other publication type e.g., conference abstracts or reviews (n = 18); (2) outcome not of interest (n = 7); (3) full-text not available (n = 5, 3 of them published before 2000); (4) lack of details for exposure (n = 5); (5) correlation exposure-outcome not possible (n = 3); (6) duplicate population (n = 2); (7) unhealthy population (n = 1); (8) study design not eligible (n = 1). Eventually, 28 studies (Ahmed et al., 2022; Andezhath et al., 2005; Bachinsky et al., 1985; Barberio et al., 2017; Cui et al., 2020; Day and Powell-Jackson, 1972; Du et al., 2021; Eltom et al., 1984; Hall et al., 2023; Hong et al., 2008; Jooste et al., 1999; Karademir

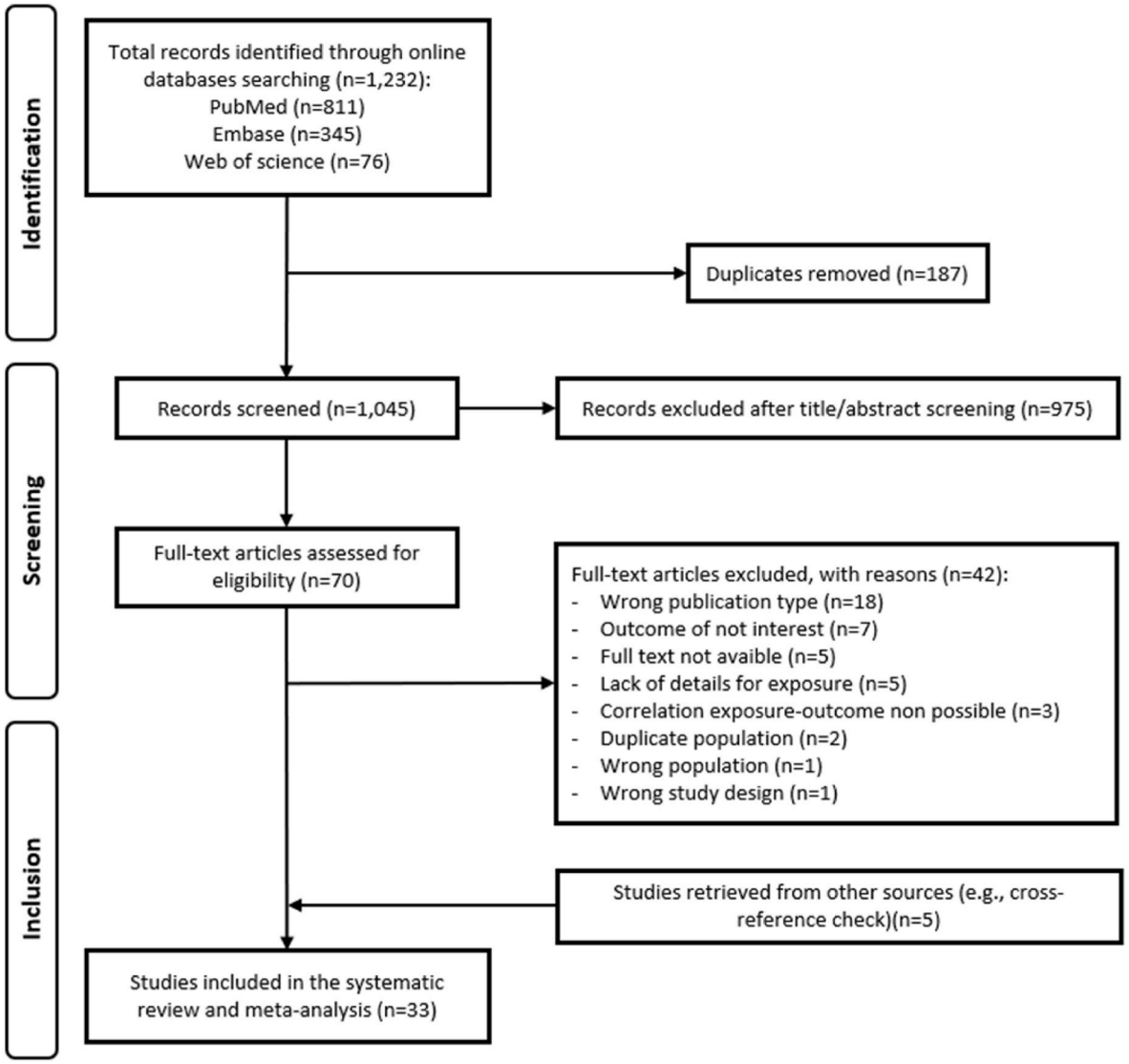

Fig. 1. PRISMA flow-chart of the inclusion process.

et al., 2011; Khandare et al., 2017, 2018; Kheradpisheh et al., 2018; Kumar et al., 2018; Kutlucan et al., 2013; Lathman and Grech, 1967; Michael et al., 1996; Peckham et al., 2015; Siddiqui, 1960; Szczuko et al., 2019; Wang et al., 2020, 2022; Xu et al., 2022; Yang et al., 2008; Yasmin et al., 2013; Zhang et al., 2015) were eligible for inclusion, to which we added 5 articles retrieved through citation chasing (Hosur et al., 2012; Shaik et al., 2019; Singh et al., 2014; Zulfiqar et al., 2019, 2020), resulting in 33 studies eventually included in our meta-analysis and suitable to assess the association between fluoride exposure and thyroid outcomes.

3.2. Characteristics of included studies

The key characteristics of the studies included in our meta-analysis are summarized in Table 1. Overall, these studies were published between 1960 and 2023, with 23 (70%) out of 33 being published from 2010 to 2023. The included studies had a pooled sample of nearly 45,000 participants (data missing for the large study by Peckham et al. (2015)), with an age between 6 and 79 years. Twenty-seven studies had a cross-sectional design, 5 case-control and 1 cohort study which was also the only report in pregnant women. Twenty-five studies were conducted in Asia (of which 10 in India and 8 in China), 3 studies in Europe, 3 in Africa, and 2 in Canada. All studies investigated different populations, with the partial exception of (Zulfiqar et al. 2019, 2020), which shared some participants. According to the available data, the exclusive evaluation of children was carried out in 22 studies (all but 1 from Asia), 7 studies involved both children and adults, and the remaining 4 studies included adult populations only. Almost half of the studies (n = 14) measured fluoride exposure through multiple modalities of assessment, such as drinking water, urinary, serum concentrations, or daily fluoride intake. Eleven studies were based solely on drinking water levels, 7 studies on urinary fluoride, and 1 on serum fluoride. Average fluoride concentrations in drinking water (n = 25) ranged from 0.08 to 25.10 mg/L (median: 0.80 and IQR: 2.04) and urinary fluoride (n = 19) ranged from 0.06 to 4.57 mg/L (median: 0.82 and IQR: 1.51). Lastly, the median ± IQR value of fluoride found in serum (n = 11) was 0.065 ± 0.17 mg/L (range: 0.03–0.395). Most studies (n = 24) reported data on thyroid function by measuring thyroid-related hormones in the blood (n = 24 for TSH, n = 22 for T4, and n = 21 for T3), 9 studies reported data on thyroid disease diagnosis (7 on goiter, 1 on hypothyroidism, and 1 on unspecified thyroid disease, respectively) and 4 papers studied thyroid volume through ultrasound methods.

About half of the studies (n = 16) did not assess iodine status of study participants, 8 studies investigated subjects with rather homogeneous and adequate iodine status (4 assessing it at an ecologic level, 4 at the individual level by including only subjects with optimal iodine intake (n = 3) or not too high intake (n = 1), 1 of them also adjusting for urinary iodine in the multivariable analysis), 9 studies simply determined iodine exposure (8 in each of the fluoride exposure category, 1 independently from fluoride assessment). Among these 10 studies assessing iodine exposure, 1 study analyzed absorption of ^{131}I over 24 h by the thyroid, 2 studies reported iodine intake, 4 measured iodine exposure through drinking water concentration, and 3 by urinary concentration. In most of these studies, iodine and fluoride values increased (n = 4) or decreased (n = 3) in the same direction, while the remaining studies (n = 3) did not show a specific trend.

3.3. Risk of bias assessment

The results of the quality assessment study by Risk of Bias are reported in Supplementary Table S3. Overall, 5 studies were considered to be at "Very high" risk of bias, 10 at "High" risk of bias, 17 at "Some concerns", and 1 at "Low" risk. Six studies were considered at "Low" risk of bias due to confounding, reporting adjustments for age, sex and iodine status; 22 were deemed to be at "Some concerns" of bias, since 9 of them adjusted for two variables among age, sex, and iodine status, and 13 adjusted only for age; finally, 5 studies were considered at "Very high" risk of bias, since all of them did not report any adjustments. For one third of the studies the selection of eligible participants was not related to fluoride exposure, being at "Low" risk of bias for the related domain, while for 16 studies the selection of eligible participants was related to fluoride exposure, but they were recruited using the same inclusion-exclusion criteria, therefore, being considered at "Some concerns" for bias. Many studies (n = 20) were deemed at "Some concerns" of risk of bias in exposure classification, whereas 2 studies have not reported the exposure assessment method, making them at "High" risk of bias. All of the studies were considered at "Low risk" for bias in departure from intended exposure domain, because the exposure dose was always reported. Regarding bias due to missing data, 7 studies were considered at "High" risk, since the potential loss of subjects was not adequately documented and addressed, and 1 at "Some concerns", because 19% of participants were excluded due to missing data. Moreover, 1 paper resulted at "High" risk of outcome measurement bias, because it had a self-reported assessment method of thyroid disease, and 6 studies were deemed at "Some concerns" of bias, since outcome assessment was based on clinical examination with no subsequent validation. Finally, most of the studies (n = 20) were considered at "Low" risk of selective reporting bias, and in 3 studies no protocol was available and a prior plan was not outlined in the methods, making them at "High" risk of bias.

3.4. Quantitative analysis

The study-specific and summary mean differences of TSH levels in children (age from 6 to 18 years), comparing the highest versus the lowest fluoride categories and according to the type of exposure assessment methodology, are displayed in Fig. 2. With regards to exposure to fluoride in drinking water (n = 13), the weighted mean TSH difference between the highest and the lowest exposure category was 1.17 μIU/mL, with some inconsistencies across the different studies that were, however, characterized by high heterogeneity in the exposure categories compared and in their differences. Summary mean differences for urinary fluoride (n = 15) and serum fluoride (n = 8) in the highest exposure category, compared to the bottom one, were 0.97 μIU/mL and 1.46 μIU/mL. Similar results were seen after we performed sensitivity analyses excluding the studies with "High" and "Very high" risk of bias (Supplementary Fig. S1), with 1.06, 0.52 and 1.09 μIU/mL mean TSH differences for water (n = 8), urinary (n = 11), and serum (n = 5) fluoride exposure, respectively. Furthermore, there was little evidence of publication bias regarding these outcomes, except for urinary fluoride exposure (Supplementary Fig. S2).

Analyses for thyroid hormones in children are shown in Supplementary Figs. S3–S5. The summary total T4 mean difference for water fluoride exposure category (n = 6) was 0.63 μg/dL, while for urinary fluoride (n = 7) and serum fluoride (n = 2) the summary estimate was close to zero. Similarly, slight overall mean differences emerged for free T4, with values of −0.05 ng/dL for water fluoride (n = 3), −0.01 ng/dL for urinary fluoride (n = 8), and 0.04 ng/dL for serum fluoride (n = 5). Regarding analyses for total T3 levels, all summary mean differences between the highest and the lowest fluoride exposure category were almost null (−0.01 ng/mL for water fluoride in 6 studies, 0.00 ng/mL for urinary fluoride in 7 studies, and 0.02 ng/mL for serum fluoride in 2 studies), whereas for free T3 the difference was −0.10 pg/mL for water fluoride (n = 7), 0.20 pg/mL for urinary fluoride (n = 8), and 0.28 pg/mL for serum fluoride (n = 5). Study-specific and summary mean differences of thyroid volume and echobody index (total thyroid volume/body surface area) were possible only for urinary fluoride (Supplementary Fig. S4) due to limited data availability for other exposure metrics, and resulted in overall mean differences of −0.08 mL (n = 4) and 0.46 mL/m^2 (n = 1), respectively. Similar results can be seen excluding studies with "High" and "Very high" risk of bias (Supplementary Figs. S6–S8), for thyroid volume parameters and all hormones,

Table 1
Characteristics of the studies included in the review.

Reference	Study design	Country	Population characteristics at baseline	Exposure categories (unit)	Exposure assessment method	Outcome	Outcome assessment method	Assessment of iodine status	Adjustments
Ahmed et al. (2022)	Cross-sectional	Pakistan	242 subjects aged >12 years	Water [F] (mg/L): 0.30; 7.00	Fluoride Ion Selective Electrode (FISE)	Mean (SD) free T3 (μg/dL): 2.04 (0.49); 2.59 (0.56) Mean (SD) free T4 (nmol/L): 8.28 (2.16); 10.43 (2.34) Mean (SD) TSH (mIU/L): 1.94 (1.29); 2.59 (1.69)	Semiautomatic immunoassay analyzer (Immulite, 2000; Elecy, 2010)	No assessment	NR
Andezhath et al. (2005)	Cross-sectional	India	111 children aged 7–18 years	Water [F] (mg/L): 0.14–0.81; 0.14–0.73 Urinary [F] (mg/L): 0.09–1.32; 0.10–4.20 Serum [F] (mg/L): 0.02–0.14; 0.02–0.29	Fluoride ion specific electrode method (Ion 85 Analyzer)	Range free T3 (pg/mL): 1.80–2.80; 2.00–3.85 Range free T4 (ng/dL): 1.10–1.80; 0.97–1.50 Range TSH (μIU/mL): 1.20–2.10; 0.20–3.82	Immuno Chemiluminescence Microparticle Assay (ICMA)	No assessment	Age
Bachinsky et al. (1985)	Case-control	Ukraine	47 adults aged 19–59 years	Water [F] (μmol/L): 52; 122 Urinary [F] (μmol/L): 78; 124 Serum [F] (μmol/L): 11; 13	Fluoride selective electrode (EFU1)	Mean (SD) total T3: (nmol/L): 2.8 (0.3); 2 (0.2) Mean (SD) total T4 (nmol/L): 97 (8); 94 (6) Mean (SD) TSH (mIU/L): 2.4 (0.2); 4.3 (0.6)	TRIK-PEG test kit for T3, SPAC test kit for T4, and TSHKPR test kit for TSH	Determination of the intrathyroidal phase of iodine metabolism (absorption of ^{131}I by the thyroid) and fluoride levels in the study areas and population. Absorption of ^{131}I over 24 h by thyroid (%): 24; 33	NR
Barberio et al. (2017)	Cross-sectional	Canada	12,180 subjects aged 7–79 years	Urinary [F] (μmol/L): 33.55; 29.81	Fluoride ion selective electrode (Orion)	Thyroid condition diagnosis (cases/participants): 87/2530; 150/2671	Self-reported diagnosis of a thyroid condition	No assessment	Age and sex
Cui et al. (2020)	Cross-sectional	China	498 children aged 7–12 years	Urinary [F] (mg/L): <1.6; 1.6–2.5; ≥2.5	Fluoride ion selective electrode method (Chinese standard WS/T 89–2015)	Median (Q1-Q3) TSH (μIU/mL): 2.81 (2.21–3.81); 2.82 (2.01–3.82); 3.29 (2.30–4.48)	Electrochemical luminescence method	Assessment of iodine levels was independent of assessment of fluoride levels.	Age
Day and Powell-Jackson (1972)	Cross-sectional	Nepal	648 subjects (500 children and 148 adults)	Water [F] (ppm): <0.1; 0.13; 0.13; 0.12; 0.19; 0.24; 0.22; 0.28; 0.21; 0.19; 0.36; 0.23; 0.23	Ion selective electrode	Goiter diagnosis (cases/participants): 2/38; 6/48; 4/31; 7/40; 10/50; 9/35; 10/36; 19/53; 40/85; 45/89; 23/43; 29/48; 36/52	Clinical examination according established guidelines	17 villages were visited and 736 people examined. 4 villages had relatively high water-iodine levels and were excluded. Our study is based upon data from the remaining 13 (648 people) which had a water-iodine concentration of 0.001 ppm or less.	Age, sex, and iodine status

(*continued on next page*)

Table 1 (*continued*)

Reference	Study design	Country	Population characteristics at baseline	Exposure categories (unit)	Exposure assessment method	Outcome	Outcome assessment method	Assessment of iodine status	Adjustments
Du et al. (2021)	Cross-sectional	China	446 children (209 girls and 237 boys) aged 7–12 years	Urinary [F] (mg/L): 0.15–0.70; 0.71–1.35; 1.36–1.93; 1.94–5.18	Fluoride ion selective electrode method	β (95% CI) total T3 (nmol/L): ref; −0.08 (−0.21, 0.05); −0.15 (−0.29, −0.01); −0.21 (−0.37, −0.04) β (95% CI) total T4 (nmol/L): ref; 6.07 (−0.60, 12.73); 7.60 (0.41, 14.80); 9.75 (1.39, 18.11) β (95% CI) TSH (μIU/mL): ref; 0.32 (−0.01, 0.65); −0.06 (−0.41, 0.30); −0.19 (−0.60, 0.23) β (95% CI) Tvol (cm^3): ref; 0.33 (0.13, 0.53); 0.56 (0.35, 0.77); 0.54 (0.30, 0.78)	Radiation immunoassay for thyroid hormones, and B-mode ultrasound method for thyroid volume	All children had sufficient iodine intake, therefore, children with urinary iodine <100 μg/L (only 3 children) were excluded from our study. Adjustment for urinary iodine in the multivariable linear regressions analyses.	Age, sex, body mass index, maternal education, urinary creatinine, and urinary iodine
Eltom et al. (1984)	Cross-sectional	Sudan	13,682 subjects (mainly children)	Water [F] (mg/L): 0.30; 0.53	Radiometer electrode according to the Swedish standard (S.I.S. 0281 35)	Goiter diagnosis (cases/participants): 1039/7697; 2907/3353	Clinical examination according established guidelines	Determination of iodine and fluoride levels in the study areas and population. Iodine intake (μg/day): 0.10; - (not assessed)	Age and sex
Hall et al. (2023)	Cohort (Maternal-Infant Research on Environmental Chemicals "MIREC" Study)	Canada	1508 pregnant women aged ≥18 years, with <14 weeks gestation	Water [F] (mg/L): 0.41; 0.42; 0.48) Urinary [F] (mg/L): 0.59; 0.57; 0.62 F daily intake (mg/day): 0.65; 0.67; 0.78	Average water fluoride levels reported by the municipal WTPs (water treatment plant) for Water [F]; Ion selective electrode (modification of the hexamethyldisiloxane microdiffusion method) for Urinary [F]; Self-reported questionnaire for F intake	Mean (SD) free T4 (pg/mL): 13.50 (1.70); 13.10 (1.50); 14.00 (3.70) Mean (SD) total T4 (ng/mL): 105.80 (19.80); 107.10 (18.90); 109.30 (26.60) Mean (SD) TSH (μIU/mL): 1.20 (0.55): 3.10 (0.66); 3.20 (2.80)	Gold standard equilibrium dialysis isotope dilution mass spectrometry (ED-ID-MS) for T4, and a commercial immunoassay for TSH	Determination of iodine and fluoride levels in the study areas and population. Iodine intake (μg/day): 440.9; 458; 470	Age and sex
Hong et al. (2008)	Cross-sectional	China	205 children aged 8–14 years	Water [F] (mg/L): 0.48; 0.75; 2.85; 2.90; 2.94	Fluoride ion selective electrode method	Goiter diagnosis (cases/participants): 9/28; 1/32; 4/32; 1/85; 12/28	Clinical examination according to the International 2 grades classification system	Determination of iodine and fluoride levels in the study areas and population. Water iodine (μg/L): 0.75; 150; 1150; - (not assessed); 0.91	Age
Hosur et al. (2012)	Case-control	India	75 children (27 boys and 38 girls) aged 7–18 years	Water [F] (ppm): <1.00; 0.50–4.00	Fluoride ion selective electrode method	Range free T3 (pg/mL): 2.72–3.79; 1.90–4.14 Range free T4 (ng/	Competitive chemiluminescent immunoassays for T3 and T4, and ultra-sensitive sandwich	No assessment	Age

(*continued on next page*)

Table 1 (*continued*)

Reference	Study design	Country	Population characteristics at baseline	Exposure categories (unit)	Exposure assessment method	Outcome	Outcome assessment method	Assessment of iodine status	Adjustments
						dL): 0.83–1.36; 0.83–1.25 Range TSH (μIU/mL): 0.94–11.00; 0.43–5.91	chemiluminescent immunoassay method for TSH		
Jooste et al. (1999)	Cross-sectional	South Africa	671 children aged 6–15 years	Water [F] (ppm): 0.30; 0.50; 0.90; 1.10; 1.70; 2.60	NR	Goiter diagnosis (cases/participants): 13/85; 22/127; 16/87; 5/95; 26/94; 53/183	Clinical examination according established guidelines	Determination of iodine and fluoride levels in the study areas and population. Water iodine (μmol/L): 0.83; >1.58; 1.00; -(not assessed); >1.58; 1.13	Age
Karademir et al. (2011)	Cross-sectional	Turkey	61 children aged 7–16 years	Urinary [F] (ppm): 0.20; 0.74; 0.90	Ion specific electrode method	Median (range) free T3 (pg/mL): 3.62 (2.69–4.41); 3.46 (2.74–4.14); 3.42 (2.69–4.41) Median (range) free T4 (ng/dL): 1.11 (0.85–1.64); 1.11 (0.63–1.53); 0.96 (0.85–1.11) Median (range) TSH (μIU/mL): 1.81 (0.80–3.43); 2.14 (0.82–3.47); 1.56 (1.21–3.52)	Chemiluminescent micro-particle immunoassay (CMIA)	No assessment	Age
Khandare et al. (2017)	Case-control	India	824 children aged 8–15 years	Water [F] (mg/L): 1.13; 1.85 Urinary [F] (mg/L): 1.91; 3.28	Fluoride specific ion electrode (Orion 9609)	Mean (SD) total T3 (ng/mL): 0.68 (0.35); 0.63 (0.24) Mean (SD) total T4 (μg/dL): 16.90 (1.60); 16.10 (2.90) Mean (SD) TSH (mIU/L): 3.40 (0.50); 2.90 (0.60)	DiaSorin kits for T3 and T4, and Immunoradiometric assay (IRMA) kit for TSH	No assessment	Age
Khandare et al. (2018)	Cross-sectional	India	1934 children (904 boys and 130 girls) aged 8–14 years	Water [F] (mg/L): 0.87; 2.53; 3.77; <1.00	Fluoride specific ion electrode (Orion 9609)	Mean (SD) total T3 (ng/mL): 2.17 (0.42); 1.57 (0.36); 1.34 (0.32); 1.46 (0.38) Mean (SD) total T4 (ng/mL): 112.69 (21.97); 116.03 (17.32); 132.8 (21.90); 84.40 (21.61) Mean (SD) TSH (μIU/mL): 1.66 (0.49); 1.85 (0.46); 3.65 (1.04); 1.58 (0.44)	Radioimmunoassay (RIA) method	No assessment	Age

(*continued on next page*)

Table 1 (*continued*)

Reference	Study design	Country	Population characteristics at baseline	Exposure categories (unit)	Exposure assessment method	Outcome	Outcome assessment method	Assessment of iodine status	Adjustments
Kheradpisheh et al. (2018)	Case-control	Iran	228 adults aged 20–60 years	Water [F] (mg/L): 0.00–0.29; 0.30–0.50	SPADANS method	Median (IQR) total T3 (ng/dL): 135.00 (18.40); 138.50 (21.60) Median (IQR) total T4 (μg/dL): 8.50 (1.20); 8.60 (1.20) Median (IQR) TSH (mIU/L): 2.20 (0.95); 2.80 (0.90)	Radioimmunoassay (RIA) method	No assessment	NR
Kumar et al. (2018)	Cross-sectional	India	400 children aged 8–15 years	Water [F] (ppm): 0.94–1.08; 1.50–5.00; 1.80–5.80 Urinary [F] (ppm): 0.22–1.07; 0.27–8.60; 0.60–7.64 Serum [F] (ppm): 0.03–0.10; 0.05–0.71; 0.05–0.71	Fluoride specific ion electrode	Range free T3 (pg/mL): 1.84–4.08; 1.20–4.21; 1.50–4.68 Range free T4 (ng/dL): 0.79–1.53; 0.96–1.82; 0.70–1.50 Range TSH (μIU/mL): 0.99–3.39; 1.34–8.31; 1.88–10.14	Immuno Chemiluminescence Microparticle Assay with Autoanalyzer	No assessment	Age
Kutlucan et al. (2013)	Cross-sectional	Turkey	559 children aged 10–15 years	Urinary [F] (mg/L): 0.22; 0.48	Fluoride selective electrode (Orion)	Mean (SD) Tvol (mL): 8.73 (2.75); 8.60 (3.11) Mean (SD) Ecobody index (mL/m^2): 6.48 (1.53); 6.94 (2.14)	Ultrasonography for thyroid volume	Determination of iodine and fluoride levels in the study areas and population. Urinary iodine (μg/L): 98.41; 93.12	Age and sex
Lathman and Grech (1967)	Cross-sectional	Tanzania	1243 children and adults	Water [F] (ppm): 18.60; 14.40; 25.10; 6.20; 2.60; 1.90	NR	Goiter diagnosis (cases/participants): 99/380; 10/30; 42/244; 26/121; 37/163; 126/305	Clinical examination	No assessment	NR
Michael et al. (1996)	Cross-sectional	India	500 subjects	Water [F] (ppm): 0.64; 2.70 Serum [F] (ppm): 0.04; 0.28	Ion selective electrode (Orion 701 A)	Mean (SE) total T3 (ng/mL): 1.50 (0.14); 1.53 (0.08) Mean (SE) total T4 (ng/mL): 9.16 (0.63); 14.77 (0.51) Mean (SE) TSH (μU/mL): 2.56 (0.36); 2.55 (0.37)	Radioimmunoassay (RIA) method for T3 and T4, and immunoradiometric assay method (IRMA) for TSH	No assessment	NR
Peckham et al. (2015)	Cross-sectional	UK	Subjects at 7935 General Practices in England (NR number of subject)	Water [F] (mg/L): 0.30–0.70; >0.70	[F] was provided by the Drinking Water Inspectorate (DWI)	ORs of upper tertile hypothyroidism prevalence: T1: reference; T2: 1.37 (1.12–1.68); T3: 1.62 (1.34–1.90)	Hypothyroidism prevalence obtained for all GP practices from the QOF data set	While iodine intake is a key determinant of thyroid status, the major source of iodine in the UK is from the diet and it is unlikely that there are	Proportion of women registered with the practice, proportion of patients over 40 years old registered

(*continued on next page*)

Table 1 (*continued*)

Reference	Study design	Country	Population characteristics at baseline	Exposure categories (unit)	Exposure assessment method	Outcome	Outcome assessment method	Assessment of iodine status	Adjustments
								significant differences between people residing in fluoridated and non-fluoridated areas.	with the practice, Index of Multiple Deprivation. Also, iodine status
Shaik et al. (2019)	Double-blinded observational trial (cross-sectional)	India	293 children aged 9–13 years	Water [F] (ppm): 0.22; 0.89; 1.44 Serum [F] (ppm): 0.03; 0.04; 0.05	Fluoride Ion Selective Electrode (F-ISE)	Mean (SD)[1] total T3: 140.80 (23.69); 156.84 (84); 158.36 (22.13) Mean (SD)[1] total T4: 8.79 (1.69); 9.29 (1.89); 9.54 (1.73) Mean (SD) TSH (mIU/L): 3.21 (1.78); 3.28 (1.30); 3.87 (1.48)	Competitive chemiluminescent immunoassay (CLIA) for T3 and T4 and ultra-sensitive sandwich CLIA for TSH	The present study was carried out among children with normal nutritional status and optimal iodine intake.	Age and iodine status
Siddiqui (1960)	Cross-sectional	India	2008 subjects (1495 children and 513 adults)	Water [F] (mg/L) 5.4; 6.1; 10.7	Thorium nitrate titration method	Goiter diagnosis (cases/participants): 21/1100; 24/813; 4/95	Clinical examination according established guidelines	Determination of iodine and fluoride levels in the study areas and population. Water iodine (μg/L): 175.30; 44.00; 14.40	Age and sex
Singh et al. (2014)	Cross-sectional	India	70 children aged 8–15 years	Water [F] (ppm): 1.60–5.10; 1.60–5.50; 0.98–1.00 Urinary [F] (ppm): 0.24–8.90; 0.40–7.79; 0.19–1.01 Serum [F] (ppm): 0.02–0.77; 0.03–0.75; 0.02–0.09	Fluoride ion specific electrode (Ion 85 Analyzer)	Range free T3 (pg/mL): 1.10–4.39; 1.20–4.57; 1.90–4.13 Range free T4 (ng/dL): 0.94–1.98; 0.80–1.70; 0.87–1.67 Range TSH (μIU/mL): 1.41–8.46; 1.92–10.99; 0.96–3.54	Immuno Chemiluminescence Microparticle Assay (ICMA)	No assessment	Age
Szczuko et al. (2019)	Case-control	Poland	40 women aged 18–38 years with PCOS (Polycystic ovary syndrome)	Serum [F] (ppm): 0.19; 0.25	Fluoride ion selective electrode (Orion 9409 BN)	Mean (SD) TSH (mIU/mL): 1.68 (0.81); 2.18 (0.60)	Electro-chemiluminescence immunoassay (ECLIA)	No assessment	Age and sex
Wang et al. (2020)	Cross-sectional	China	571 children aged 7–13 years	Water [F] (mg/L): 0.56; 0.85; 1.45; 2.28 Urinary [F] (mg/L): 0.12; 0.28; 1.35; 2.74	Fluoride ion selective electrode (ion analyzer EA940)	Water [F]: β (95% CI) total T3 (ng/mL): ref; −0.03 (−0.08, 0.02); 0.03 (−0.01, 0.07); 0.02 (−0.03, 0.06) β (95% CI) free T3 (pg/mL): ref; −0.02 (−0.11, 0.07); 0.09 (0.02, 0.16); 0.05 (−0.03, 0.12) β (95% CI) total T4 (μg/dL): ref; −0.38	Chemiluminescent microparticle immunoassay (ARCHITECT i4000SR)	None of the study sites was delimitated into endemic areas of iodine deficiency disorders which were determined by thyroid status examination and the median urinary iodine concentration in the population.	Age, sex, body mass index, paternal education, maternal education, household income, and low birth weight. Also, iodine status

(*continued on next page*)

Table 1 (*continued*)

Reference	Study design	Country	Population characteristics at baseline	Exposure categories (unit)	Exposure assessment method	Outcome	Outcome assessment method	Assessment of iodine status	Adjustments
						(−0.69, −0.07); −0.44 (−0.69, −0.19); −0.27 (−0.52, −0.02) β (95% CI) free T4 (ng/dL): ref; −0.03 (−0.06, 0.003); −0.03 (−0.05, −0.001); −0.04 (−0.06, −0.01) β (95% CI) TSH (μIU/mL): ref; −0.15 (0.52, 0.21); 0.24 (−0.01, 0.52); 0.31 (0.01, 0.60) Urinary [F]: β (95% CI) total T3 (ng/mL): ref; 0.04 (−0.001, 0.09); 0.08 (0.04, 0.13); 0.04 (−0.01, 0.08) β (95% CI) free T3 (pg/mL): ref; 0.05 (−0.03, 0.12); 0.14 (0.06, 0.21); 0.08 (0.001, 0.16) β (95% CI) total T4 (μg/dL): ref; 0.04 (−0.23, 0.31); −0.25 (−0.51, 0.02); −0.21 (−0.48, 0.06) β (95% CI) free T4 (ng/dL): ref; −0.002 (−0.03, 0.03); −0.03 (−0.06, 0.001); −0.02 (−0.05, 0.005) β (95% CI) TSH (μIU/mL): ref; 0.02 (−0.29, 0.33); 0.23 (−0.08, 0,54); 0.36 (0.05, 0.67)			
Wang et al. (2022)	Cross-sectional	China	413 children (198 boys and 215 girls) aged 7–12 years	Urinary [F] (mg/L): 0.78; 1.46	Fluoride ion selective electrode method	Median (Q1-Q3) free T3 (pmol/L): 6.49 (6.08, 6.93); 6.59 (6.23, 7.15) Median (Q1-Q3) free T4 (pmol/L): 18.39 (16.94, 19.77); 17.30 (16.07, 18.60) Median (Q1-Q3)	Electrochemiluminescence method for thyroid hormones, and B-mode ultrasound for thyroid volume (Tvol)	According to the data of children's urinary iodine published annually by the Tianjin Health Commission, Beichen and Xiqing are not iodine malnutrition areas. Determination of iodine and fluoride levels in the study areas and	Age, sex, and iodine status

(*continued on next page*)

Table 1 (*continued*)

Reference	Study design	Country	Population characteristics at baseline	Exposure categories (unit)	Exposure assessment method	Outcome	Outcome assessment method	Assessment of iodine status	Adjustments
						TSH (μIU/mL): 2.90 (2.24, 3.82); 2.75 (2.13, 4.21) Median (Q1-Q3) Tvol (ml): 3.63 (2.66, 4.44); 2.52 (2.02, 3.27)		population. Urinary iodine (μg/L): 167.00; 159.20	
Xu et al. (2022)	Cross-sectional	China	424 children (226 boys and 198 girls) aged 7–12 years	Urinary [F] (mg/L): 0.80; 1.51	Fluoride ion selective electrode method	Mean (SD) total T3 (nmol/L): 2.58 (0.45); 2.59 (0.59) Mean (SD) total T4 (nmol/L): 135.29 (25.42); 134.13 (26.20) Median (IQR) TSH: (μIU/L): 1.53 (1.18, 2.05); 1.58 (1.28, 2.03) Mean (SD) Tvol (cm3): 2.78 (1.11); 3.16 (1.16)	Radiation immunoassay (RIA) method for thyroid hormones, and B-mode ultrasound for thyroid volume (Tvol)	Almost all children had appropriate iodine intake, which may be due to the diversity of food and the implementation of iodized salt policy. Determination of iodine and fluoride levels in the study areas and population. Urinary iodine (μg/L): 351.70; 346.70	Age, sex, and iodine status
Yang et al. (2008)	Cross-sectional	China	1518 children aged 8–14 years	Water [F] (mg/L): 0.50; 2.97 Urinary [F] (mg/L): 0.82; 2.08	Fluoride ion selective electrode method	Mean (SD) total T3 (μg/dL): 0.74 (0.43); 0.76 (0.36) Mean (SD) total T4 (ng/dL): 128.46 (38.12); 147.83 (88.31) Mean (SD) TSH (μIU/mL): 0.82 (0.51); 3.37 (2.16) Goiter diagnosis (cases/ participants): 2/ 416; 42/1102	Radioimmunoassay (RIA) method for thyroid hormones and clinical examination according to national standards for endemic goiter control	Determination of iodine and fluoride levels in the study areas and population. Water iodine (μg/L): 128.6; 1100	Age
Yasmin et al. (2013)	Cross-sectional	India	145 subjects (43 children, 54 men and 48 women)	Water [F] (mg/L): 0.49; 2.82 Urinary [F] (mg/L): 0.32; 0.70 Serum [F] (mg/L): 0.05; 0.09	Fluoride ion selective electrode (Orion 9690)	Mean (SE) total T3 (pg/dL): Men: 1.12 (0.08); 1.85 (0.12) Women: 1.14 (0.09); 1.94 (0.12) Children: 0.65 (0.05); 2.65 (0.22) Mean (SE) total T4 (μg/L): Men: 9.59 (0.45); 10.35 (0.78) Women: 10.44 (0.80); 10.41 (0.82) Children: 9.25 (0.63); 13.93 (0.92) Mean (SE) TSH (mIU/L):	ELISA reader (ERBA Lisa scan EM)	No assessment	Age and sex

(*continued on next page*)

Table 1 (*continued*)

Reference	Study design	Country	Population characteristics at baseline	Exposure categories (unit)	Exposure assessment method	Outcome	Outcome assessment method	Assessment of iodine status	Adjustments
						Men: 2.75 (0.47); 2.42 (0.29) Women: 2.58 (0.29); 2.64 (0.28) Children: 0.47 (0.08); 3.63 (0.28)			
Zhang et al. (2015)	Cross-sectional	China	180 children aged 10–12 years	Water [F] (mg/L): 0.63; 1.40 Urinary [F] (mg/L): 1.10; 2.40 Serum [F] (mg/L): 0.06; 0.18	Fluoride Ion selective electrode method (ion analyzer EA940)	Mean (SD) total T3 (ng/mL): 2.15 (0.42); 2.22 (0.36) Mean (SD) total T4 (ng/mL): 90.79 (18.85); 85.65 (16.80) Median (IQR) TSH (μIU/mL): 2.59 (2.24–3.16); 3.11 (2.60–4.09)	Standard radioimmunoassay (RIA)	None of the study areas 2 sites was delimitated into endemic areas of iodine deficiency disorders.	Age, sex, and iodine status
Zulfiqar et al. (2019)	Cross-sectional	Pakistan	134 children aged 7–18 years	Water [F] (ng/L): 0.54; 4.66 Urinary [F] (ng/L): 1.52; 2.59 Serum [F] (ng/L): 0.05; 0.06	Fluoride ion selective electrode (F-ISE)	Mean (SD) free T3 (pmol/L): 5.09 (0.53); 5.06 (0.60) Mean (SD) free T4 (pmol/L): 17.55 (3.43); 18.30 (2.47) Mean (SD) TSH (mIU/L): 2.36 (1.06); 3.20 (2.20)	Competitive Radioimmunoassay (RIA) for T3 and T4, and sandwich-type Immunoradiometric assay (IRMA) for TSH	No assessment	Age
Zulfiqar et al. (2020)	Cross-sectional	Pakistan	190 children aged 7–18 years	Water [F] (mg/L): 0.54; 6.23 Urinary [F] (mg/L): 1.52; 3.38 Serum [F] (mg/L): 0.05; 0.21	Fluoride ion selective electrode (F-ISE)	Mean (SD) free T3 (pmol/L): 5.09 (0.53); 5.57 (1.01) Mean (SD) free T4 (pmol/L): 17.55 (3.43); 16.64 (3.78) Mean (SD) TSH (mIU/L): 2.36 (1.06); 4.41 (2.29)	Competitive Radioimmunoassay (RIA) for T3 and T4, and sandwich-type Immunoradiometric assay (IRMA) for TSH	No assessment	Age

Abbreviations: β = mean difference; CI 95% = confidence interval at 95%; Ecobody index = Tvol/body surface area (mL/m^2); F = fluoride; [F] = fluoride concentration; IQR = interquartile range; Q = quartile; NR = not reported; ref = reference; SD = standard deviation; SE = standard error; T3 = triiodothyronine; T4 = thyroxine or tetraiodothyronine; TSH = thyroid-stimulating hormone; Tvol = thyroid volume; [1] Such information has been considered in the study but the related data were not suitable for inclusion in the analyses due to missing units of measurement of outcome.

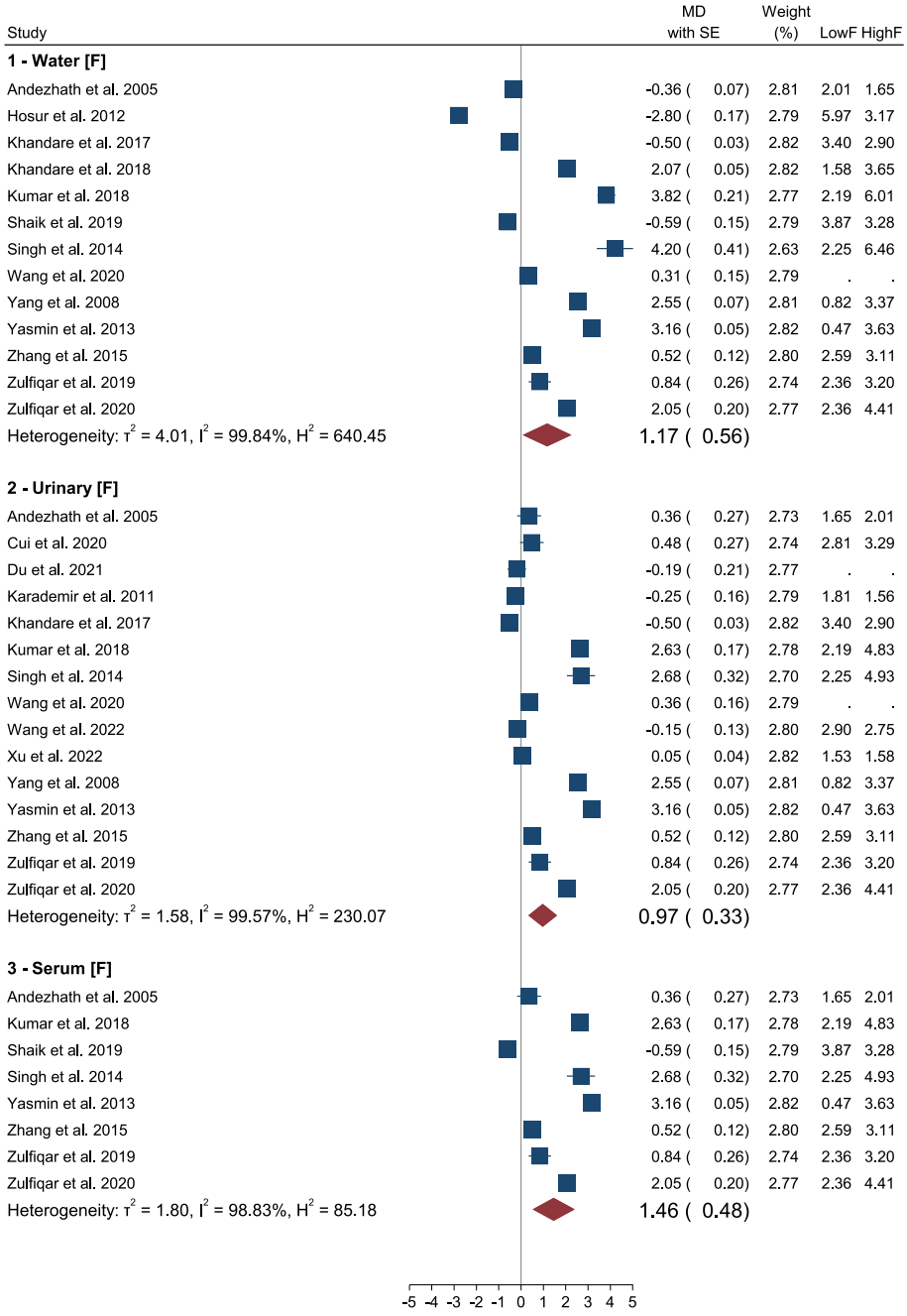

Fig. 2. Forest plot of the included studies regarding differences of TSH (μIU/mL) in children according to the highest and the lowest fluoride (F) exposure category (mg/L). The squares represent mean differences (MD) between the highest and the lowest exposure category and horizontal lines their standard errors (SE). The LowF and HighF columns report mean TSH values in the lowest and the highest fluoride exposure category, respectively. The area of each square is proportional to the weight of the study in the meta-analysis. The diamonds represent the summary mean difference, and the solid vertical line represents null value. Inverse-variance estimation method used for study weighting.

except for total T4, for which the summary mean differences were 0.82, −0.17, −0.51 μg/dL for drinking water (n = 4), urinary (n = 5), and serum (n = 1) fluoride exposure, respectively. Finally, there was some evidence of publication bias regarding thyroid hormones, both free and total (Supplementary Figs. S9–S12).

We also computed study-specific and summary mean differences of TSH and thyroid hormones in adults (≥19 years), comparing the highest versus the lowest fluoride categories and according to the type of exposure assessment method, which are shown in Supplementary Figs. S13–S16. TSH mean difference was 0.82 μIU/mL for water fluoride (n = 4), 0.89 μIU/mL for urinary fluoride (n = 3), 0.53 μIU/mL for serum fluoride (n = 3) and 2.00 μIU/mL for daily fluoride intake (n = 1), while differences for thyroid hormones across exposure categories were considerably lower. Total T3 mean differences were −0.12 ng/mL (n = 3), −0.17 ng/mL (n = 2), −0.17 ng/mL (n = 2) for water, urinary and serum fluoride, respectively, while calculation of study-specific and summary mean differences of free T3 could not be performed due to limited data availability. Moreover, summary total T4 mean differences were close to zero for water (n = 3), urinary (n = 3), and serum fluoride (n = 2), while for daily fluoride intake (n = 1) was 0.35 μg/day. Data on free T4 in adults were reported only by Hall et al. (2023), resulting in summary mean differences close to zero. The study-specific and

summary mean differences of TSH and thyroid hormones in adults, considering only "Low" and "Some concerns" risk of bias studies (n = 2), are displayed in Supplementary Fig. S17 and one of the studies in this forest plot is the one published by Hall et al. (2023), which is the only study in our meta-analysis having a cohort design and pregnant women as the studied population. Regarding publication bias for TSH in adults (Supplementary Fig. S18), there seemed to be some evidence of publication bias.

Finally, in Supplementary Fig. S19, we report the forest plot of the 2 studies regarding differences of TSH and thyroid hormones in the general population (without distinction between children and adults), according to the highest and the lowest fluoride exposure category, stratified by exposure and outcome. Considering the analyses for thyroid diseases (Supplementary Fig. S20), summary RRs for goiter in children was well above unity, i.e., 1.79 (95% CI: 1.18; 2.73) for the highest versus the lowest drinking water fluoride category (n = 3), and 7.93 (95% CI: 1.93; 32.59) for urinary fluoride (n = 1). RRs for thyroid disease in adults were computed only for water fluoride exposure due to lack of data for other exposure types, and were 7.38 (95% CI: 1.17; 46.53) for goiter (n = 1) and 1.62 (95% CI: 1.36; 1.93) for

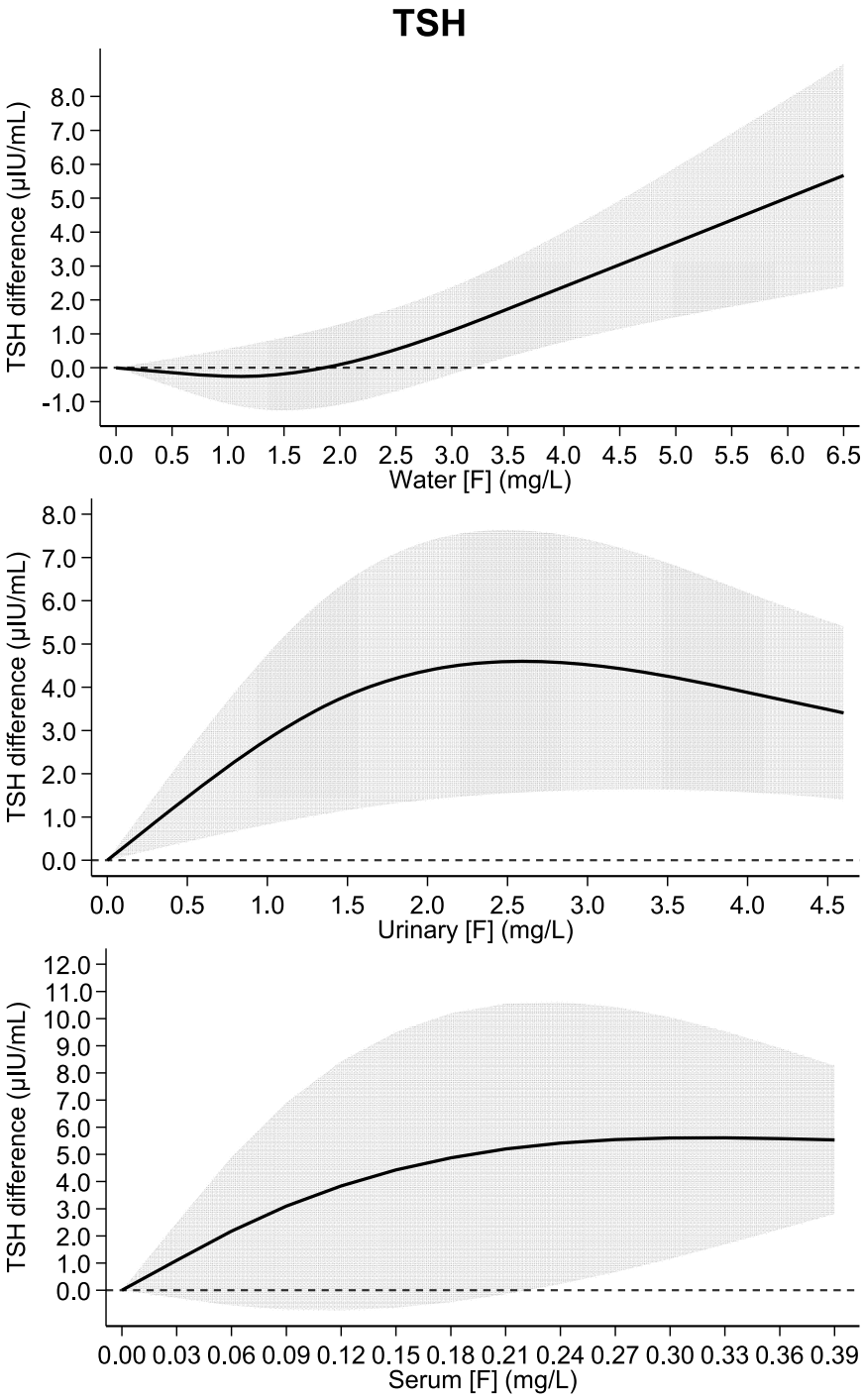

Fig. 3. Dose-response meta-analysis of TSH concentrations and exposure to fluoride (F) from drinking water (n = 13), urinary fluoride (n = 15) and serum fluoride (n = 8) in children. TSH difference curve (black solid line) with 95% confidence interval (grey area).

hypothyroidism (n = 1). Regarding the studies that considered both adults and children as population, without any distinction, the RR for goiter with exposure to fluoride in water (n = 4) was 2.63 (95% CI: 0.63; 10.91), while a RR of 0.09 (95% CI: 0.07; 0.11) was reported in the only study addressing 'unspecified' thyroid condition. Similar positive results were also observed excluding studies with the highest risk of bias, i.e., "High" and "Very high" risk of bias studies (Supplementary Fig. S21).

The dose-response meta-analysis based on a non-linear restricted cubic spline regression model was only conducted for studies assessing TSH levels (Fig. 3) and total T3 and T4 (Fig. 4) in children, given the limited number of studies available for other age groups and for other endpoints. The dose-response curve for water fluoride exposure (n = 13) showed no change in TSH concentrations in the lowest exposure range, while at a drinking water fluoride concentration around 2.0 mg/L, TSH levels started to increase, with a positive and approximately linear pattern. Compared with the analysis based on water fluoride concentrations, the shapes of the dose-response curves based on both urinary (n = 15) and serum fluoride (n = 8) showed moderately different patterns. TSH levels were positively associated with fluoride already at very low exposure and up to 2.5 mg/L for urinary and 0.2 mg/L for serum,

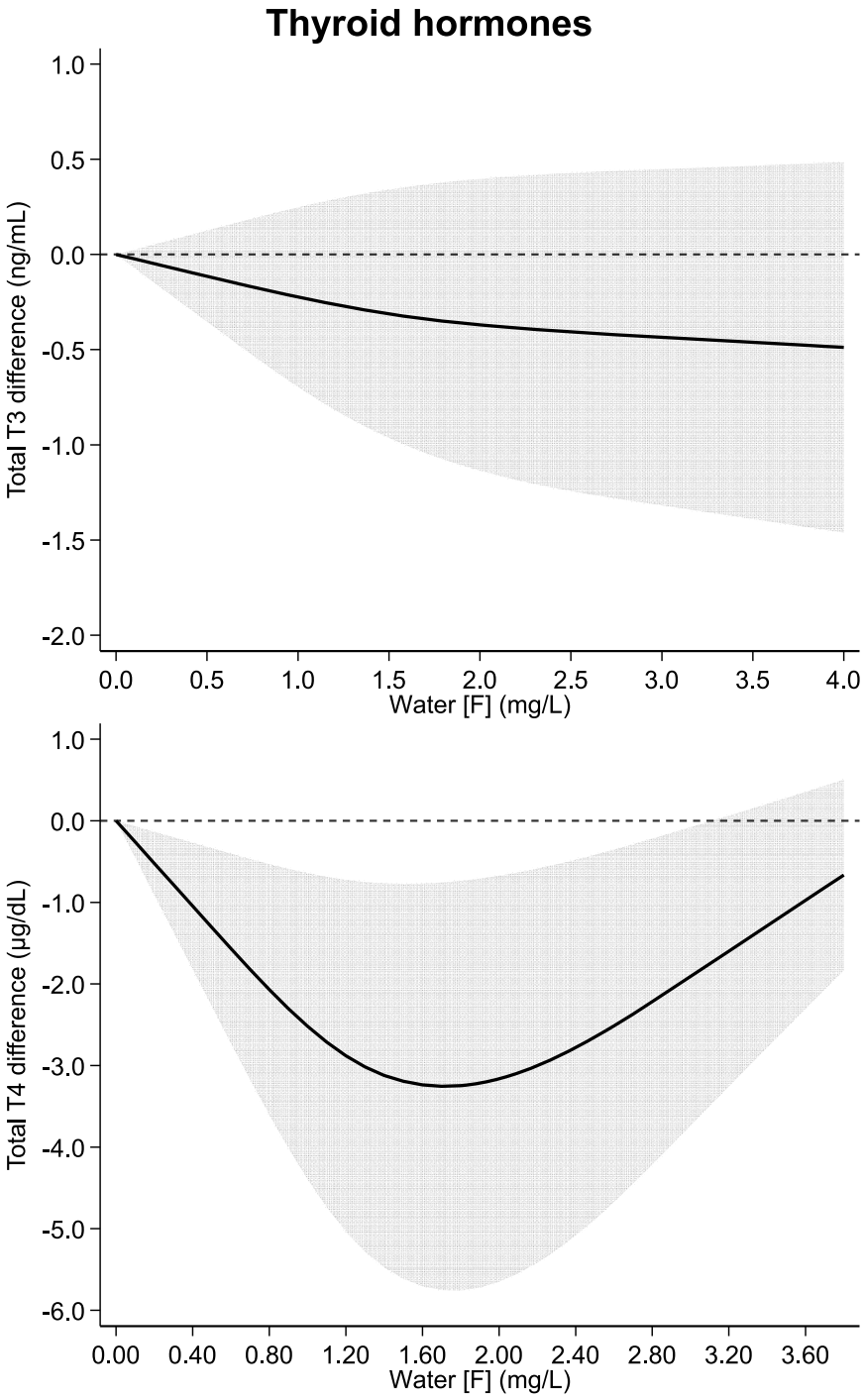

Fig. 4. Dose-response meta-analysis of total T3 (n = 6) and total T4 (n = 6) concentrations and exposure to fluoride (F) from drinking water in children. Difference curve (black solid line) with 95% confidence interval (grey area).

when both curves started to flatten, with even an indication of a downward pattern for urinary fluoride. The dose-response curves for total T3 (n = 6) and total T4 (n = 6) concentrations in children exposed to fluoride from drinking water are displayed in Fig. 4. Fluoride exposure was negatively associated with T3 and T4 concentrations already at very low levels and up to 2.0 mg/L, when the T3 curve started to flatten, while for T4 there was an upward pattern, resulting in a U-shaped curve.

We also performed sensitivity analyses excluding the studies belonging to the two categories with the highest risk of bias, i.e., "High" and "Very high". Such subgroup analyses for TSH in children (Fig. 5) revealed some changes in the shape of the spline curves for all the three indicators of fluoride exposure. The curve for water fluoride had a less steep slope, as well as a slightly higher threshold level (2.5 mg/L instead than the around 2.0 mg/L of the overall analysis), above which TSH started to increase. Conversely, the curve based on urinary fluoride flattened above 2.5 mg/L of the biomarker but lacked a downward inflection, and the one for serum fluoride had an almost linear, positive pattern. Fig. 6 displays sensitivity analyses with "Low" and "Some concerns" risk of bias studies for total T3 (n = 4) and total T4 (n = 4) concentrations in children exposed to fluoride from drinking water. Both

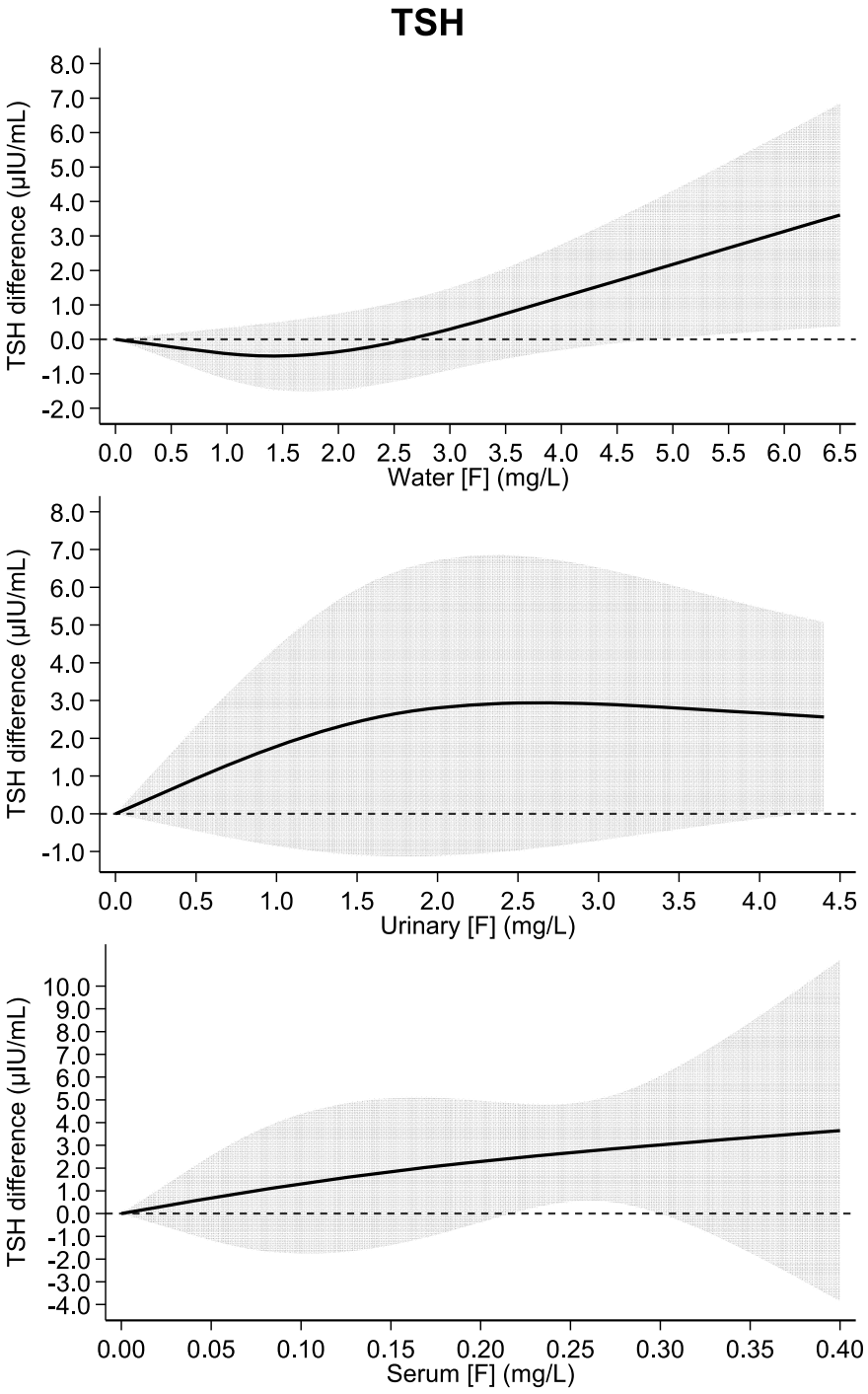

Fig. 5. Dose-response meta-analysis of TSH concentrations and exposure to fluoride (F) from drinking water (n = 8), urinary fluoride (n = 11) and serum fluoride (n = 5) in children, only with "Low" and "Some concerns" risk of bias studies. TSH difference curve (black solid line) with 95% confidence interval (grey area).

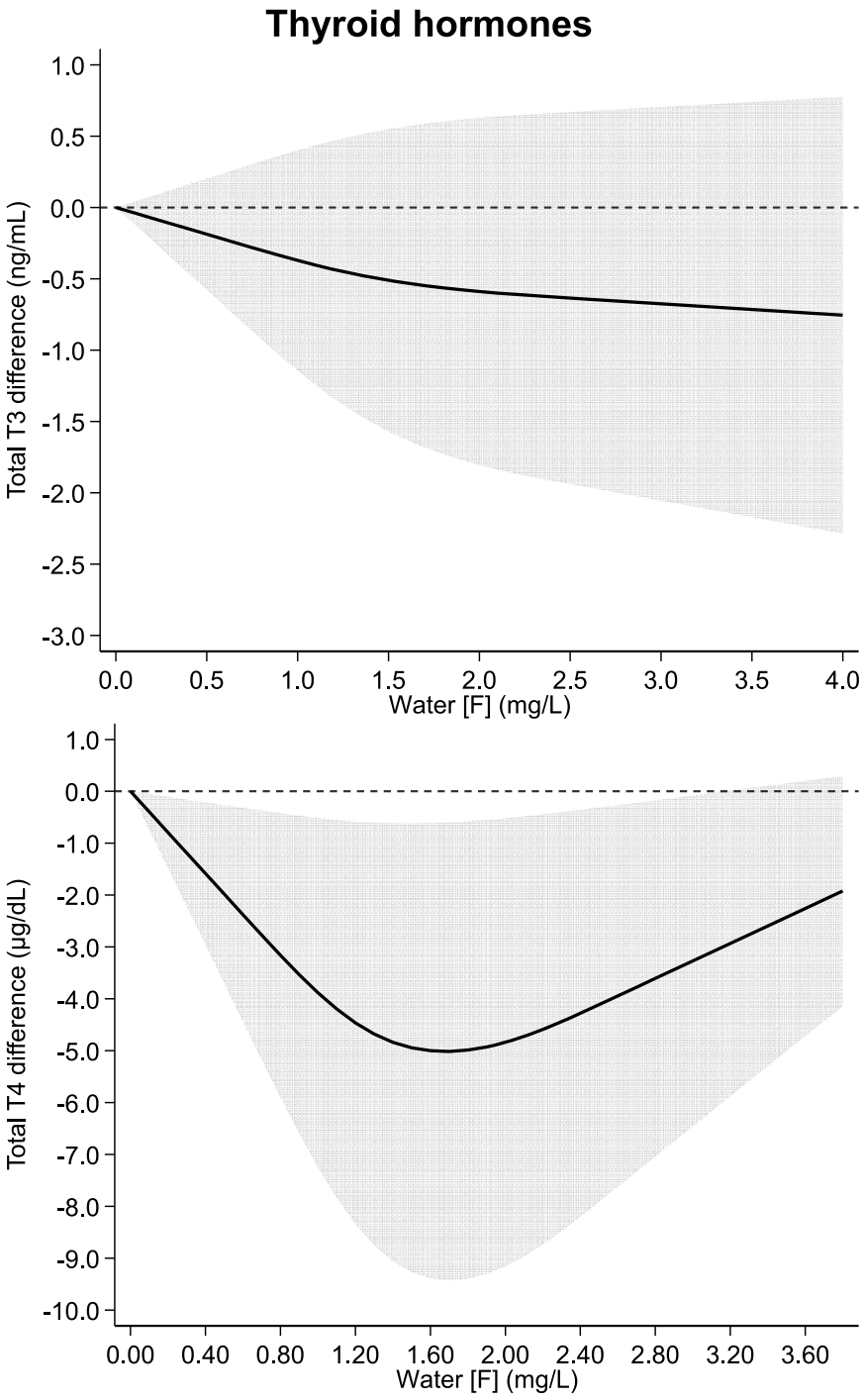

Fig. 6. Dose-response meta-analysis of total T3 (n = 4) and total T4 (n = 4) concentrations and exposure to fluoride (F) from drinking water in children, only with "Low" and "Some concerns" risk of bias studies. Difference curve (black solid line) with 95% confidence interval (grey area).

spline curves seem to have similar shapes to the ones from the overall analyses, which can be explained by the small number of studies excluded due to high risk of bias. With reference to study-specific dose-response curves for TSH concentrations in children (Supplementary Fig. S22), the dose-dependent patterns for water fluoride exposure showed substantial homogeneity, with comparable trends among them and for the overall curve, with only a few lines (n = 5) exceeding the confidence interval of the summary estimate. Conversely, analyses for both urinary and serum fluoride showed high heterogeneity, with different shapes and trends, and few of the urinary fluoride study-specific lines included within the confidence interval of the overall curve. Similarly, the study-specific lines for total T3 concentration in children exposed to fluoride in drinking water (Supplementary Fig. S23) showed high heterogeneity while those for total T4 had U-shape trends, but with different depths of their nadirs. For both analyses, no study-specific line was outside the confidence interval.

4. Discussion

In this systematic review and dose-response meta-analysis on thyroid

function as related to fluoride exposure, we found a clear pattern of association between fluoride content in drinking water consumed by the study participants and their circulating TSH concentrations. However, this occurred only above 2 mg/L of water fluoride (2.5 mg/L when the studies with the best quality were considered), thus confirming the hypothesis of a non-linear, dose-dependent pattern of association, something suggested to occur also for the adverse effects of drinking water fluoride on children's intelligence quotient (Veneri et al., 2023b). However, in the current pooled analysis on thyroid function, the removal of studies with the lowest methodological quality had a limited impact on the results, suggesting that the effect of biases on the thyroid effect estimates was not relevant. This positive but non-linear relation between exposure to fluoride through drinking water and circulating TSH concentrations was remarkably consistent across the many studies carried out about this association and included in this dose-response analysis (Andezhath et al., 2005; Hosur et al., 2012; Khandare et al., 2017, 2018; Kumar et al., 2018; Shaik et al., 2019; Singh et al., 2014; Wang et al., 2020; Yang et al., 2008; Yasmin et al., 2013; Zhang et al., 2015; Zulfiqar et al., 2019, 2020), further strengthening the possibility of a causal link.

Conversely, studies on TSH as related to biomarkers of fluoride exposure, i.e., urinary and serum fluoride, did not yield a pattern of association similar to that for water fluoride. The shape of the curve indicates a no-threshold positive association starting from the lowest exposure levels, and flattening at the highest exposure range, with the exception of studies of the highest methodological quality based on serum fluoride, for which the pattern of association was almost linear. Such discrepancies between fluoride exposure through drinking water and biomarkers could be due to different reasons, including the lower number of studies available for serum fluoride, possible differences among the analytical methods used in the included studies, or more importantly the lower reliability of studies based on biomarkers in reflecting real internal exposure (particularly for urinary fluoride), due to individual changes in fluoride absorption, metabolism and excretion. In addition, assessing fluoride exposure through a long-term, stable, and major determinant of intake, such as fluoride in drinking water, could be more reliable than considering more unstable and short-term indicators like urinary and serum fluoride levels (Hall et al., 2023), unless for very high exposures, when biomarkers tend to be considerably associated with the environmental sources of exposure. In fact, while residential drinking water fluoride level and water consumption tend to be substantially stable over time, fluoride intake with foods, beverages, or dental products can considerably vary. This may induce non-differential misclassification of antecedent exposure when using short-term biomarkers, biasing the risk and effect estimates towards the null. Demographic factors such as age and sex, lifestyle factors such as smoking, alcohol consumption and regular tooth-brushing, and dietary and metabolic factors influencing fluoride absorption and metabolism may also modify the association between fluoride exposure (as that occurring through drinking water or other sources) and biomarkers such as serum and urinary fluoride concentrations (Kishor et al., 2023; Maguire and Zohoori, 2013; Riddell et al., 2021). This is also reflected by the null or low correlation between fluoride exposure and fluoride biomarkers found in some of the studies (Carwile et al., 2020; Lavalle-Carrasco et al., 2023; Saad et al., 2022; Zohoori et al., 2019). Overall, the limitations of biomarkers (unless based on repetitive samples) in adequately reflecting long-term fluoride exposure could explain the different patterns of the dose-response associations for individual studies based on urinary or serum fluoride concentrations, as compared to the substantially homogeneous dose-response curves across studies on drinking water fluoride levels, and the different results for the various exposure indicators within single epidemiologic studies (Hall et al., 2023; Malin et al., 2018; Riddell et al., 2019; Yu et al., 2018b) as does the current meta-analysis. Interestingly, a dose-response pattern comparable to that found in our pooled analysis emerged from a Chinese cross-sectional study (Yu et al., 2018b) assessing the relation of water and urinary fluoride with dental fluorosis, where a threshold was identified of slightly less than 1 mg/L of water fluoride before detecting a positive association, while the association started already at the lowest urinary fluoride concentrations (from 0.01 mg/L). Consistently, in a recently published cross-sectional analysis of a cohort study in Canada (Hall et al., 2023), no linear association emerged between urinary fluoride and risk of hypothyroidism, while such association was positive with fluoride intake through beverages (OR: 1.25; 95% CI: 0.99–1.57), and particularly with drinking water fluoride concentrations (OR: 1.65; 95% CI: 1.04–2.60).

Despite the strong association between fluoride exposure and TSH, we were unable to confirm such a clear association (expected on the opposite direction) for circulating thyroid hormones, either total or free. This may have been due to the limited number of studies that precluded a reliable analysis through a spline-based non-linear model, or to the real absence of such effects, despite the forest plot indicated some slight changes, mostly a decrease, in the circulating concentrations of these hormones. In addition, such possible effects on circulating thyroid hormones should be better appreciated at the individual level, particularly in subjects characterized by subtle thyroid hormone deficiency or located at the lowest range of such levels, these likely being the individuals most susceptible to fluoride-induced adverse effects on thyroid function. Finally, it cannot be excluded that different analytical methods for thyroid hormones might have affected comparisons across studies.

With reference to thyroid disease risk, we could retrieve too few studies for a dose-response meta-analysis. Results of such studies (Barberio et al., 2017; Day and Powell-Jackson, 1972; Eltom et al., 1984; Hong et al., 2008; Jooste et al., 1999; Lathman and Grech, 1967; Peckham et al., 2015; Siddiqui, 1960; Yang et al., 2008), either in children or in adults or in mixed populations, generally indicated a higher (in some cases much higher) risk of goiter, hypothyroidism, or thyroid diseases overall considered, with the exception of the few observations in the mixed population, showing a very inconsistent overall picture. Overall, the evidence available for thyroid disease risk as associated with fluoride exposure is too limited to draw conclusions, though there is a general indication of detrimental effects of exposure to the highest levels of fluoride occurring in the included studies. This appears also supported by biological plausibility, since laboratory and animal studies have suggested that excess fluoride exposure may decrease thyroid hormone synthesis and increase circulating TSH levels (Skórka-Majewicz et al., 2020). Fluoride may damage the proper functioning of the thyroid, interfering with Na/K-ATPase and with iodothyronine deiodinase, disrupting sensitive G-proteins of hormone receptors, mimicking TSH, and impairing T4 conversion into to T3, through effects on the enzymes catalyzing deiodination i.e., iodothyronine deiodinases (Bürgi et al., 1984; Hong-liang et al., 2014; Peeters and Visser, 2000; Shashi and Singla, 2013; Skórka-Majewicz et al., 2020; Spittle, 2016; Strunecka and Strunecky, 2020; Susheela and Toteja, 2018; Waugh, 2019). Fluoride could also damage thyroid cells by determining mitochondrial swelling and disintegration, and by inducing DNA-RNA damage (Peeters and Visser, 2000; Wei et al., 2022). It may also have adverse effects on thyroid cells and thyroid follicular morphology, altering thyroid structure (Liu et al., 2016; Sarkar and Pal, 2014; Yu et al., 2018a).

We must outline a few limitations of our assessment. First, some the epidemiologic studies did not provide the data to assess the dose-response relation between fluoride exposure and thyroid function and disease (for instance reporting only linear regression coefficients for the overall association). Secondly, it is known that both excess and deficient iodine intake may affect thyroid function and morphology, as well as could interact with fluoride in modifying the impact of both elements (Jiang et al., 2016; Malin et al., 2018). However, such potential confounding effect and interactions have not been generally addressed in the included studies, with some exceptions. Some studies investigated subjects with homogeneous iodine exposure while others determined iodine exposure in the same categories of fluoride exposure, most of which finding changes in the same direction of iodine and fluoride

levels. Only one study (Du et al., 2021) adjusted for urinary iodine in the multivariable analysis, finding no association between urinary fluoride and TSH. In addition, there is some evidence of susceptibility of pregnant women to fluoride-induced hypothyroidism but based on one study only (Hall et al., 2023). More data are clearly required to better investigate the relation between fluoride exposure and thyroid function and disease risk, considering the overall nutritional status in relation to iodine and other trace elements as well as environmental pollutants possibly associated with thyroid function and disease (Chamot et al., 2023; Ge et al., 2023; Kim et al., 2022; Loomba et al., 2020; Urbano et al., 2022a, 2022b). In addition, fluoride toxicity on thyroid function, disease risk, and morphology could theoretically be enhanced in individuals carrying some functional alterations and impairment of the gland, or at an early stage of thyroid disease, and such a possible higher susceptibility should be investigated in-depth in epidemiologic and clinical studies. Genetic factors should also be taken into consideration when assessing the risk of any environmental exposure, including fluoride (Chakraborty et al., 2022; Cui et al., 2018; González-Casamada et al., 2022), and the availability of data with reference to fluoride overexposure and its effects on the thyroid gland is unfortunately very poor, being limited to few studies with inconclusive results (Wang et al., 2022; Xu et al., 2022; Zhang et al., 2015). Finally, since all the included studies are observational, the potential impact of unmeasured confounding cannot be ruled out, adding caution to the interpretation of the individual studies and of our pooled analysis. However, most of the studies appear to have considered major factors affecting TSH levels, such as age and sex (Xing et al., 2021).

Our findings are of considerable public health relevance given the importance of thyroid function for human health (including cognitive neurodevelopment), and the rather frequent occurrence of high fluoride concentrations in groundwaters in several regions of the world, including Africa (Rift Valley like Tanzania, Kenya and Ethiopia), Asia, and America. The World Health Organization (WHO) has estimated that around 260 million people inhabit locations having excessive fluoride levels in drinking water (>1.5 mg/L) globally (Jha and Tripathi, 2021). Our findings are also relevant to the public health issue of water fluoridation in order to prevent dental disease and caries in particular, given the uncertainties and the controversies surrounding this topic and the need of a comprehensive risk assessment of such type of intervention (Till and Green, 2021).

In conclusion, our systematic review and dose-response meta-analysis showed that at the highest levels of naturally occurring fluoride exposure there are detrimental effects on thyroid function and possibly thyroid disease risk, whose most evident and consistent finding is a dose-dependent increase in TSH concentrations associated with consumption of drinking water above 2.5 mg/L of fluoride.

Funding

This study did not receive any specific funding.

CRediT authorship contribution statement

Inga Iamandii: Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Lisa De Pasquale:** Data curation, Methodology, Writing - original draft, Writing - review & editing. **Maria E. Giannone:** Methodology, Writing - review & editing. **Federica Veneri:** Conceptualization, Writing - review & editing. **Luigi Generali:** Writing - review & editing. **Ugo Consolo:** Writing - review & editing. **Linda S. Birnbaum:** Writing - review & editing. **Jacqueline Castenmiller:** Writing - review & editing. **Thorallur I. Halldorsson:** Writing - review & editing. **Tommaso Filippini:** Conceptualization, Data curation, Methodology, Supervision, Writing - original draft, Writing - review & editing. **Marco Vinceti:** Conceptualization, Methodology, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117759.

References

Ahmad, S., Singh, R., Arfin, T., Neeti, K., 2022. Fluoride contamination, consequences and removal techniques in water: a review. Adv. Environ. Sci. 1, 620–661. https://doi.org/10.1039/D1VA00039J.

Ahmed, I., Ghuman, F., Salman, S., Fatima, I., 2022. Does drinking water with raised fluoride content affect the thyroid hormone status: a study from Tharparker Pakistan. J. Pakistan Med. Assoc. 72, 228–230. https://doi.org/10.47391/jpma.481.

Andezhath, S., Bhatnagar, M., Vig, K., Mondal, N.K., 2005. Excess fluoride ingestion and thyroid hormone derangements in children living in Delhi, India. Fluoride 3838, 98–108.

Bachinsky, P.P., Gutsalenko, O.A., Naryzhnyuk, N.D., 1985. Fluorine effect on function of the pituitary-thyroid system in the body of healthy persons and patients with thyropathies. Probl. Endokrinol. 31, 25–29. https://doi.org/10.14341/probl198531625-29.

Barberio, A.M., Hosein, F.S., Quiñonez, C., McLaren, L., 2017. Fluoride exposure and indicators of thyroid functioning in the Canadian population: implications for community water fluoridation. J. Epidemiol. Community Health 71, 1019–1025. https://doi.org/10.1136/jech-2017-209129.

Bürgi, H., Siebenhüner, L., Miloni, E., 1984. Fluorine and thyroid gland function: a review of the literature. Klin. Wochenschr. 62, 564–569. https://doi.org/10.1007/BF01728174.

Carwile, J.L., Ahrens, K.A., Seshasayee, S.M., Lanphear, B., Fleisch, A.F., 2020. Predictors of plasma fluoride concentrations in children and adolescents. Int. J. Environ. Res. Publ. Health 17, 9205. https://doi.org/10.3390/ijerph17249205.

CDC, 2020. Community Water Fluoridation. fluoridation/index.html?CDC_AA_refVal=.cdc.gov%2Ffluoridation%2Findex.htm. (Accessed 29 October 2023).

Chaitanya, N., Karunakar, P., Allam, N.S.J., Priya, M.H., Alekhya, B., Nauseen, S., 2018. A systematic analysis on possibility of water fluoridation causing hypothyroidism. Indian J. Dent. Res. 29, 358–363. https://doi.org/10.4103/ijdr.IJDR_505_16.

Chakraborty, A., Pramanik, S., Datta, K., Goswami, R., Saha, D., Majumdar, K.K., Sikdar, N., 2022. Possible association between polymorphisms in ESR1, COL1A2, BGLAP, SPARC, VDR, and MMP2 genes and dental fluorosis in a population from an endemic eegion of West Bengal. Biol. Trace Elem. Res. 200, 4641–4653. https://doi.org/10.1007/s12011-021-03072-8.

Chamot, S., Al-Salameh, A., Petit, P., Bonneterre, V., Cance, C., Decocq, G., Boullier, A., Braun, K., Desailloud, R., 2023. Does prenatal exposure to multiple airborne and tap-water pollutants increase neonatal thyroid-stimulating hormone concentrations? Data from the Picardy region. France. Sci. Total Environ. 905, 167089 https://doi.org/10.1016/j.scitotenv.2023.167089.

Crippa, A., Discacciati, A., Bottai, M., Spiegelman, D., Orsini, N., 2019. One-stage dose-response meta-analysis for aggregated data. Stat. Methods Med. Res. 28, 1579–1596. https://doi.org/10.1177/0962280218773122.

Cui, Y., Zhang, B., Ma, J., Wang, Y., Zhao, L., Hou, C., Yu, J., Zhao, Y., Zhang, Z., Nie, J., Gao, T., Zhou, G., Liu, H., 2018. Dopamine receptor D2 gene polymorphism, urine fluoride, and intelligence impairment of children in China: a school-based cross-sectional study. Ecotoxicol. Environ. Saf. 165, 270–277. https://doi.org/10.1016/j.ecoenv.2018.09.018.

Cui, Y., Yu, J., Zhang, B., Guo, B., Gao, T., Liu, H., 2020. The relationships between thyroid-stimulating hormone and/or dopamine levels in peripheral blood and IQ in children with different urinary iodine concentrations. Neurosci. Lett. 729, 134981 https://doi.org/10.1016/j.neulet.2020.134981.

Day, T.K., Powell-Jackson, P.R., 1972. Fluoride, water hardness, and endemic goitre. Lancet 299, 1135–1138. https://doi.org/10.1016/S0140-6736(72)91370-0.

Di Federico, S., Filippini, T., Whelton, P.K., Cecchini, M., Iamandii, I., Boriani, G., Vinceti, M., 2023. Alcohol intake and blood pressure levels: a dose-response meta-analysis of nonexperimental cohort studies. Hypertension 80, 1961–1969. https://doi.org/10.1161/hypertensionaha.123.21224.

Du, Y., Zhou, G., Gong, B., Ma, J., An, N., Gao, M., Yang, M., Ma, Q., Huang, H., Zuo, Q., Ba, Y., 2021. Iodine modifies the susceptibility of thyroid to fluoride exposure in school-age children: a cross-sectional study in Yellow River basin, Henan, China. Biol. Trace Elem. Res. 199, 3658–3666. https://doi.org/10.1007/s12011-020-02519-8.

Duan, Q., Jiao, J., Chen, X., Wang, X., 2018. Association between water fluoride and the level of children's intelligence: a dose-response meta-analysis. Publ. Health 154, 87–97. https://doi.org/10.1016/j.puhe.2017.08.013.

Egger, M., Davey Smith, G., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ 315, 629–634. https://doi.org/10.1136/bmj.315.7109.629.

Eltom, M., Hofvander, Y., Torelm, I., Fellström, B., 1984. Endemic goitre in the Darfur region (Sudan). Epidemiology and aetiology. Acta Med. Scand. 215, 467–475. https://doi.org/10.1111/j.0954-6820.1984.tb17680.x.

Fewtrell, L., Smith, S., Kay, D., Bartram, J., 2007. An attempt to estimate the global burden of disease due to fluoride in drinking water. J. Water Health 4, 533–542. https://doi.org/10.2166/wh.2006.045.

Filippini, T., Malavolti, M., Whelton, P.K., Naska, A., Orsini, N., Vinceti, M., 2021. Blood pressure effects of sodium reduction: dose-response meta-analysis of experimental studies. Circulation 143, 1542–1567. https://doi.org/10.1161/circulationaha.120.050371.

Filippini, T., Wise, L.A., Vinceti, M., 2022. Cadmium exposure and risk of diabetes and prediabetes: a systematic review and dose-response meta-analysis. Environ. Int. 158, 106920 https://doi.org/10.1016/j.envint.2021.106920.

Fiore, G., Veneri, F., Di Lorenzo, R., Generali, L., Vinceti, M., Filippini, T., 2023. Fluoride exposure and ADHD: a systematic review of epidemiological studies. Medicina 59, 797. https://doi.org/10.3390/medicina59040797.

Ge, X., He, J., Lin, S., Bao, Y., Zheng, Y., Cheng, H., Cai, H., Feng, X., Yang, W., Hu, S., Wang, L., Liao, Q., Wang, F., Liu, C., Chen, X., Zou, Y., Yang, X., 2023. Associations of metal mixtures with thyroid function and potential interactions with iodine status: results from a cross-sectional study in MEWHC. Environ. Sci. Pollut. 30, 105665–105674. https://doi.org/10.1007/s11356-023-29682-4.

Ghosh, A., Mukherjee, K., Ghosh, S.K., Saha, B., 2013. Sources and toxicity of fluoride in the environment. Res. Chem. Intermed. 39, 2881–2915. https://doi.org/10.1007/s11164-012-0841-1.

González-Casamada, C., Nevarez-Rascón, M., Nevarez-Rascón, A., González-Galván, M., Isiordia-Espinoza, M.A., Bologna-Molina, R., Sánchez-Pérez, L., Molina-Frechero, N., 2022. Single nucleotide polymorphisms and dental fluorosis: a systematic review. Dent. J. 10, 211. https://doi.org/10.3390/dj10110211.

Hall, M., Lanphear, B., Chevrier, J., Hornung, R., Green, R., Goodman, C., Ayotte, P., Martinez-Mier, E.A., Zoeller, R.T., Till, C., 2023. Fluoride exposure and hypothyroidism in a Canadian pregnancy cohort. Sci. Total Environ. 869, 161149 https://doi.org/10.1016/j.scitotenv.2022.161149.

Higgins, J., Morgan, R., Rooney, A., Taylor, K., Thayer, K., Silva, R., Lemeris, C., Akl, A., Arroyave, W., Bateson, T., Berkman, N., Demers, P., Forastiere, F., Glenn, B., Hróbjartsson, A., Kirrane, E., LaKind, J., Luben, T., Lunn, R., McAleenan, A., McGuinness, L., Meerpohl, J., Mehta, S., Nachman, R., Obbagy, J., O'Connor, A., Radke, E., Savović, J., Schubauer-Berigan, M., Schwingl, P., Schunemann, H., Shea, B., Steenland, K., Stewart, T., Straif, K., Tilling, K., Verbeek, V., Vermeulen, R., Viswanathan, M., Zahm, S., Sterne, J., 2023. Risk of bias in non-randomized studies - of exposure (ROBINS-E). https://www.riskofbias.info/welcome/robins-e-tool. (Accessed 17 September 2023).

Higgins, J.P., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. BMJ 327, 557–560. https://doi.org/10.1136/bmj.327.7414.557.

Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A., 2019. Cochrane Handbook for Systematic Reviews of Interventions. John Wiley & Sons, Chichester (UK).

Hong, F., Cao, Y., Wangb, H., 2008. Research on the effects of fluoride on child intellectual development under different environmental conditions. Fluoride 41, 156–160.

Hong-liang, L., Qiang, Z., Yu-shan, C., Lei, Z., Gang, F., Chang-chun, H., Liang, Z., Aiguoc, W., 2014. Fluoride-induced thyroid cell apoptosis. Fluoride 47, 161–169.

Hosur, M.B., Puranik, R.S., Vanaki, S., Puranik, S.R., 2012. Study of thyroid hormones free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH) in subjects with dental fluorosis. Eur. J. Dermatol. 6, 184–190. https://doi.org/10.1055/s-0039-1698949.

Jha, P.K., Tripathi, P., 2021. Arsenic and fluoride contamination in groundwater: a review of global scenarios with special reference to India. Groundw. Sustain. Dev. 13, 100576 https://doi.org/10.1016/j.gsd.2021.100576.

Jiang, Y., Guo, X., Sun, Q., Shan, Z., Teng, W., 2016. Effects of excess fluoride and iodide on thyroid function and morphology. Biol. Trace Elem. Res. 170, 382–389. https://doi.org/10.1007/s12011-015-0479-0.

Jooste, P.L., Weight, M.J., Kriek, J.A., Louw, A.J., 1999. Endemic goitre in the absence of iodine deficiency in schoolchildren of the Northern Cape Province of South Africa. Eur. J. Clin. Nutr. 53, 8–12. https://doi.org/10.1038/sj.ejcn.1600671.

Karademir, S., Akçam, M., Kuybulu, A.E., Olgar, S., Oktem, F., 2011. Effects of fluorosis on QT dispersion, heart rate variability and echocardiographic parameters in children. Anadolu Kardiyol. Derg. 11, 150–155. https://doi.org/10.5152/akd.2011.038.

Khandare, A.L., Gourineni, S.R., Validandi, V., 2017. Dental fluorosis, nutritional status, kidney damage, and thyroid function along with bone metabolic indicators in school-going children living in fluoride-affected hilly areas of Doda district, Jammu and Kashmir, India. Environ. Monit. Assess. 189, 579. https://doi.org/10.1007/s10661-017-6288-5.

Khandare, A.L., Validandi, V., Gourineni, S.R., Gopalan, V., Nagalla, B., 2018. Dose-dependent effect of fluoride on clinical and subclinical indices of fluorosis in school going children and its mitigation by supply of safe drinking water for 5 years: an Indian study. Environ. Monit. Assess. 190, 110. https://doi.org/10.1007/s10661-018-6501-1.

Kheradpisheh, Z., Mirzaei, M., Mahvi, A.H., Mokhtari, M., Azizi, R., Fallahzadeh, H., Ehrampoush, M.H., 2018. Impact of drinking water fluoride on human thyroid hormones: a case-control study. Sci. Rep. 8, 2674. https://doi.org/10.1038/s41598-018-20696-4.

Kim, K., Argos, M., Persky, V.W., Freels, S., Sargis, R.M., Turyk, M.E., 2022. Associations of exposure to metal and metal mixtures with thyroid hormones: results from the NHANES 2007-2012. Environ. Res. 212, 113413 https://doi.org/10.1016/j.envres.2022.113413.

Kishor, K., Singsit, J.S., Pittman, C.U., Mohan, D., 2023. Demographic study of fluoride excretion vs intake: influence of variables. J. Hazard Mater. 10, 100312 https://doi.org/10.1016/j.hazadv.2023.100312.

Kumar, V., Chahar, P., Kajjari, S., Rahman, F., Bansal, D.K., Kapadia, J.M., 2018. Fluoride, thyroid hormone derangements and its correlation with tooth eruption pattern among the pediatric population from endemic and non-endemic fluorosis areas. J. Contemp. Dent. Pract. 19, 1512–1516. https://doi.org/10.5005/jp.journals-10024-2458.

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

Lathman, M.C., Grech, P., 1967. The effects of excessive fluoride intake. Am. J. Public Health Nation's Health 57, 651–660. https://doi.org/10.2105/ajph.57.4.651.

Lavalle-Carrasco, J., Vergara-Onofre, M., González-González, R., Bologna-Molina, R., Isiordia-Espinoza, M.A., Gaona, E., Molina-Frechero, N., 2023. A systematic review and meta-analysis of the relationship between the severity of dental fluorosis and fluoride biomarkers in endemic areas. Biol. Trace Elem. Res. 201, 1051–1062. https://doi.org/10.1007/s12011-022-03227-1.

Liu, H., Hou, C., Zeng, Q., Zhao, L., Cui, Y., Yu, L., Wang, L., Zhao, Y., Nie, J., Zhang, B., Wang, A., 2016. Role of endoplasmic reticulum stress-induced apoptosis in rat thyroid toxicity caused by excess fluoride and/or iodide. Environ. Toxicol. Pharmacol. 46, 277–285. https://doi.org/10.1016/j.etap.2016.08.007.

Liu, H.-l., Zeng, Q., Cui, Y.-s., Zhang, L., Fu, G., Hou, C.-c., Zhao, L., Wang, A., 2014. Fluoride-induced thyroid cell apoptosis. Fluoride 47, 161–169.

Loomba, R., Filippini, T., Chawla, R., Chaudhary, R., Cilloni, S., Datt, C., Singh, S., Dhillon, K.S., Vinceti, M., 2020. Exposure to a high selenium environment in Punjab, India: effects on blood chemistry. Sci. Total Environ. 716, 135347 https://doi.org/10.1016/j.scitotenv.2019.135347.

Lubojanski, A., Piesiak-Panczyszyn, D., Zakrzewski, W., Dobrzynski, W., Szymonowicz, M., Rybak, Z., Mielan, B., Wiglusz, R.J., Watras, A., Dobrzynski, M., 2023. The safety of fluoride compounds and their effect on the human body-A narrative review. Materials 16, 1242. https://doi.org/10.3390/ma16031242.

Maguire, A., Zohoori, F.V., 2013. Fluoride balance in infants and young children in the U. K. and its clinical relevance for the dental team. Br. Dent. J. 214, 587–593. https://doi.org/10.1038/sj.bdj.2013.531.

Malin, A.J., Riddell, J., McCague, H., Till, C., 2018. Fluoride exposure and thyroid function among adults living in Canada: effect modification by iodine status. Environ. Int. 121, 667–674. https://doi.org/10.1016/j.envint.2018.09.026.

Michael, M., Barot, V., Chinoy, N., 1996. Investigations of soft tissue functions in fluorotic individuals of north Gujarat. Fluoride 29, 63–71.

Morgan, R.L., Whaley, P., Thayer, K.A., Schünemann, H.J., 2018. Identifying the PECO: a framework for formulating good questions to explore the association of environmental and other exposures with health outcomes. Environ. Int. 121, 1027–1031. https://doi.org/10.1016/j.envint.2018.07.015.

Murad, M.H., Verbeek, J., Schwingshackl, L., Filippini, T., Vinceti, M., Akl, E., Morgan, R.L., Mustafa, R.A., Zeraatkar, D., Senerth, E., Street, R., Lin, L., Falck-Ytter, Y., Guyatt, G., Schunemann, H.J., Group, G.W., 2023. GRADE guidance 38: updated guidance for rating up certainty of evidence due to a dose-response gradient. J. Clin. Epidemiol. 164, 45–53. https://doi.org/10.1016/j.jclinepi.2023.09.011.

Orsini, N., Larsson, S.C., Salanti, G., 2022. Dose–response meta-analysis. Systematic reviews in health research 258–269. https://doi.org/10.1002/9781119099369.ch14.

Ouzzani, M., Hammady, H., Fedorowicz, Z., Elmagarmid, A., 2016. Rayyan—a web and mobile app for systematic reviews. Syst. Rev. 5, 210. https://doi.org/10.1186/s13643-016-0384-4.

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., Moher, D., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372, n71. https://doi.org/10.1136/bmj.n71.

Peckham, S., Awofeso, N., 2014. Water fluoridation: a critical review of the physiological effects of ingested fluoride as a public health intervention. Sci. World J. 2014, 293019 https://doi.org/10.1155/2014/293019.

Peckham, S., Lowery, D., Spencer, S., 2015. Are fluoride levels in drinking water associated with hypothyroidism prevalence in England? A large observational study of GP practice data and fluoride levels in drinking water. J. Epidemiol. Community Health 69, 619–624. https://doi.org/10.1136/jech-2014-204971.

Peeters, R.P., Visser, T.J., 2000. Metabolism of thyroid hormone. In: Feingold, K.R., Anawalt, B., Blackman, M.R., Boyce, A., Chrousos, G., Corpas, E., de Herder, W.W., Dhatariya, K., Dungan, K., Hofland, J., Kalra, S., Kaltsas, G., Kapoor, N., Koch, C., Kopp, P., Korbonits, M., Kovacs, C.S., Kuohung, W., Laferrère, B., Levy, M., McGee, E.A., McLachlan, R., New, M., Purnell, J., Sahay, R., Shah, A.S., Singer, F., Sperling, M.A., Stratakis, C.A., Trence, D.L., Wilson, D.P. (Eds.), Thyroid Disease Manager. MDText.com, Inc.,, South Dartmouth (MA).

Riddell, J.K., Malin, A.J., Flora, D., McCague, H., Till, C., 2019. Association of water fluoride and urinary fluoride concentrations with attention deficit hyperactivity disorder in Canadian youth. Environ. Int. 133, 105190. https://doi.org/10.1016/j.envint.2019.105190.

Riddell, J.K., Malin, A.J., McCague, H., Flora, D.B., Till, C., 2021. Urinary fluoride levels among Canadians with and without community water fluoridation. Int. J. Environ. Res. Publ. Health 18, 6203. https://doi.org/10.3390/ijerph18126203.

Saad, H., Escoube, R., Babajko, S., Houari, S., 2022. Fluoride intake through dental care products: a systematic review. Front. Oral Health 3, 916372. https://doi.org/10.3389/froh.2022.916372.

Sarkar, C., Pal, S., 2014. Ameliorative effect of resveratrol against fluoride-induced alteration of thyroid function in male wistar rats. Biol. Trace Elem. Res. 162, 278–287. https://doi.org/10.1007/s12011-014-0108-3.

Shahab, S., Mustafa, G., Khan, I., Zahid, M., Yasinzai, M., Ameer, N., Asghar, N., Ullah, I., Nadhman, A., Ahmed, A., Munir, I., Mujahid, A., Hussain, T., Ahmad, M.N., Ahmad, S., 2017. Effects of fluoride ion toxicity on animals, plants, and soil health: a review. Fluoride 50, 393–408.

Shaik, N., Shanbhog, R., Nandlal, B., Tippeswamy, H.M., 2019. Fluoride and thyroid function in children resident of naturally fluoridated areas consuming different levels of fluoride in drinking water: an observational study. Contemp. Clin. Dent. 10, 24–30. https://doi.org/10.4103/ccd.ccd_108_18.

Shashi, A., Singla, S., 2013. Clinical and biochemical profile of deiodinase enzymes and thyroid function hormones in patients of fluorosis. Aust. J. Basic Appl. Sci. 7, 100–107.

Siddiqui, A.H., 1960. Incidence of simple goitre in areas of endemic fluorosis. J. Endocrinol. 20, 101–105. https://doi.org/10.1677/joe.0.0200101.

Singh, G., Kumari, B., Sinam, G., Kriti Kumar, N., Mallick, S., 2018. Fluoride distribution and contamination in the water, soil and plants continuum and its remedial technologies, an Indian perspective– a review. Environ. Pollut. 239, 95–108. https://doi.org/10.1016/j.envpol.2018.04.002.

Singh, N., Verma, K.G., Verma, P., Sidhu, G.K., Sachdeva, S., 2014. A comparative study of fluoride ingestion levels, serum thyroid hormone & TSH level derangements, dental fluorosis status among school children from endemic and non-endemic fluorosis areas. SpringerPlus 3, 7. https://doi.org/10.1186/2193-1801-3-7.

Skórka-Majewicz, M., Goschorska, M., Żwierełło, W., Baranowska-Bosiacka, I., Styburski, D., Kapczuk, P., Gutowska, I., 2020. Effect of fluoride on endocrine tissues and their secretory functions – review. Chemosphere 260, 127565. https://doi.org/10.1016/j.chemosphere.2020.127565.

Spittle, B., 2016. Short stature, bone deformities, cognitive impairment, delayed dental eruption, and dental fluorosis as examples of fluoride-induced developmental disorders involving disturbed thyroid hormone metabolism and sonic hedgehog signalling. Fluoride 49, 95–101.

Strunecka, A., Strunecky, O., 2020. Mechanisms of fluoride toxicity: from enzymes to underlying integrative networks. Appl. Sci. 10, 7100. https://doi.org/10.3390/app10207100.

Susheela, A.K., Toteja, G.S., 2018. Prevention & control of fluorosis & linked disorders: developments in the 21(st) century - reaching out to patients in the community & hospital settings for recovery. Indian J. Med. Res. 148, 539–547. https://doi.org/10.4103/ijmr.IJMR_1775_18.

Szczuko, M., Splinter, J., Zapałowska-Chwyć, M., Ziętek, M., Maciejewska, D., 2019. Fluorine may intensify the mechanisms of polycystic ovary syndrome (PCOS) development via increased insulin resistance and disturbed thyroid-stimulating hormone (TSH) synthesis even at reference levels. Med. Hypotheses 128, 58–63. https://doi.org/10.1016/j.mehy.2019.05.007.

Till, C., Green, R., 2021. Controversy: the evolving science of fluoride: when new evidence doesn't conform with existing beliefs. Pediatr. Res. 90, 1093–1095. https://doi.org/10.1038/s41390-020-0973-8.

Ullah, R., Sohail Zafar, M., Shahani, N., 2017. Potential fluoride toxicity from oral medicaments: a review. Iran. J. Basic Med. Sci. 20, 841–848. https://doi.org/10.22038/ijbms.2017.9104.

Urbano, T., Filippini, T., Wise, L.A., Lasagni, D., De Luca, T., Sucato, S., Polledri, E., Malavolti, M., Rigon, C., Santachiara, A., Pertinhez, T.A., Baricchi, R., Fustinoni, S., Vinceti, M., 2022a. Associations of urinary and dietary cadmium with urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine and blood biochemical parameters. Environ. Res. 210, 112912 https://doi.org/10.1016/j.envres.2022.112912.

Urbano, T., Zagnoli, F., Malavolti, M., Halldorsson, T.I., Vinceti, M., Filippini, T., 2022b. Dietary intake of potentially toxic elements and children's chemical exposure. Curr Opin Environ Sci Health 30, 100393. https://doi.org/10.1016/j.coesh.2022.100393.

Veneri, F., Iamandii, I., Vinceti, M., Birnbaum, L.S., Generali, L., Consolo, U., Filippini, T., 2023a. Fluoride exposure and skeletal fluorosis: a systematic review and dose-response meta-analysis. Curr Environ Health Rep. https://doi.org/10.1007/s40572-023-00412-9.

Veneri, F., Vinceti, M., Generali, L., Giannone, M.E., Mazzoleni, E., Birnbaum, L.S., Consolo, U., Filippini, T., 2023b. Fluoride exposure and cognitive neurodevelopment: systematic review and dose-response meta-analysis. Environ. Res. 221, 115239 https://doi.org/10.1016/j.envres.2023.115239.

Vinceti, M., Filippini, T., Crippa, A., de Sesmaisons, A., Wise, L.A., Orsini, N., 2016. Meta-analysis of potassium intake and the risk of stroke. J. Am. Heart Assoc. 5, e004210 https://doi.org/10.1161/jaha.116.004210.

Wang, M., Liu, L., Li, H., Li, Y., Liu, H., Hou, C., Zeng, Q., Li, P., Zhao, Q., Dong, L., Zhou, G., Yu, X., Liu, L., Guan, Q., Zhang, S., Wang, A., 2020. Thyroid function, intelligence, and low-moderate fluoride exposure among Chinese school-age children. Environ. Int. 134, 105229 https://doi.org/10.1016/j.envint.2019.105229.

Wang, Y., Cui, Y., Zhang, D., Chen, C., Hou, C., Cao, L., 2022. Moderating role of TSHR and PTPN22 gene polymorphisms in effects of excessive fluoride on thyroid: a school-based cross-sectional study. Biol. Trace Elem. Res. 200, 1104–1116. https://doi.org/10.1007/s12011-021-02753-8.

Waugh, D.T., 2019. Fluoride exposure induces inhibition of sodium/iodide symporter (NIS) contributing to impaired iodine absorption and iodine deficiency: molecular mechanisms of inhibition and implications for public health. Int. J. Environ. Res. Publ. Health 16, 1086. https://doi.org/10.3390/ijerph16061086.

Wei, M., Ye, Y., Ali, M.M., Chamba, Y., Tang, J., Shang, P., 2022. Effect of fluoride on cytotoxicity involved in mitochondrial dysfunction: a review of mechanism. Front. Vet. Sci. 9, 850771 https://doi.org/10.3389/fvets.2022.850771.

Xing, D., Liu, D., Li, R., Zhou, Q., Xu, J., 2021. Factors influencing the reference interval of thyroid-stimulating hormone in healthy adults: a systematic review and meta-analysis. Clin. Endocrinol. 95, 378–389. https://doi.org/10.1111/cen.14454.

Xu, K., Feng, Z., Afrim, F.K., Ma, J., Yang, S., Zhang, X., Niu, Z., An, N., Du, Y., Yu, F., Zhou, G., Ba, Y., 2022. Interaction of fluoride exposure and CREB1 gene polymorphisms on thyroid function in school-age children. Chemosphere 303, 135156. https://doi.org/10.1016/j.chemosphere.2022.135156.

Yang, Y., Wang, X., Guo, X., Hu, P., 2008. The effects of high levels of fluoride and iodine on intellectual ability and the metabolism of fluoride and iodine. Fluoride 41 (4), 336–339.

Yasmin, S., Ranjan, S., null, H., D'Souza, D., 2013. Effect of excess fluoride ingestion on human thyroid function in Gaya region, Bihar, India. Toxicol. Environ. Chem. 95, 1235–1243. https://doi.org/10.1080/02772248.2013.847619.

Yu, L.Y., Cui, Y.S., Liu, H.L., 2018a. Effects of endoplasmic reticulum stress-induced apoptosis in thyroid injury caused by fluoride in rat. Zhonghua Yufang Yixue Zazhi 52, 1182–1187. https://doi.org/10.3760/cma.j.issn.0253-9624.2018.11.017.

Yu, X., Chen, J., Li, Y., Liu, H., Hou, C., Zeng, Q., Cui, Y., Zhao, L., Li, P., Zhou, Z., Pang, S., Tang, S., Tian, K., Zhao, Q., Dong, L., Xu, C., Zhang, X., Zhang, S., Liu, L., Wang, A., 2018b. Threshold effects of moderately excessive fluoride exposure on children's health: a potential association between dental fluorosis and loss of excellent intelligence. Environ. Int. 118, 116–124. https://doi.org/10.1016/j.envint.2018.05.042.

Zhang, S., Zhang, X., Liu, H., Qu, W., Guan, Z., Zeng, Q., Jiang, C., Gao, H., Zhang, C., Lei, R., Xia, T., Wang, Z., Yang, L., Chen, Y., Wu, X., Cui, Y., Yu, L., Wang, A., 2015. Modifying effect of COMT gene polymorphism and a predictive role for proteomics analysis in children's intelligence in endemic fluorosis area in Tianjin, China. Toxicol. Sci. 144, 238–245. https://doi.org/10.1093/toxsci/kfu311.

Zohoori, F.V., Omid, N., Sanderson, R.A., Valentine, R.A., Maguire, A., 2019. Fluoride retention in infants living in fluoridated and non-fluoridated areas: effects of weaning. Br. J. Nutr. 121, 74–81. https://doi.org/10.1017/s0007114518003008.

Zulfiqar, S., Rehman, S.u., Ajaz, H., Elahi, S., Zaman, W.u., Batool, N., Yasmeen, F., 2019. Correlation of water fluoride with body fluids, dental fluorosis and FT4, FT3 –TSH disruption among children in an endemic fluorosis area in Pakistan. Open Chem. 17, 465–474. https://doi.org/10.1515/chem-2019-0055.

Zulfiqar, S., Ajaz, H., Rehman, S.u., Elahi, S., Shakeel, A., Yasmeen, F., Altaf, S., 2020. Effect of excess fluoride consumption on urine-serum fluorides, dental state and thyroid hormones among children in "Talab Sarai" Punjab Pakistan. Open Chem. 18, 119–128. https://doi.org/10.1515/chem-2020-0012.