Environmental Health

RESEARCH Open Access

Human biomonitoring in support of the Minamata Convention: a case of phasing out dental amalgam

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Abstract
This study analysed urinary mercury (U-Hg) concentrations in 1412 Slovenian children across four human biomonitoring campaigns conducted between 2007 and 2024. Median U-Hg levels declined from 0.76 ng mL^{-1} (0.72 µg g^{-1} creatinine) in the 2007 PHIME cohort to 0.22 ng mL^{-1} (0.21 µg g^{-1} creatinine) in the 2018–2024 SLO-HBM-II cohort, paralleling a decrease in the prevalence (from 65 to 3%) and the average number of dental amalgam fillings in children. Multilevel mixed-effects models showed a consistent temporal decline in U-Hg that persisted after adjustment for demographic and environmental covariates. In contrast, the inclusion of the amalgam number substantially attenuated the time trend, indicating that reduced amalgam use likely contributed to the observed decrease. As amalgam prevalence fell, other sources of exposure, such as fish consumption, became relatively more prominent predictors of U-Hg, while children living in historically Hg-contaminated areas showed persistently higher levels. Although Slovenia had already phased down dental amalgam in children before ratifying the Minamata Convention, these long-term biomonitoring data illustrate how changes in exposure sources are reflected in internal Hg levels. The study demonstrates the value of repeated national HBM programmes for identifying dominant exposure pathways, investigating their evolution over time, and providing evidence relevant to effectiveness-evaluation frameworks under Article 22 of the Minamata Convention.

Highlights
- Phase-out of dental amalgam in children is reflected in decreasing urinary mercury
- Predominant exposure sources have shifted from dental amalgam towards dietary and environmental
- HBM is a key tool for evaluating exposure trends and informing policy decisions
- Continued HBM is essential for exposure assessment and health protection

Keywords Human biomonitoring, Dental amalgam, Mercury, Minamata Convention, Children

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Introduction

Mercury (Hg) is a naturally occurring environmental pollutant that is toxic to humans and wildlife [6, 18]. It is recognised by the World Health Organisation as one of the top ten chemicals of major public health concern due to its persistence, bioaccumulation, and adverse health effects [59]. In the environment, it exists in three main forms: elemental mercury (Hg^0), inorganic mercury (Hg^{+1} and Hg^{+2}), and organic mercury species such as methylmercury (MeHg) and dimethylmercury (Me_2Hg), each exhibiting distinct toxicokinetic properties [6, 18, 32]. MeHg and Hg vapor are of particular concern, as they readily cross the blood–brain and placental barrier and can affect the central nervous system [27, 62]. Fetuses and young children are especially vulnerable, as early-life exposure can result in irreversible neurodevelopmental damage [2, 9, 20, 22, 25]. Global exposure to Hg varies geographically and demographically, with the general population exposed to MeHg primarily through fish and seafood, and to Hg^{2+} and Hg^0 through consumer products and dental amalgam [6, 9, 10, 18, 24, 25, 32, 62].

Amalgam has been in clinical use for more than 150 years, largely due to its durability and low cost. However, its composition, of approximately 50% Hg^0, has raised longstanding concerns about human exposure to Hg vapor released during the placement, use, and removal of amalgam fillings, affecting not only patients but also dental workers who may experience occupational exposures during these procedures [33, 45]. The extent of exposure depends on the number and surface area of restorations, individual oral habits, the body's ability to excrete Hg, the age and quality of the amalgams, as well as the frequency of consuming hot food and drinks [12, 21, 44, 62]. Hg vapor is continuously released from amalgam fillings, particularly during chewing and tooth brushing, and may accumulate in various organs, especially the kidneys and brain [4, 41]. For most individuals, daily Hg exposure from amalgam is estimated to be below 5 µg, although reported intakes vary between 1 and 27 µg per day [33, 63].

Beyond individual exposure, the dental sector is a non-negligible contributor to environmental Hg pollution. Globally, it consumes 270–340 tons of Hg annually, of which 70–100 tons (20–30%) enter the waste stream through disposal, incineration, and wastewater discharges [23, 55]. This highlights the dual challenge of amalgam use: potential health effects from direct exposure to Hg vapor and its broader environmental footprint.

To address these and other risks, the international community adopted the Minamata Convention on Mercury in 2013, which entered into force in 2017. This legally binding treaty mandates a range of actions to reduce Hg use and emissions, including bans on new Hg mining, phase-outs of Hg-added products, and controls on emissions. In dentistry, it calls for a phase-down of amalgam use, especially in children under 15 years, pregnant women, and nursing mothers, while promoting Hg-free alternatives and environmentally sound waste management [14, 56, 58, 60, 62].

Recognizing the need to monitor the effectiveness of regulatory measures, Article 22 of the Minamata Convention calls for the use of human biomonitoring (HBM) to assess exposure trends over time. At present, however, no universally agreed methodology, standardization framework, or timeline for such monitoring has been established under the Convention. HBM typically involves measuring total Hg or its species in biological matrices such as urine, blood, or hair, providing reliable indicators of cumulative exposure, with the relative contribution of different exposure routes depending on the matrix analyzed [9, 17, 47]. For Hg^0 exposure from amalgam, urinary total mercury (U-Hg) is considered the most appropriate biomarker, reflecting long-term inhalation exposure to Hg vapor [29, 42]. However, U-Hg is also influenced by dietary MeHg intake, since a fraction of ingested MeHg undergoes demethylation to inorganic $Hg2^+$, which is subsequently excreted in urine [4, 42, 49]. Thus, U-Hg reflects not only inorganic and elemental Hg exposure (e.g., from dental amalgam), but also, to a lesser extent, MeHg exposure from fish and seafood. This dual origin is particularly relevant in the interpretation of biomonitoring data and is one of the reasons why fish consumption is included as a covariate in statistical models.

Slovenia has aligned its legislation with the European Union (EU) Mercury Regulation (EU 2017/852), which prohibits the use of bulk amalgam and allows only predosed, encapsulated forms [37]. In 2021, the Slovenian Ministry of Health adopted a national plan for the complete phase-out of amalgam use by January 2025 [21, 51]. In line with these commitments and the Minamata Convention, Slovenia has also implemented a national HBM programme within the framework of the Chemicals Act, aimed at tracking environmental exposure trends and evaluating the effectiveness of health and environmental policies [43, 52].

Despite restrictions on amalgam use in children, pregnant and breastfeeding women, dental amalgam is still used in about 30% of restorations in Slovenia, primarily in adults, as it remains the only filling material fully reimbursed by the public health insurance system [14, 21, 44, 61]. However, population-based studies on Hg exposure from dental amalgam are still scarce. To date, the only Slovenian investigation was focused on dental personnel and was conducted more than three decades ago [35].

In response to this gap, and in line with the evaluation requirements of the Minamata Convention, this study uses human biomonitoring data on U-Hg in Slovenian children to characterise long-term exposure trends and major determinants, and to illustrate how such information can support future assessments of amalgam phase-down measures in settings where changes occur in the post-ratification period. Special attention is given to children because of their susceptibility and because they represent a key target group of the Convention's provisions. The present work aims to evaluate children's overall Hg exposure by analysing U-Hg, describe temporal trends in U-Hg from 2007 to 2024, examine the association between U-Hg and the presence and number of dental amalgam fillings, explore geographical variation by comparing children from historically contaminated, urban and rural areas, and assess the contribution of dietary factors, particularly fish consumption, as amalgam use declines. By addressing these objectives, this work provides empirical evidence on trends in Hg exposure among children and shifts in the relative importance of exposure sources, and illustrates how national HBM data can inform effectiveness evaluations under Article 22 of the Minamata Convention.

Methods

Study population

The target population consisted of school-aged children (6–11 years) living in Slovenia. Participants were recruited through four different cross-sectional biomonitoring studies conducted between 2007 and 2024: PHIME (2007), DEMOCOPHES (2011–2012), CROME (2016), and SLO-HBM-II (2018–2024). Basic characteristics of each study are summarised in Table 1, additional details in the Supplementary Material Table S1.

The pooled dataset comprised 1412 children; 1388 had valid urinary Hg (U-Hg) measurements and 1386 had valid creatinine-adjusted values. All sampling campaigns were designed and conducted by the same research group at the Department of Environmental Sciences, Jožef Stefan Institute, and all urinary Hg analyses were performed in the same laboratory.

Children were recruited through primary schools in predefined urban, rural, and historically mercury-contaminated towns, using structured convenience sampling with approximately balanced sex distributions and at least three years of residence in the area. This non-probability design was intended to capture key exposure settings rather than to obtain a nationally representative sample. Most towns were sampled only once (within a single study period), whereas the capital city of Ljubljana (urban) and the town of Idrija (former Hg mining area), were included in multiple campaigns. Initial contact was established with school headmasters, followed by informational meetings with parents, and ultimately, children. Written informed consent was obtained from a parent or guardian for each participant, and oral consent was obtained from each child prior to sampling.

Parents completed detailed questionnaires covering dietary habits, dental health, socioeconomic background, residential conditions, and basic medical history. For the present work, the following variables were extracted:

Table 1 Overview of Slovenian child cohorts included in the present analysis. More detailed information is available in the Supplementary Table S1

	PHIME[a]	DEMOCOPHES[b]	CROME[c]	SLO-HBM-II[d]
Study characteristics				
Sampling year(s)	2007	2011–2012	2016	2018–2024
Study area(s)	Urban ($n=1$), rural ($n=1$), Hg-contaminated ($n=1$)	Urban ($n=1$), rural ($n=1$)	Urban ($n=1$)	Urban ($n=7$), rural ($n=1$), (potentially) Hg-contaminated ($n=3$)
N of children[e]	180	155	178	899[e]
References	[1–4]	[5]	[6]	[7]
Analytical method				
Sample	Spot morning urine	Spot morning urine	Spot random urine	Spot morning urine
Sample preparation	Acid digestion (hot plate)	Acid digestion (hot plate)	Acid digestion (microwave)	Acid digestion (microwave)
THg determination	CVAAS	CVAAS	ICP-MS	ICP-MS
LOD (ng mL^{-1})	0.10	0.05	0.06	0.04
% samples < LOD	3.0	0.0	9.7	4.5
Reference materials	ClinCheck L1	ClinCheck L1	ClinCheck L1 Seronorm Urine L1, L2 SRM 3668 L1, L2	ClinCheck L1 Seronorm Urine L1, L2
References	[8–11]	[8–11]	[12]	[12]

1] Kobal et al., 2017 [36]; [2] Hruba et al, 2012 [31]; [3] Hruba et al., 2023 [30]; [4] Snoj Tratnik et al., 2019 [53]; [5] Den Hond et al., 2015 [17]; [6] Bravo et al., 2020 [11]; [7] Stajnko et al., 2020 [54]; [8] Akagi, 1997 [1]; [9] Horvat et al., 1991 [28]; [10] Miklavčič et al., 2011 [39]; [11] Miklavčič et al., 2013 [40]; [12] Kek et al, 2025 [34]

[a]Public Health Impact of Long-term, Low-level Mixed Element Exposure in Susceptible Population Strata

[b]Demonstration of a European Human Biomonitoring System

[c]Cross-Mediterranean Environment and Health Network

[d]Second National Human Biomonitoring Programme, Slovenia

[e]Refers to the total number of recruited children. For SLO-HBM-II, a subsample consisting of children aged 6–9

child's age, sex, height, weight, residence area type, presence and number of dental amalgam fillings, and fish consumption habits.

To create a harmonised dataset, variable definitions were aligned across studies. Anthropometric and demographic data were collected consistently, whereas fish consumption questions differed. Therefore, fish intake was harmonised into a three-level categorical variable representing the highest common resolution: low (< 1 meal per month), moderate (1–3 meals per month), and high (> 3 meals per month). It should be emphasised that this categorisation was created specifically for the purpose of this study and is not intended to represent standardised definitions of low, medium or high fish consumption in a broader nutritional context beyond this study. A standardised portion size of ~ 150 g was assumed. Other key variables, including the number of dental amalgam fillings, were collected comparably across studies and required no additional harmonisation.

All studies were approved by the National Medical Ethics Committee of the Republic of Slovenia (approval numbers listed in Table S1). As these were observational biomonitoring studies, clinical trial registration was not applicable.

Urine collection, storage and analysis

Participants of the PHIME, DEMOCOPHES, and SLO-HBM-II studies provided a first-morning spot urine sample at their local primary school visit. Samples were collected into nitric-acid solution pre-cleaned vessels (or sterile vessels for PHIME) provided prior to the sampling. Participants of the CROME study provided a random spot sample (not first-morning) at their appointed visit to the Paediatric Clinic. All samples were transported to the clinical laboratory within 2 h at 2 °C, aliquoted, and stored at −20°C (PHIME, DEMOCOPHES) or −80°C (CROME, SLO-HBM-II) until analysis. In all studies, urinary creatinine concentrations were determined at the University Medical Centre Ljubljana using the compensated Jaffé reaction (Dimension biochemical analyser, Dade Behring) to account for urine dilution.

All measurements of total Hg in urine were performed at the Jožef Stefan Institute, Department of Environmental Sciences, however, the analytical methodology evolved over the 17 years (Table 1). In the initial cohorts (PHIME, DEMOCOPHES), total Hg was analysed using cold vapor atomic absorption spectrometry (CVAAS) after acid digestion on a hot plate. The limits of detection (LODs) were 0.10 ng mL^{-1} and 0.05 ng mL^{-1}, respectively. In later cohorts (CROME, SLO-HBM-II), measurements were performed using triple quadrupole inductively coupled plasma mass spectrometry (QQQ-ICP-MS) after microwave acid digestion. The respective LODs were 0.06 ng mL^{-1} and 0.04 ng mL^{-1} for CROME and SLO-HBM-II, respectively.

Throughout all four study cohorts, analytical reliability and long-term data comparability were ensured by rigorous, consistent Quality Assurance (QA) protocols, that required inclusion of at least one reference material in every analytical batch, monitoring reference material recoveries within the certified uncertainty range, verification that within-batch precision met predefined targets, and repetition of batches that did not fulfil the acceptance criteria. The reference materials used over the span of 17 years include ClinCheck Urine Control for Trace Elements L1 (RECIPE), Seronorm Urine L1 and L2 (SERO AS), and SRM 3668 L1 and L2, NIST. Importantly, ClinChek Urine L1 was consistently measured across all cohorts, providing essential indirect cross-calibration between the older CVAAS method and the newer ICP-MS method. These procedures, along with continuous participation in external interlaboratory comparison schemes, such as the German External Quality Assessment Scheme (G-EQUAS), confirmed that all results consistently fell within assigned acceptance ranges.

Statistical analysis

Descriptive statistics

Geometric means, minimum, maximum, and percentiles for urinary Hg were calculated for each study cohort using all participants with valid U-Hg measurements. Values below the limit of detection (LOD) were replaced by LOD/2. Differences between cohorts were evaluated using the Kruskal–Wallis test, with Dunn's post-hoc comparisons (Bonferroni-adjusted). Associations between the number of dental amalgam fillings and U-Hg levels were investigated using Spearman's correlation (ρ).

Assessment of temporal trends and determinants

Temporal trends in U-Hg levels were investigated on the pooled dataset using linear mixed-effects models (LMMs). This approach was selected to account for the hierarchical and unbalanced structure of the data, especially the nesting of individual children within towns and the repeated sampling of certain towns (Ljubljana, Idrija) across multiple years. A random intercept for town was included to capture residual spatial heterogeneity and unmeasured local context shared by children residing in the same place. The sampling year was included as a fixed effect to estimate the overall temporal trend across the entire dataset.

The outcome variable was natural log-transformed (ln(U-Hg)). Fixed-effect covariates included age, sex, BMI, urinary creatinine, and residence area type (urban as reference; rural; potentially contaminated, including the former Hg-mining town of Idrija and two towns with potential industrial Hg contamination). The primary predictors were sampling year (continuous), dental amalgams (binary or numeric, depending on the model), and fish consumption (three frequency levels: low, moderate, high). The final model specification was chosen based on likelihood ratio tests and favoured parsimony. For modelling purposes, BMI was standardised to BMI z-scores using the WHO growth reference for school-aged children and adolescents [15]. All continuous predictors were grand-mean centred. To characterise the temporal trend in U-Hg and identify its drivers, a two-stage modelling strategy was applied:

Stage 1: Reduction of the year effect (Primary Analysis)

A stepwise sequence of models was fitted to assess how much of the temporal decline in U-Hg was explained by reduced amalgam exposure. The following models were fitted:

- Model 1 (base model): sampling year as fixed effect and a random intercept for town (unadjusted time trend).
- Model 2: Model 1 additionally adjusted for demographic and contextual covariates (age, sex, BMI, urinary creatinine, residence area type, fish consumption).
- Model 3: Model 2 further adjusted for the number of amalgam fillings to test their mediating role.

The contribution of dental amalgams to the time trend was quantified by calculating the percent reduction of the year coefficient from Model 1 to Model 3, using the formula: % reduction = $(\beta_{Model1} — \beta_{Model3})/\beta_{Model1} \times 100$.

Stage 2: Effect Modification (Secondary Analysis)

Interaction terms were added to Model 3 to assess whether the temporal trend varied across subgroups. For this purpose, additional models were fitted:

- Model 4: amalgam included as a binary variable; the year × fish consumption interaction included to test whether the temporal trend differed by fish intake.
- Model 5: Model 4 with the addition of year × binary amalgam interaction to determine whether the difference between amalgam carriers and non-carriers changed over time.
- Model 6: Model 5 but with amalgam number instead of binary amalgam, to test whether the per-filling effect changed over time.

Missing data and sensitivity analysis

The overall data missingness was low: 94.1% of children (N = 1329) had complete data on every model predictor and were retained in the complete-case analysis (Table S2). As a sensitivity check, the same mixed-effects models were fitted to 20 multiply imputed data sets and to complete cases only. The parallel analyses produced similar inferences, therefore, the complete-case estimates are presented.

To formally compare effects between the earliest (PHIME) and most recent (HBM-II) cohort, we fitted multiple regression models to the pooled data, including study × amalgam and study × fish consumption interaction terms. A statistically significant interaction term indicates that the corresponding effect differs between cohorts.

All analyses and plotting were performed using R Statistical Software (version 4.4.1 (2024–06–14 ucrt)). The statistical significance level was set at $p < 0.05$.

Results

Urinary Hg concentrations (both in ng mL^{-1} and μg g^{-1} creatinine) varied significantly between the four study cohorts (Kruskal–Wallis, $p < 0.001$; Table S3). The highest median concentrations (both unadjusted and creatinine-adjusted) were observed in the earliest cohort (PHIME) and the lowest in the most recent, SLO-HBM-II cohort (Table 2; Table S4). When using creatinine-adjusted concentrations, the results of some pairwise comparisons shifted (Table S4). These discrepancies likely reflect variability in urinary creatinine concentrations, due to differences in physiological factors and sampling protocols (e.g., unlike other studies, CROME urine samples were not first-morning samples; [5, 8, 13, 48]). A consistent downward trend over time was evident within towns (Idrija and Ljubljana) sampled over multiple cohorts (Fig. 1 and Figure S2; Table S5). Across the pooled dataset, the number of amalgam fillings showed a significant positive correlation with U-Hg concentrations (Spearman's $\rho = 0.315$ and 0.340 for U-Hg in ng mL^{-1} and in μg g^{-1} creatinine; both $p < 0.001$; Figure S1).

The observed decline in U-Hg levels from the earliest to the latest cohort closely paralleled the reduction in amalgam prevalence in children. The proportion of children with at least one amalgam filling decreased over time, from 65% in PHIME, 37% in DEMOCOPHES, 12% in CROME, to only 3% in SLO-HBM-II. Moreover, even among amalgam carriers, the average number of fillings decreased across successive cohorts (Table 3; Fig. 1; Figure S3). In contrast, the overall prevalence of fish consumption remained consistently high across cohorts, while the proportion of children classified as 'high' consumers (> 3 fish meals per month) increased markedly in recent cohorts from 19.7% in PHIME, 48.9% in CROME, to 67.5% in SLO-HBM-II. Similar trends were observed in Idrija and Ljubljana over the years (Fig. 1; Figure S2; Table S5).

To formally assess temporal trends, a multilevel modelling approach with random intercepts for town was

Table 2 Summary of U-Hg concentrations (in ng mL^{-1} and in µg g^{-1} creatinine) by study cohort and year

Study cohort	Year	N[a]	Urinary Hg (ng mL^{-1})						Urinary Hg (µg g^{-1} creatinine)					
			GM	Min[b]	p25	p50	p75	Max	GM	Min	p25	p50	p75	Max
PHIME	2007	164	0.75	<LOD	0.38	0.76	1.54	12.4	0.78	0.04	0.38	0.72	1.46	10.3
DEMOCOPHES	2011	95	0.31	<LOD	0.13	0.27	0.58	4.79	0.26	0.05	0.14	0.22	0.53	3.80
	2012	60	0.34	<LOD	0.11	0.25	0.79	13.6	0.29	0.05	0.13	0.20	0.68	8.16
	Total	155	0.32	<LOD	0.13	0.25	0.59	13.6	0.27	0.05	0.13	0.22	0.56	8.16
CROME	2016	176	0.23	<LOD	0.09	0.23	0.55	5.04	0.49	0.02	0.25	0.45	0.84	11.7
SLO-HBM-II	2018	130	0.20	<LOD	0.13	0.21	0.33	3.19	0.21	0.05	0.13	0.20	0.31	3.97
	2019	233	0.18	<LOD	0.10	0.17	0.28	2.01	0.15	0.01	0.09	0.15	0.26	1.71
	2020	205	0.18	<LOD	0.11	0.18	0.32	4.07	0.18	0.01	0.10	0.18	0.29	4.05
	2021	37	0.22	<LOD	0.15	0.22	0.36	0.75	0.27	0.07	0.18	0.26	0.44	1.38
	2022	51	0.27	0.08	0.18	0.28	0.37	5.99	0.25	0.09	0.17	0.26	0.34	4.81
	2023	25	0.24	0.06	0.16	0.22	0.36	2.81	0.22	0.05	0.13	0.25	0.34	1.29
	2024	212	0.33	<LOD	0.21	0.33	0.53	2.85	0.31	0.04	0.18	0.31	0.51	2.98
	Total	893	0.22	<LOD	0.13	0.22	0.38	5.99	0.21	0.01	0.13	0.21	0.34	4.81

GM = geometric mean; Min = minimum; p25 = 25th percentile; p50 = 50th percentile (median); p75 = 75th percentile; Max = maximum

[a] Number of individuals with available urinary Hg concentration data. Missing data in µg g^{-1} creatinine: in 2007 ($n = 1$); in 2016 ($n = 1$)

[b] The LODs for the PHIME, DEMOCOPHES, CROME, and SLO-HBM-II were: 0.1 ng mL^{-1}, 0.05 ng mL^{-1}, 0.06 ng mL^{-1}, and 0.04 ng mL^{-1}, respectively

applied to the pooled dataset (2007 – 2024). The results of the models are presented in Tables 4 and 5. In the base model, Model 1, a significant decreasing trend in U-Hg was observed, with a geometric mean ratio (GMR) of 0.945 per year (95% CI: 0.929, 0.962; $p < 0.001$), indicating an average annual decline of approximately 5.5%. To ensure that this trend was not driven by changes in population characteristics, demographic and dietary confounders (urinary creatinine, age, sex, BMI z-score, residence area type) were added in Model 2. The time trend observed in the base model remained virtually unchanged after adjustment (GMR = 0.947). Regarding covariates, residence in potentially contaminated towns was associated with significantly higher U-Hg compared to urban areas (GMR = 1.986, $p = 0.004$). U-Hg was positively associated with urinary creatinine (GMR = 1.937, $p < 0.001$) and negatively associated with BMI z-score (GMR = 0.960, $p = 0.010$). Finally, to test the mediating role of dental amalgams, the number of amalgam fillings was added in Model 3. The addition of the number of dental amalgams substantially reduced the year effect, increasing the GMR to 0.977 (95% CI: 0.961, 0.994; $p = 0.008$). This corresponds to a 59% reduction in the magnitude of the temporal trend relative to the base model. It further implies that a large portion of the decline in U-Hg in children over time can be explained by the concurrent reduction in the prevalence and/or number of dental amalgams they had.

To further characterise the temporal trend and evaluate whether it differed across population subgroups, interaction terms were introduced in Models 4 – 6 (Table 5). Across all models, there was a significant interaction between year and fish consumption (Table 5). The interaction terms for moderate and high fish consumption were consistently > 1.0 (1.053–1.055 in Models 4 – 6 for high vs. low, all $p < 0.001$), indicating that, after adjustment for amalgam status and other covariates, the annual decline in U-Hg was substantially slower among children with higher fish intake. This further implies that, as dental amalgam prevalence decreases, the relative contribution of dietary sources to U-Hg becomes more prominent.

Regarding the interaction between dental amalgams effect and time, the results depended on whether amalgams were modelled as a binary or numerical variable. In Model 5, where amalgam status was included as a binary indicator (yes/no), the interaction of amalgam and time indicated a reduction in the difference between children with and without fillings over time (GMR = 0.938, $p < 0.001$). In other words, the difference in U-Hg levels between children with and without fillings narrowed significantly over time. However, when the exact number of fillings was used in the model instead (Model 6), the interaction term was not significant ($p = 0.800$), which suggests that the Hg contribution per individual filling

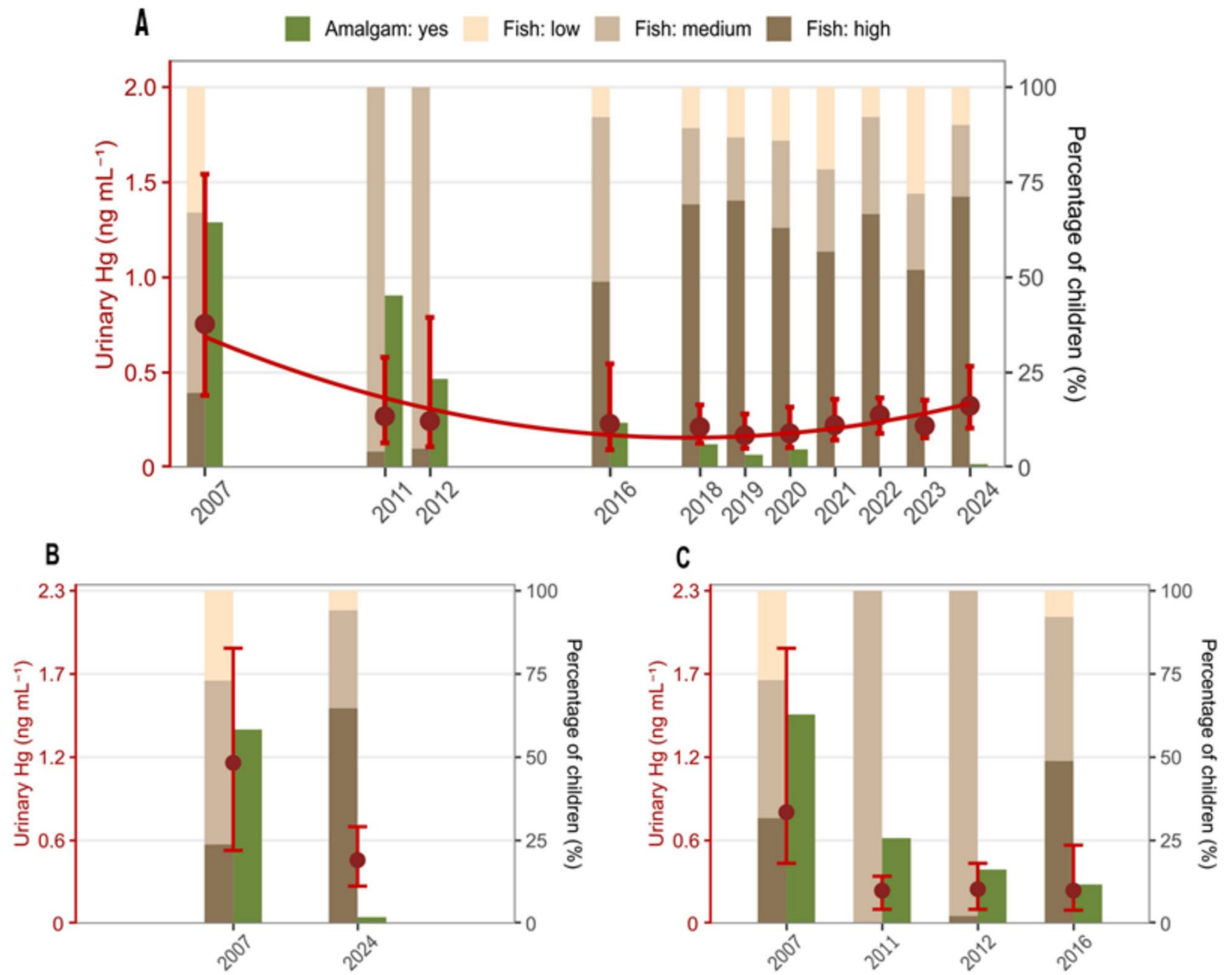

Fig. 1 Urinary mercury concentrations and exposure-related characteristics in the recruited Slovenian children. **A** All study sites combined: urinary Hg (ng mL.$^{-1}$; median and interquartile range, left y-axis) and, on the right y-axis, the percentage of participants with at least one dental amalgam and the percentages in low-, moderate-, and high-fish consumption groups by year (2007–2024). **B** Same metrics restricted to children from Idrija (data available for two sampling years). **C** Same metrics restricted to children from Ljubljana (data available for four sampling years)

remained essentially stable over time. Consequently, the narrowing difference observed in the binary model is consistent with a reduction in the number of amalgam fillings per child in more recent cohorts (rather than, for example, a change in the amount of Hg released per filling).

The findings from multilevel modelling were confirmed by the multiple regression model fitted on the pooled PHIME and HBM-II datasets. In both the binary- and numeric-amalgam models, HBM-II children showed significantly stronger associations between fish intake and U-Hg compared with PHIME, as indicated by significant positive interaction terms for HBM-II × fish consumption (Supplementary Material, Tables S6 – 7), indicating that fish consumption played a larger role in later years. In contrast, the interaction between study and amalgam status (yes/no) was negative and borderline significant ($\beta \approx -0.37$, $p = 0.085$), suggesting a weaker difference between amalgam carriers and non-carriers in HBM-II than in PHIME, but with confidence intervals including no effect. When the actual number of fillings was used instead of the binary amalgam variable, the interaction with study was small and not statistically significant, indicating that the per-filling contribution to U-Hg was stable across cohorts.

Discussion

This study represents the first investigation in Slovenia to assess the relationship between U-Hg concentrations in children and their exposure to Hg via dental amalgam fillings. Overall, U-Hg concentrations observed in Slovenian children were low and comparable to those reported in other European countries, with most values below 3 ng mL^{-1}, typical for general background populations [6]. The highest median U-Hg concentration was recorded in the earliest cohort, PHIME (0.76 ng mL^{-1}

Table 3 Summary of basic participant characteristics by study cohort and year

Study cohort	Year	N[a]	Age[b] ($\bar{x} \pm SD$)	Male (%)	BMI[c] ($\bar{x} \pm SD$)	Amalgam[d]		Fish consumption[e]		
						Yes (%)	N ($\bar{x} \pm SD$)	Low (%)	Moderate (%)	High (%)
PHIME	2007	180	9.2 ± 1.2	50.6	17.5 ± 3.39	64.5	3.02 ± 2.21	32.9	47.4	19.7
DEMOCOPHES	2011	95	8.6 ± 1.7	44.2	17.1 ± 2.71	45.3	2.33 ± 1.61	0.00	95.8	4.20
	2012	60	9.2 ± 2.0	53.3	16.9 ± 3.04	23.3	2.57 ± 1.70	0.00	95.0	5.00
	Total	155	8.8 ± 1.8	47.7	17.1 ± 2.83	36.8	2.39 ± 1.62	0.00	95.5	4.50
CROME	2016	178	7.7 ± 0.5	50.0	16.6 ± 6.93	11.8	2.19 ± 1.29	7.90	43.3	48.9
SLO-HBM-II	2018	130	8.2 ± 0.9	41.5	17.3 ± 3.68	6.15	2.62 ± 1.30	10.8	20.0	69.2
	2019	236	7.9 ± 0.9	50.0	16.6 ± 2.78	3.39	1.57 ± 0.79	13.2	16.6	70.2
	2020	208	8.1 ± 0.9	60.1	16.7 ± 2.65	4.81	1.56 ± 0.73	14.1	22.9	63.0
	2021	37	8.1 ± 0.9	45.9	16.3 ± 1.94	0.00	-	21.6	21.6	56.8
	2022	51	8.0 ± 0.9	43.1	16.3 ± 2.24	0.00	-	7.80	25.5	66.7
	2023	25	8.3 ± 0.8	52.0	16.6 ± 2.57	0.00	-	28.0	20.0	52.0
	2024	212	8.1 ± 0.8	44.8	17.4 ± 2.78	0.94	1.0 ± 0.00	9.90	18.9	71.2
	Total	899	8.1 ± 0.9	49.4	16.9 ± 2.86	3.11	1.85 ± 1.05	12.7	19.8	67.5

$\bar{x}$ = arithmetic mean; SD = standard deviation; BMI = body mass index; Amalgam Yes = indicator that child has dental amalgam filling(s); fish consumption: low = < 1 fish meal per month; moderate = 1–3 fish meals per month; high = > 3 fish meals per month

[a]Total number of recruited individuals

[b]Missing data on age: in 2016 (*n* = 1)

[c]Missing data on BMI: in 2007 (*n* = 12); in 2016 (*n* = 1); in 2020 (*n* = 1)

[d]Missing data on amalgam presence/number: in 2007 (*n* = 28)

[e]Missing data on fish consumption: in 2007 (*n* = 7); in 2019 (*n* = 1); in 2020 (*n* = 16)

Table 4 Multilevel modelling of the temporal trend in urinary Hg concentrations (2007–2024). Year effect reduction (%) represents the percentage of the temporal decline explained by the inclusion of the dental amalgam number relative to the base model (Model 1)

	Model 1		Model 2		Model 3	
	Estimate (SE)[a]	*p*-value	Estimate (SE)[a]	*p*-value	Estimate (SE)[a]	*p*-value
Fixed effects						
Intercept	0.301 (0.031)	<.001	0.224 (0.027)	<.001	0.220 (0.024)	<.001
Year (ratio of change per 1 year)	0.945 (0.008)	<.001	0.947 (0.008)	<.001	0.977 (0.008)	0.008
Amalgam number (per filling)					1.256 (0.029)	<.001
Fish: moderate vs low			1.058 (0.084)	0.479	1.032 (0.079)	0.680
Fish: high vs low			1.395 (0.104)	<.001	1.365 (0.098)	<.001
Age (per 1 year)			0.998 (0.023)	0.915	1.018 (0.022)	0.406
Sex: F vs M			0.916 (0.043)	0.059	0.927 (0.042)	0.090
BMI z-score (per 1 unit)			0.960 (0.015)	0.010	0.961 (0.015)	0.009
Urinary creatinine						
Rural vs urban			0.985 (0.195)	0.938	1.014 (0.184)	0.942
Potentially contaminated vs urban			1.986 (0.410)	0.004	1.803 (0.342)	0.006
Random effects						
Variance within towns (Residual)	0.870		0.700		0.652	
Variance between towns	0.135		0.068		0.055	
Model fit						
AIC	3624		3346		3251	
BIC	3645		3409		3319	
ICC	0.135		0.088		0.078	
Year effect reduction (%)	Ref		2.40		59.0	

[a]The regression coefficients (β) from models with ln(U-Hg) as the outcome were exponentiated to obtain multiplicative effects (ratios) for U-Hg. For continuous predictors, an estimate < 1 indicates a decrease, and an estimate > 1 an increase, in U-Hg associated with a one-unit increase in the predictor. For categorical predictors, estimates represent the ratio of U-Hg in each category relative to the reference category (estimate < 1 indicates lower uHg and estimate > 1 higher U-Hg compared with the reference)

Table 5 Multilevel mixed-effects models assessing effect modification of the temporal trend in urinary Hg (2007–2024). Models include interaction terms (year × predictor) to test if temporal trends differ across subgroups

	Model 4		Model 5		Model 6	
	Estimate (SE)a	*p*-value	Estimate (SE)a	*p*-value	Estimate (SE)a	*p*-value
Fixed effects						
Intercept	0.199 (0.021)	<.001	0.192 (0.020)	<.001	0.219 (0.024)	<.001
Year (ratio of change per 1 year)	0.937 (0.012)	<.001	0.949 (0.012)	<.001	0.940 (0.012)	<.001
Amalgam number (per filling)					1.277 (0.060)	<.001
Amalgam (Yes vs No)	2.187 (0.173)	<.001	1.642 (0.176)	<.001		
Fish: moderate vs low	1.049 (0.081)	0.535	1.049 (0.081)	0.535	1.058 (0.082)	0.462
Fish: high vs low	1.336 (0.099)	<.001	1.336 (0.099)	<.001	1.333 (0.099)	<.001
Age (per 1 year)	1.016 (0.022)	0.469	1.020 (0.022)	0.360	1.017 (0.022)	0.436
Sex: F vs M	0.937 (0.042)	0.146	0.935 (0.042)	0.129	0.930 (0.041)	0.103
BMI z-score (per 1 unit)	0.958 (0.015)	0.005	0.957 (0.015)	0.004	0.960 (0.015)	0.007
Urinary creatinine	1.939 (0.073)	<.001	1.934 (0.073)	<.001	1.935 (0.073)	<.001
Rural vs urban area	0.909 (0.157)	0.590	0.931 (0.158)	0.680	0.995 (0.175)	0.980
Pot. contaminated vs urban	1.763 (0.320)	0.005	1.707 (0.305)	0.007	1.743 (0.321)	0.007
Year × Fish (moderate vs low)	1.051 (0.014)	<.001	1.056 (0.014)	<.001	1.050 (0.014)	<.001
Year x Fish (high vs low)	1.053 (0.015)	<.001	1.053 (0.015)	<.001	1.055 (0.015)	<.001
Year × Amalgam (Yes vs No)			0.938 (0.015)	<.001		
Year × Amalgam number					1.001 (0.006)	0.800
Random effects						
Variance within towns (resid.)	0.648		0.641		0.643	
Variance between towns	0.049		0.047		0.050	
Model fit						
AIC	3247		3233		3240	
BIC	3325		3316		3323	
ICC	0.070		0.068		0.072	

[a]The regression coefficients (β) from models with ln(U-Hg) as the outcome were exponentiated to obtain multiplicative effects (ratios) for U-Hg. For continuous predictors, an estimate < 1 indicates a decrease, and an estimate > 1 an increase, in U-Hg associated with a one-unit increase in the predictor. For categorical predictors, estimates represent the ratio of U-Hg in each category relative to the reference category (estimate < 1 indicates lower uHg and estimate > 1 higher U-Hg compared with the reference)

or 0.72 μg g^{-1} creatinine), and the lowest in the latest, SLO-HBM-II (0.22 ng mL^{-1} or 0.21 μg g^{-1} creatinine). For health risk assessment, the German Human Biomonitoring Commission has established an HBM-I value of 7 ng mL^{-1} for urinary Hg in children, below which no adverse effects are expected [3]. In our pooled dataset, only six children exceeded this value, five of those in PHIME and one in DEMOCOPHES.

Consistent with international evidence, our findings confirm that dental amalgams are a major determinant of U-Hg in children. We observed a significant positive correlation between the number of dental amalgam fillings and U-Hg (Figure S1), in agreement with previous reports of a dose-dependent relationship between amalgam burden and urinary Hg [19, 38, 62].

A clear downward trend in U-Hg was observed from 2007 to 2024. Our results suggest that much of this decline can be attributable to the reduction of amalgam use in children. The proportion of children with at least one dental amalgam filling decreased from 65% in PHIME to only 3% in SLO-HBM-II, and the mean number of fillings among amalgam carriers also decreased from an average of 3.02 in 2007, to 1.00 in 2024 (Table 3). When the amalgam number was added to the time trend model (Table 4, Model 3), the estimated year effect was substantially decreased, indicating that reduced amalgam use appears to account for a large share of the observed time trend. Moreover, the interaction results showed that the difference between children with and without amalgams narrowed over time when amalgam was modelled as a binary (yes/no) variable (Table 5, Model 5), but not when modelled as the actual number of fillings (Table 5, Model 6). This suggests that the Hg contribution per filling remained stable, and the difference between the groups (amalgam carriers and non-carriers) shrank because children in more recent cohorts had fewer fillings.

On the other hand, the relative importance of fish consumption increased over time. Although the overall fish consumption prevalence (i.e., number of children eating at least one fish meal per month) remained high across cohorts, the proportion of children reporting frequent consumption (> 3 fish meals per month) increased from ~ 20% in 2007 to 71% in 2024, with similar observations in the two towns sampled multiple times over the years (Idrija and Ljubljana). Interaction analyses suggest that the decreasing trend of U-Hg was slower among frequent fish consumers (Table 5, Models 4 – 6, interactions of fish x year), indicating increasing relative contribution of dietary Hg as exposure from amalgams decreased. Multiple regression models comparing PHIME and SLO-HBM-II confirmed stronger fish consumption and U-Hg associations in the recent cohort (Tables S6 – 7). This, however, does not imply increased absolute dietary exposure, but rather a redistribution of sources as one major source of exposure (amalgam) decreases. Similar patterns have been reported in other HBM datasets [4, 6, 30, 42, 49].

The environmental context also contributed to variability in exposure. Children living in historically Hg-contaminated areas, such as Idrija (a former Hg-mining town), and in certain industrial zones had higher U-Hg levels (Tables 4 and 5, Models 2 – 6), consistent with previous work demonstrating persistent impacts of legacy pollution on local populations [4, 36]. These findings underline the importance of interpreting HBM data within both environmental and policy contexts, and they support the need for ongoing environmental surveillance and targeted remediation. This is in line with recommendations from European HBM coordination initiatives to systematically include legacy contamination sites in national monitoring frameworks [17, 50].

An important methodological consideration concerns creatinine adjustment. Although creatinine correction is widely used to account for urine dilution, differences in sampling time and inter-individual variability in creatinine excretion (e.g. due to age, sex, muscle mass, hydration, and diet) can introduce bias [5, 48]. In our dataset, the afternoon sampling protocol used in CROME likely contributed to discrepancies between unadjusted and creatinine-corrected U-Hg results. This highlights the need to carefully consider sampling protocols and creatinine variability when comparing U-Hg across studies, as these factors may influence the conclusions.

Article 22 of the Minamata Convention establishes a framework for the global evaluation of the treaty's effectiveness, relying on indicators such as environmental monitoring, human biomonitoring, and information on regulatory implementation. These evaluations occur at the international level, nonetheless, national human biomonitoring programs represent a key source of evidence for tracking exposure trends and understanding changes in dominant exposure pathways. In this context, our study provides insight into how a major Hg^0 exposure source, dental amalgam, declined in Slovenia over nearly two decades, and how this decline was reflected in biomarker concentrations in children.

Slovenia ratified the Minamata Convention in 2018, however, our data demonstrates that the reduction in dental amalgam use among children began well before the Convention entered into force, with only 12% of the sampled children reporting having dental amalgams in 2016, compared to over 64% in 2007. This pattern likely reflects evolving clinical guidelines, improved preventive oral health programs, increased availability of composite restorative materials, and changing preferences among both clinicians and patients [61]. Slovenia is not unique in this regard, as several countries had already phased down or banned dental amalgam independently of Minamata, including Norway (2008), Sweden (2009), and others with partial or full restrictions (e.g., Japan, Switzerland, France, Germany, Finland, Austria, Canada) [26, 57].

Because the major decline in amalgam use in Slovenian children occurred before Minamata implementation, our findings cannot be interpreted as a direct assessment of Convention effectiveness in Slovenia. However, they provide an example of how HBM data can support effectiveness evaluations elsewhere by illustrating the type of evidence directly relevant for Article 22: long-term trends in internal exposure, shifts in dominant sources linked to policy or practice changes, quantification of how specific reductions relate to biomarker declines, and identification of residual exposure pathways (e.g., dietary, or environmental factors). By linking long-term trends in U-Hg to changes in dental amalgam prevalence and burden, and by quantifying how much of the temporal decline is explained by reduced amalgam use, our results suggest that declining dental amalgam use likely contributed to lower internal Hg levels in children. At the same time, as amalgam use decreases, dietary and other sources account for an increasing share of the residual exposure. This type of combined information on exposure trends, sources and determinants is directly relevant for the effectiveness evaluation framework.

Even though the use of dental amalgams in Slovenian children at the present moment is no longer concerning, amalgam-related exposure is still relevant in adults, especially among socioeconomically vulnerable groups, as it remains the only restorative material fully reimbursed by the national health insurance system [14, 44, 61]. Dental professionals are also directly exposed to Hg vapor during amalgam handling, making them an additional group that would benefit from the full transition to amalgam-free dentistry [45].

It is also worth noting that an important value of human biomonitoring is its participatory dimension. By involving citizens directly in the monitoring process, HBM raises awareness of exposure sources and empowers individuals to make informed decisions to reduce their personal risks [16, 46].

This study has several limitations. First, the analyses are based on repeated cross-sectional, observational data rather than longitudinal follow-up, so the temporal patterns in U-Hg reflect population-level changes and cannot be interpreted as causal at the individual level. The samples of children included in the present study were not designed to be fully nationally representative, therefore, the findings reflect the characteristics of the sampled populations rather than the entire national child population. Next, although we adjusted for key covariates (age, sex, BMI, creatinine, residence area type, fish intake), residual confounding by unmeasured factors such as oral health practices, socioeconomic status, and interindividual differences in Hg metabolism cannot be excluded. It also relies on self-reported amalgam and dietary data, and lacks detailed fish species information. Moreover, most towns were sampled only once, and only two were repeatedly sampled across cohorts, which limits the ability to fully disentangle temporal from spatial variability.This further means that, while the direction of the amalgam–U-Hg association and its contribution to the temporal decline appear robust, the precise magnitude of effect should be interpreted with caution.

Conclusions

Over the past 17 years, urinary Hg concentrations in Slovenian children have decreased significantly, from the median of 0.76 ng mL^{-1} (0.72 μg g^{-1} creatinine) in PHIME (2007) to 0.22 ng mL^{-1} (0.21 μg g^{-1} creatinine) in SLO-HBM-II (2018–2024). Over the same period, the prevalence of dental amalgam fillings in children dropped from 64% to around 3%, with a parallel reduction in the number of fillings among carriers. Multilevel models confirmed that dental amalgams remain a significant determinant of U-Hg, and their inclusion in the models explained a significant portion of the observed time trend, showing that reduced amalgam prevalence may explain part of the decline observed during the study period. Decreased use of amalgams allowed for the influence of other exposure sources (such as dietary) to become more apparent.

These findings demonstrate the value of long-term human biomonitoring for tracking changes in exposure sources and for informing policy. Although most of the decline in dental amalgam use in Slovenia occurred before ratification of the Minamata Convention, the patterns observed here, such as declining internal exposure, identification of major contributing sources, and quantification of their changing influence, provide the type of evidence that directly supports effectiveness-evaluation frameworks under Article 22. Continued investment in national human biomonitoring studies will be essential to monitor residual exposures, assess the impact of remaining sources (including dietary and environmental), and guide future public health strategies aimed at minimising mercury exposure.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12940-025-01255-7.

Supplementary Material 1.

Acknowledgements

The authors acknowledge the financial support from the Slovenian Research and Innovation Agency (ARIS) programme group P1-0143 and the MultiPart project (J7-60123).

The authors are grateful to all children who participated in this study and the personnel of the Regional Health Centres, who collaborated in recruiting participants and collecting biological samples.

Authors' contributions

V.U. and A.A.O. contributed equally to the study. V.U. and A.A.O. designed the research, performed data analysis, and drafted the manuscript. J.S.T., M.J.H., and D.M. contributed to data acquisition, interpretation, and critical review of the manuscript. D.Ko. and D.Ko. (Kontić) assisted in data curation, model development, and policy contextualization. M.H. supervised the study, provided methodological guidance, and contributed to the final manuscript revision. All authors reviewed and approved the final version of the manuscript.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request. The biomonitoring data were obtained from the national and European HBM studies (PHIME, DEMOCOPHES, CROME, and SLO-HBM-II) conducted between 2007 and 2024. Access to the raw data is subject to ethical and data protection regulations. Clinical trial number: not applicable.

Declarations

Competing interests

The authors declare no competing interests.

Received: 5 October 2025 / Accepted: 21 December 2025
Published online: 05 January 2026

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